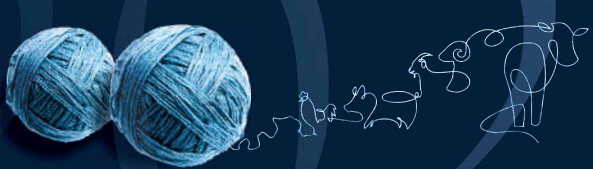
13th
INTERNATIONAL
SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

2021



PROCEEDINGS

6 - 8 October 2021, Belgrade, Serbia

Institute for Animal Husbandry

Belgrade - Zemun, SERBIA

13th INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

2021

PROCEEDINGS

6 -8 October 2021, Belgrade, Serbia

PATRON
Ministry of Education, Science and Technological Development of the Republic of Serbia

ORGANIZER
Institute for Animal Husbandry
Autoput 16, P. Box. 23,
11080, Belgrade-Zemun, Serbia
Tel: +381 11 2691 611; +381 11 2670 121; +381 11 2670 541;
Fax: + 381 11 2670 164;

PUBLISHER
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Editor-in-Chief
Čedomir Radović, PhD, Senior Research associate
Director of the Institute for Animal Husbandry, Belgrade-Zemun

Editor
Zdenka Škrbić, PhD, Principal Research Fellow
Institute for Animal Husbandry, Belgrade-Zemun

The Proceedings is printed by the Institute for Animal Husbandry, Belgrade, 2021

biotechnology.izs@gmail.com
www.istocar.bg.ac.rs
Circulation 100 copies.

ISBN 978-86-82431-77-0

INTERNATIONAL SCIENTIFIC COMMITTEE

CHAIRMAN

Prof. Dr. **Giacomo Biagi,**
Department of Veterinary Medical Sciences, University of Bologna, Italy

SECRETARY

Dr. **Čedomir Radović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Dr. **Zdenka Škrbić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Veselin Petričević,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Marjeta Čandek-Potokar,**
Agricultural Institute of Slovenia, Slovenia
Dr. **Giuseppe Bee,**
Agroscope Posieux, Posieux, Switzerland
Prof. Dr. **Elena Kistanova,**
Institute of Biology and Immunology of Reproduction „Kiril Bratanov“, Sofia, Bulgaria
Prof. Dr. **Stayka Laleva ,**
Agricultural Institute, Stara Zagora, Bulgaria
Prof. Dr. **Galia Zamaratskaia,**
Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden
Dr. **Miloš Lukić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Vlada Pantelić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Maya Ignatova,**
Institute of Animal Science, Kostinbrod, Bulgaria
Dr. **Dragana Ružić-Muslić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Marina I. Selionova,**
FSBSI-All-Russian Scientific Research Institute of Sheep and Goat Breeding, Stavropol, Russia
Prof. Dr. **Radica Đedović,**
University of Belgrade, Faculty of Agriculture, Serbia

Dr. **Juan M. García Casco,**
Departamento Mejora Genética Animal, INIA, Madrid, Spain
Dr. **Slavica Stanković,**
Maize Research Institute „Zemun Polje“, Zemun Polje, Serbia
Dr. **Vesna S. Krnjaja,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Rui Miguel Carracha Charneca,**
Universidade de Évora, Escola de Ciências e Tecnologia,
Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Évora, Portugal
Dr. **Jean-Louis Peyraud,**
INRA, UMR PEGASE, France

ORGANIZING COMMITTEE

CHAIRMAN

Dr. **Čedomir Radović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

SECRETARY

Dr. **Dragan Nikšić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Dr. **Maja Petričević,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Marina Lazarević,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Nikola Delić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Ljiljana Samolovac,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Marija Gogić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

SYMPOSIUM SECRETARIAT

CHAIRMAN

Dr. **Dragan Nikšić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Slavko Maletić, grad. econ.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Olga Devečerski, grad. prof.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Nenad Mićić, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Bogdan Cekić, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Miloš Marinković, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Vladimir Živković, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Nenad Stojiljković, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Ivan Ćosić, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Tamara Stamenić, BSc,
Institute for Animal Husbandry, Belgrade-Zemun

Address:

Institute for Animal Husbandry,
Autoput 16, P. Box 23,
11080, Belgrade-Zemun, Serbia

Tel:
+381 11 2691 611
+381 11 2670 121
+381 11 2670 541
Fax:
+381 11 2670 164

E-mail: biotechnology.izs@gmail.com
www.istocar.bg.ac.rs

CONTENTS

INVITED PAPERS

ORAL PRESENTATION

POSTER PRESENTATION

NEW TRENDS IN PUBLISHING RESEARCH AND TRANSFERRING THE KNOWLEDGE ON ANIMAL PRODUCTION – presenting Animal Open Space and EUREKA project

Marjeta Čandek-Potokar[1], Giuseppe Bee[2]

[1] Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia
[2] Swine Research Unit, Agroscope Posieux, Posieux, Switzerland
Corresponding author: Giuseppe Bee, giuseppe.bee@agroscope.admin.ch
Invited paper

Abstract: Open science is not only about making research results freely available, but also about making them understandable to and reproducible for a wider public (audience) and not only to specialised scientists. It is thus influencing strongly the research environment, especially the way research results are disseminated and communicated. Many research results remain unpublished due to lack of novelty or insufficient strength of the experimental design, which is a loss to the particular knowledge community. If the research is reproducible and the associated data are available, publication can open up new opportunities and ideas for data reuse, contributing significantly to knowledge gain. Thus, the classical way of publishing research results needs alternative approaches. This paper presents two initiatives to disseminate results from animal production research and agriculture in general. The first one is the e-platform *animal open space* (ANOPES), intended for research results. It opens the possibility to publish research, methodological and data papers with faster review and is already operational. The second initiative is a proof-of-concept study of the EUREKA project to create an agricultural knowledge e-platform to strengthen the agricultural knowledge and innovation system of the European Union. For ANOPES, the scope and opportunities for publication are presented. For EUREKA, the analysis of data and knowledge objects created in a multi-actor projects and their relevance to the knowledge repository are presented.

Key words: animal production; open science; knowledge objects; e-repository

Introduction

Open science has for the objective to make scientific research results (e.g. publications, data, software) freely available to all stakeholders, amateur or professional (*Woefle et al, 2011*). Open science denotes a transparent and accessible knowledge shared and developed through the collaborative networks and generally making it easier to publish and communicate scientific knowledge (*Vicente-Saez et al., 2018*). According to *Eve (2014)*, for a piece of academic research to be called open access (OA), it must be available digitally for anybody to read at no financial cost beyond those intrinsic to using the internet; it means implementing a new system that allows free access to peer-reviewed scholarly research on the world wide web. The term also means that people should be able to reuse this material beyond the provisions of fair use enshrined in copyright law, as long as the author is credited. This policy has been rapidly spreading and has strongly affected the scientific environment, in particular the publishing. For example, the policy of open science has been implemented in European Union research programmes, and unless the results need to be protected, there is a contractual obligation for open access dissemination. Thus science becomes more transparent and accessible to society at large and can contribute to boosting the innovation potential, as suggested by *Woefle et al. (2011)* in a paper Open science is a research accelerator. OA also contributes to more efficient research and increases its impact.

It should be mentioned that open access (OA) of research results demands that FAIR data principles are respected (FAIR stands for Findable, Accessible, Interoperable and Reusable). The acronym and principles were defined in a paper that was published in 2016 the journal Scientific Data (*Wilkinson et al., 2016*) and emphasise the ability of machine-based operation i.e. the capacity of computational systems with none or minimal human intervention (FAIR principles). OA does not only refer to research papers being freely accessible, but covers the whole spectrum of research outputs like primary data produced or tools and methodologies developed in the research. The re-use of primary data is of particular interest as it contributes to avoiding additional experiments on animals, allows new ideas/analyses to be tested and used in larger context e.g. big data analysis. There is also a lot of research that has not been published due to shortcomings related to scientific soundness, insignificance of tested hypothesis or insufficient power of methodological approach which is a pity to be completely lost for the knowledge community. Knowledge community is strongly aware, that there is an urgent need to improve the infrastructure supporting the reuse of scholarly data (*Wilkinson et al., 2016*).

Besides creation of new knowledge, another issue to be considered in the context of knowledge availability is the innovation uptake, i.e. the transfer and implementation of knowledge into practice. The knowledge objects that emerge in the research community are diverse (see Fig. 1) and not always easy for various public to understand. Therefore, research papers are not and should not be the only outputs of a given research effort. While research papers are mostly produced for and intended for the scientific community, other types of knowledge objects developed by researchers are of interest and importance to practitioners. In particular those that convey a concise, condensed and practical message. When preparing or disseminating to farmers or practitioners, it is important to consider what and how to present the information - the content, the form and the communication channel.

Thus, the aim of this article is to present new trends and developments in the dissemination and communication of research results or knowledge transfer in animal production science. First, the platform for dissemination of research results related to animal production that has recently been established and put into operation is Animal Open Space. Second, the Horizon 2020 feasibility study of EUREKA project for the development of an e-platform for agricultural knowledge to facilitate knowledge transfer to end users. Both platforms respond to societal needs related to open science, FAIR principles and better research impact, and represent an innovative approach to the provision and/or transfer of knowledge (know-how) for enhanced innovation uptake in the field of animal production.

Animal Open Space – concept and scope

On June 28 2021, the animal consortium, which is a collaboration between the British Society of Animal Science (BSAS), the Institut National de la Recherche pour l’Agriculture, l’Alimentation et l’Environnement (INRAe) and the European Federation for Animal Science (EAAP) launched with the journal animal – open space a new publishing initiative. This new journal is part of a family of journals including the flagship journal animal and animal – science proceedings.

Compared to other known journal in animal science, animal - open space fully embraces Open Science. Its philosophy is that all reproducible research, the data linked to that research and the associated points of views of the authors contribute to knowledge gain. Therefore, this knowledge deserves to be rapidly published and open for comments once published. Thus, we would like to open the possibility of a discussion between author and reader using the platform PubPeer. The journal publishes articles that relate to farmed or other managed animals, leisure and companion animals and the use of insects for animal feed and human food. In animal – open space, articles can be accepted from all species if they are in, or contribute knowledge to, the aforementioned categories (e.g. cattle, sheep, pigs, poultry, horses, rabbits, fish, cats, dogs). One key element is also that lack of novelty, negative results or lack of significant treatment differences are not a barrier for publication in this journal.

The objective of the animal consortium is that animal - open space will become an essential reading for all animal scientists, stakeholders and policy makers interested in agricultural, veterinary and environmental sciences with expected impacts on animal performance and productivity, animal welfare, animal health, food security, environment, climate change, product quality, human health and nutrition, sustainability of animal agriculture, livestock systems and methodology. The impacts of the articles can be either of local or international relevance.

In the spirit of the Opens Science, animal – open space aims to publish open and reproducible research as data papers, method articles and research articles. The following characteristics are the key elements for the journal for all article types, it is mandatory 1) to deposit the complete raw dataset and the metadata describing them in an official data repository and 2) to provide a detailed description of critical methodologies, including mathematical equations and statistical models including the programming codes, that ensures that the research process and products are transparent and can be reproduced. As aforementioned there will be 3 types of articles published in animal – open space:

Data paper: What is a data paper? A data paper is a searchable metadata document, describing a dataset or a group of datasets and the circumstances of their collection, but without further analyses and interpretation of the data. Data paper provides a way for researchers to share and reuse each other's datasets by publishing datasets. Almost any piece of information can be defined as data. However, to merit publication in animal – open space data should be a set of information that are acquired/collected with a scientific method and be accurate, reusable, reproducible, replicable, and of value to the research community. What are the benefits of data papers for the research community? For instance, data sets can be used for another purpose (e.g. meta-analysis) than their original purpose. Data sets published as a data paper are accessible for a long period of time and by everyone and are easily to find. What are the advantages for the author(s)? The data is citable. If the associated research article is published in another journal (e.g. animal), a data paper might increase the traffic towards the research article and lead to more citations. Data papers may help to open doors for new research collaborations.

Method article: We know that reproducible experimental and laboratory methodologies are essential to science and animal - open space welcomes method article dedicated only to these aspects. This includes new research protocols and methods or changes to existing research protocols and methods. In most cases (but not mandatory), research protocols and methodology articles compare (at least) two methods: the proposed or alternative method and the currently used or "gold standard" method. The rapid development of non-invasive methods (e.g. based on sensors and cameras, in vitro systems) provides alternatives to more invasive research protocols and methods and these alternatives can be published in animal – open space. Method articles can also describe novel, improved or experimental extension and teaching methods in animal science used in either higher or continuing education. The authors of method articles should clearly demonstrate why the novel, improved or experimental methods were considered and provide a qualitative or quantitative assessment of the method.

Research article: Research articles correspond to all types of reproducible experimental research and include: 1) "Confirmational" research which may not be novel per se, but contributes to enlarging the knowledge that is essential to life sciences. 2) Pilot studies and proof-of-concept research for which the statistical power may be not sufficient to make clear conclusions on the outcome. 3) Applied animal research in which a management factor or solution is tested under field conditions. 4) Observational data papers in which the results are based on observations made in field conditions, and not necessarily controlled conditions. 5) Articles describing meta-analysis, modelling research or software tools are considered as research articles.

The raw data used to present in tables or graphs in research articles need to be published in data repository. Compared to a data paper, authors of a research article need to express their opinion on their results.

Compared to a "classical" journal, the articles will be subjected to an editorial review process, that is a review by an editorial staff of animal – open space. The evaluation process will focus mainly on the scope, transparency, reproducibility, clarity of writing, quality of English, ethics and the quality of the metadata. That means that Material and Method, Results and Metadata will be reviewed. The introduction and discussion/points of view of the authors are reviewed only for clarity but the responsibility of the contents is left to the authors.

With this journal we aim to bring animal - open space to a point where it is recognized as an important member of the animal family, and we hope that with the introduction of this new concept (open science, open review process), the journal will be able to initiate and foster the post-print interaction between its authors and readers.

EUREKA project – analysing knowledge supply of multi-actor projects

EUREKA is a Horizon 2020 project (https://www.h2020eureka.eu/) responding to the EU call "Reinforcing the EU agricultural knowledge base", with the aim of analysing multi-actor projects and assessing the feasibility of developing the agricultural knowledge base as an e-infrastructure and proposing options for the future, in particular to efficiently link existing communication and dissemination channels with national and regional agricultural knowledge and information systems (AKISs). The project EUREKA has several objectives, one of which is to analyse the projects from the point of view of the data generated in the multi-actor environment. In Horizon 2020 Framework Programme, the multi-actor approach (MAA) was introduced as an innovative concept to facilitate and accelerate the adoption of innovation in agriculture. The concept of MAA means addressing real problems and opportunities and actively involving different stakeholders in project activities to make the best use of complementary competencies in co-creating new knowledge and solutions and thus enhancing the practical implementation and impact of the projects.

The EUREKA project is exploring the feasibility of building an open data knowledge reservoir by

- analysing the supply of knowledge from MAA projects, and the profile of end-users
- engaging with MAA community to identify most relevant needs of end-users
- developing an e-platform to integrate knowledge and innovation in line with the FAIR principles.

In the frame of the EUREKA the funded MAA projects were analysed to identify the types of knowledge and data generated in order to recognize the knowledge supply of interest for an agricultural knowledge e-platform. The analysis included 101 MAA projects of different types and the clusters of various outputs are presented in Figure 1.

Figure 1: Clusters of outputs created in MAA projects

It was observed that there is a large diversity of outputs from MAA projects, and that outputs differ mainly according to the type of project (research and innovation action-RIA, coordination and support action-CSA, innovation action-IA). RIA projects typically put more emphasis on scientific publications, whereas CSA and IA projects are more knowledge-exploitation oriented. With respect to FAIR principles, the published outputs (scientific and technical papers) are in-line with them, whereas more efforts are needed to assure the access to other types of important outputs produced in the projects, e.g. raw data, software/applications. It has emerged that publication of raw (or primary) data papers should be encouraged for a better re-use of data. The importance of intangible benefits that MAA projects create was highlighted, as was the challenge for the sustainability of the created knowledge community. This last point is particularly important in the context of the MAA paradigm, since it helps in keeping alive the interactions between providers and users of the knowledge. It was recognised that there is a need for the "infrastructure" that would support the reuse of scholarly data and that a unique online repository could greatly increase the impact of projects.

In the interviews conducted with representatives of MAA projects it was also noted that such an e-platform should integrate raw data, knowledge objects and digital tools but at the same time enable the sustainability of networks and facilitate the knowledge community in general. With regard to end-users it should be kept in mind that different stakeholders/actors have different information needs as well as different preferred ways to obtain the information they need. It has also been noted that if we are to provide the information to farmers, we need to provide the results in a way that farmers trust, and this is where personal contact should be prioritized over written information. In relation to the importance of developing an e-platform of knowledge, it was also noted that the results created by the projects need to be applied and disseminated widely and easily. There is also a need to increase the understanding of the importance of data management, adherence to the principles of FAIR and the associated adherence to open access. This should be done not only to comply with the policy but as a part of good scientific practice. Any multi-actor project represents a knowledge community whose sustainability is at risk once the project is completed. An e-platform capable of integrating different outputs of the multi-actor projects is important for the sustainability of the knowledge community after the end of the project.

Conclusions

Open Science changes the way research results are disseminated with the aim of making them freely available, reproducible, but also more understandable to a wider audience and not only to specialised scientists. In the area of animal production, *animal open space* platform offers a novel approach for disseminating research outputs and foresees research, methodology and data papers.

A concept of multi-actor approach in a project builds a knowledge community whose sustainability is not assured once the project is completed. An e-platform capable of integrating different outputs of the multi-actor projects is important for the sustainability of the knowledge community after the end of the project. In that respect the understanding of the importance of data management, and adherence to the principles of FAIR and open access to knowledge and data created by the projects is fundamental.

Acknowledgments

The financial support of EU for project EUREKA (grant agreement No 862790) and Slovenian Research Agency core financing (program Sustainable Agriculture P4-0133) is greatly acknowledged.

Novi trendovi u objavljivanju istraživanja i prenošenju znanja o stočarstvu - predstavljanje projekat Animal Open Space i EUREKA

Marjeta Čandek-Potokar, Giuseppe Bee

Rezime

Otvorena nauka se ne sastoji samo u tome da rezultati istraživanja budu dostupni, već i u tome da ih učini razumljivim i reproducibilnim za širu javnost, a ne samo za specijalizovane naučnike. Time snažno utiče na istraživačko okruženje, posebno na način na koji se rezultati istraživanja šire i saopštavaju. Mnogi rezultati istraživanja ostaju neobjavljeni zbog nedostatka novina ili nedovoljne snage eksperimentalnog dizajna, što predstavlja gubitak za specifičnu zajednicu znanja. Ako se istraživanje može ponoviti i ako su povezani podaci dostupni, objavljivanje može otvoriti nove mogućnosti i ideje za ponovnu upotrebu podataka, značajno doprinoseći sticanju znanja. Dakle, klasičnom načinu objavljivanja rezultata istraživanja potrebni su alternativni pristupi. Ovaj rad predstavlja dve inicijative za širenje rezultata istraživanja proizvodnje u stočarstvu i poljoprivrede. Prvi je Animal Open Space na e-platformi (ANOPES), namenjen rezultatima istraživanja. Otvara mogućnost objavljivanja istraživačkih, metodoloških i podataka sa bržim pregledom i već je operativan. Druga inicijativa je studija dokazivanja koncepta u okviru projekta EUREKA za stvaranje e-platforme za jačanje znanja u poljoprivredi i inovacionog sistema Evropske unije. Za ANOPES su predstavljeni obim i mogućnosti objavljivanja. Za EUREKA projekat je predstavljena analiza podataka i objekata znanja nastalih u projektima sa više aktera i njihova relevantnost za repozitorijum znanja.

References

EVE M. (2014): Introduction, or why open access? In Open Access and the Humanities: Contexts, Controversies and the Future (pp. 1-42). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9781316161012.003

FAIR Principles. GO FAIR. https://www.go-fair.org/fair-principles/ Accessed 22-7-2021.

VICENTE-SAEZ R., MARTINEZ-FUENTES C. (2018): Open Science now: A systematic literature review for an integrated definition". Journal of Business Research. 88: 428–436.

WILKINSON M., DUMONTIER M., AALBERSBERG I. et al. (2016):The FAIR Guiding Principles for scientific data management and stewardship. Sci Data 3, 160018. https://doi.org/10.1038/sdata.2016.18

WOELFLE M., OLLIARO P., TODD M. H. (1988): Open science is a research accelerator. Nature Chemistry. 3 (10): 745–748. doi:10.1038/nchem.1149

AGRICULTURE AND ANIMAL PRODUCTION—FROM A FOUNDER OF CIVILIZATION TO A FAILURE OR SUSTAINABILITY

Vesna Gantner[1], Denis Kučević[2], Muhamed Brka[3]

[1]Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Croatia
[2]Department of Animal Science, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia
[3]Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Hercegovina
Corresponding author: Vesna Gantner, vgantner@fazos.hr
Invited paper

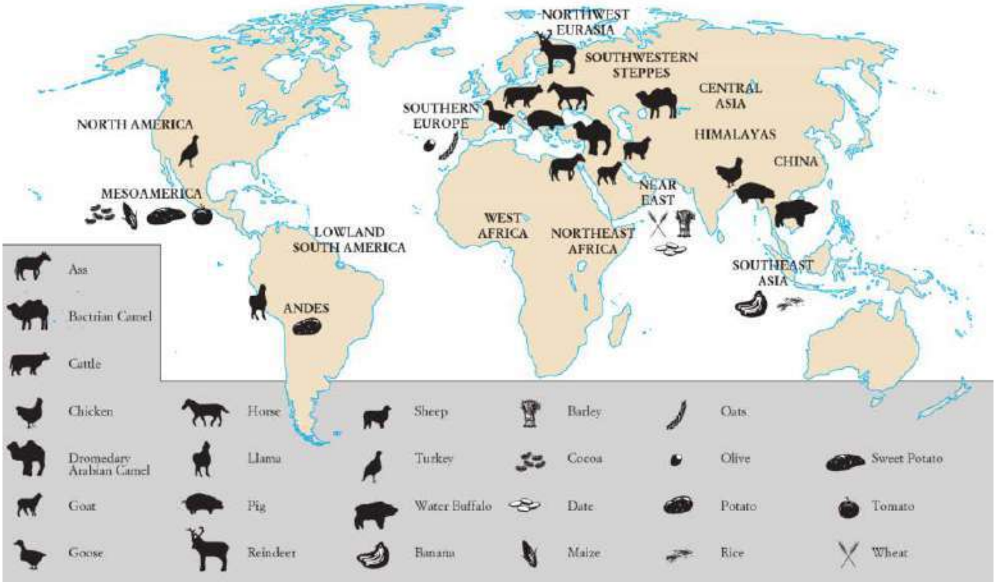
Abstract: The agricultural revolution made us who we are, the ultimate predator on Earth. This evolution breakthrough enabled a sudden jump of Homo Sapiens in the food chain, rapid population growth, creation of civilizations and rapid expansion to all parts of the planet. These days we, as agricultural experts, are facing a new task; how to produce enough food for a growing human population, in conditions of limited resources and increasingly frequent labelling as pollutants by those who clearly do not understand the basic role of agriculture for human survival, agriculture produce food!!! Research, development and innovation have made the agricultural sector in Europe competitive and effective in producing enough quantity of quality food for the citizens of Europe. Now, this sector needs to operate in the production system that ensures safe and healthy food supply, reduces environmental impact, improves resource utilization, provides ecosystem services, contributes to a sustainable economy and satisfies consumer needs in a way that society values. And finally, we need to educate the population we are feeding; we are not Farming for Failure, we are **Farming to Sustain!**

Key words: agriculture, animal production, sustainability

Agricultural Revolution

The agricultural revolution implies cultural transformations that originally enabled humans to transform from hunting and gathering resources to plant and animal domestications and consequently agricultural production. Today, more than 80% of the human diet is originated from less than a dozen crop species and several animal species domesticated thousands of years ago. Accordingly, to archaeological data, the domestication of plants and animals started in the Holocene period approximately 12 thousand years ago in separate global locations (Picture 1) possibly caused by climate change and local population increases. This transformation from hunting and gathering to agriculture production occurred gradually as humans selected plants and animals for domestication, and then continued the selection for the traits of interest. The development of agriculture represents a major turning point in the evolution of the human population. The breeding of plants and animals increased according to the distinct environmental conditions of the region, while human migration and trade enabled the global expanse of domesticated beings (plants and animals). This change in resources availability provided a surplus of plant food and consumption during the winter period, while domesticated animals were used during the entire year. These new survival strategies enable the building of homes, villages, and communities, which consequently resulted in rapid increases in the human population and finally in the appearance of civilizations.

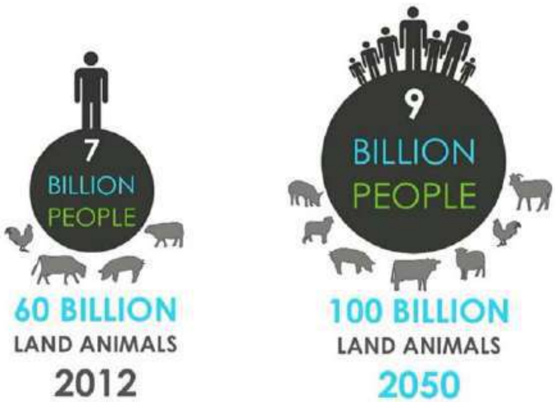
The agricultural revolution also caused many other modifications of the environment such as deforestation and irrigation. Furthermore, the agricultural revolution also initiated innovations in technologies, trade, architecture, and changes in socioeconomic relations, property ownership, and political systems. This change in the way of living provided a relatively safer existence and more time for analytical and creative thinking resulting in the development of art, religion, and science. It is necessary to emphasize that the human population, without the agricultural revolution, would still be organized into small uncivilized groups for hunting and gathering with the human population of about 500 million.



Picture 1. Origin of crops and domestic animals (*ECOCLIMAX, 2015*)

Agriculture today

Agricultural production today has to manage production in the changing world from the aspects of environmental conditions (climate change) and the population that it needs to feed (increasing food demands for growing human population). The forecasts indicate that the world human population will increase from the current 7.2 billion to 9.6 billion by the year 2050 (*Census Bureau, 2016*). Expected population growth, in combination with rising incomes and urbanization, implies great challenges to systems of food production and agriculture (*FAO, 2011*).



Picture 2. Expected increase of human and domestic animals' population (*FAO, 2011*).

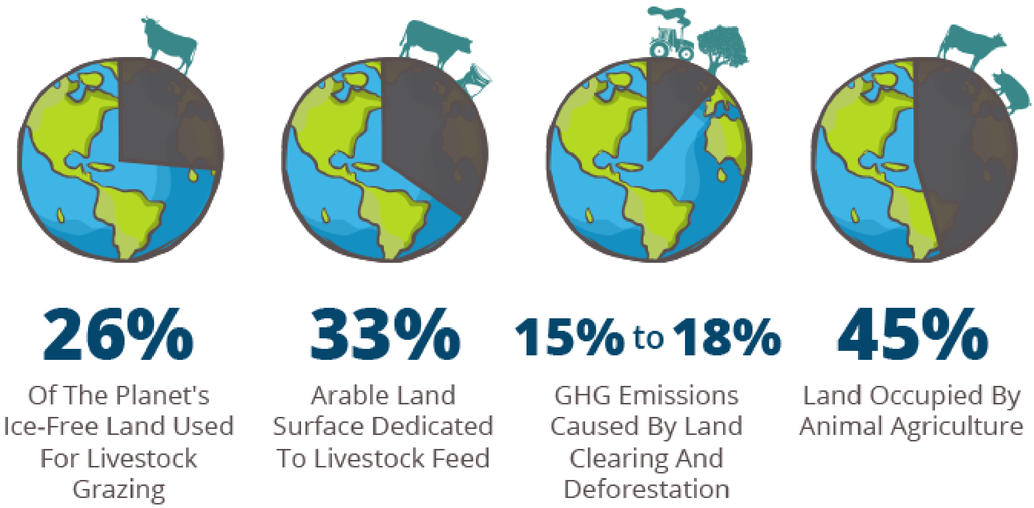
It is expected that the demand for meat and milk will increase by 73 and 58% in the year 2050, comparing the levels in the year 2010. Furthermore, the expected increase in human population and changes in feeding preferences implies the necessity of an increase of the number of domestic animals from the current 60 billion to 100 billion by the year 2050 (Picture 2). In order to prevent global food insecurity, it is necessary to enable sustainable intensification of animal production under the following conditions: increased demand for animal products, decreased available resources needed for production (agricultural land, water), and available various tools and production methods.

In these conditions, livestock intensification implies increase of animal density and decrease of the stockperson per animal ratio. In the case of increasing herd size and decreasing workforce availability the precision livestock farming imposes as optimal solution. Precision livestock farming implies usage of various sensors and big data managing in order to provide a simple score regarding the animal health, productivity, and welfare. By collecting and analyzing large amount of data the precision livestock farming can provide farmers with the information regarding the production, reproduction and welfare at the individual and herd level. That information enable farmer to optimize the management of farm and consequently realize the efficient and sustainable production system.

So, to be a successful farmer you need to manage a number of different tasks; to build adequate facilities, to select proper genetic, to control and manage environmental (microclimate) conditions, to grow and process the animal fed, to prevent animals' disorders or diseases, to answer the market tasks; to minimize the environmental footprint, so in one world, you have to be a magician.

Agriculture – From the Founder of Civilization to the Destroyer of the Planet

According to the Greenpeace analysis (2020, ominously named: Farming for Failure), farm animals and the production of animals' fed in Europe are producing more greenhouse gases every year than all of the bloc's cars and vans put together. Furthermore, *Greenpeace* (2020) continues that the increase in meat and dairy production in Europe over the past decade has made farming a significantly greater source of emissions, while on the other hand, governments invested in renewable energy and transport resulting in the reduction of emissions from energy and transport sector.



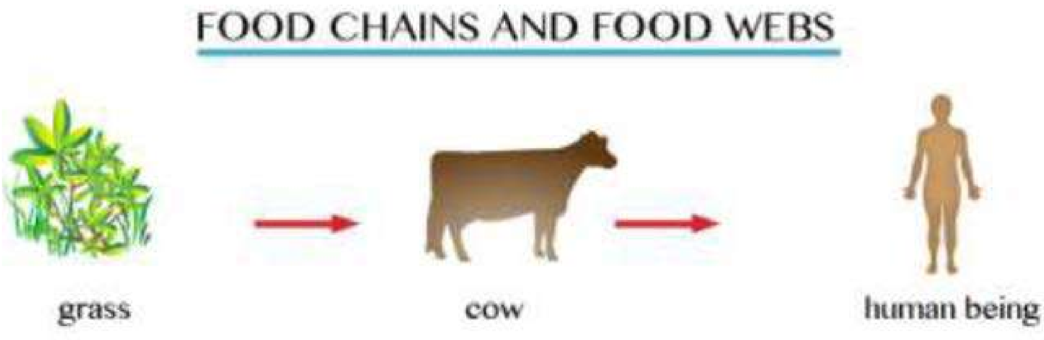
Picture 3. Global statistics on land clearing and deforestation (*ECOPEANUT, 2021*).

In a similar mood continues also *ECOPEANUT (2021)* stating that the livestock industry has a negative impact on the environment through emissions, water consumption, deforestation and land clearing, land occupation, ... In continuation, they suggested that *'if farm animals stopped landing on everyone's plates'* meaning that we supposed to switch to vegetarianism or become hunters, which raises other questions (what to hunt and what will be the emissions and do we have enough resources to produce food of plant origin for the growing human population, and of course the basic characteristic of the genus Homo that we are omnivores (Picture 4) and as such cannot survive only on foods of plant origin (*we are not ruminants*)), '*14.5% of all human-related emissions would be offset'*.

Nevertheless, the information and relevance of the information always depends on the source of the information and the intention to place the same information.

Furthermore, speaking purely theoretically, the complete extinction of domestic animals (which is ardently advocated by certain lobbies) would leave catastrophic long-term consequences on the pedological quality and structure of arable land that could not be recovered by any known alternative to manure! This would automatically and directly jeopardize existing plant production. Also, there would be an increase in the number of starving people in the world, because in some regions only traditional livestock breeding with acclimatized plant crops is possible (where wheat and corn do not grow). Moreover, there would be large population migrations, hunger, the devastation of the region, etc.

Today's agroecological systems are extremely complex and dynamic, but they are still based on biological principles. Reckless moves by those who will manage the development of agriculture in the future could lead to catastrophic consequences for the planet, and the entire living system on it. Which wouldn't even rule out the outbreak of wars!



Picture 4. The basics – food chain (*Gloster EDU, 2019*).

It could be observed that the changes in climate are changing the environmental conditions in various regions by making them not convenient for living and agricultural and livestock production in particular. FAO experts (*FAO, 2013*) stated that with the purpose to hold the increase in global temperature below 2^oC and to avoid dangerous climate change, global GHG emissions need to be significantly decreased worldwide.

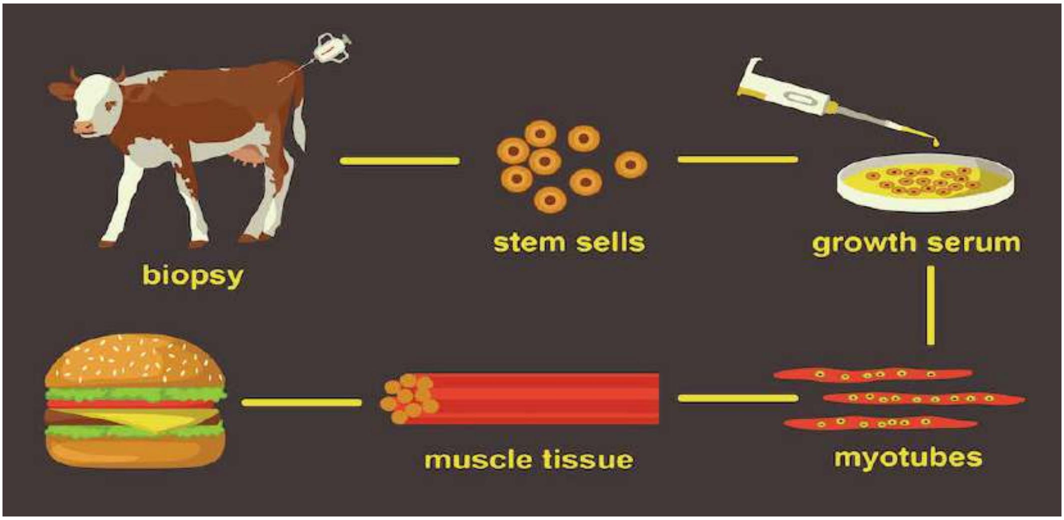
The animal production sector, within agriculture production sector, has to some extent impact on the environment. The global animal production sector significantly adds to an anthropogenic GHG emission, but on the other hand, it can also deliver a significant share of the required mitigation effort. Total GHG emissions from livestock supply chains are estimated at 7.1 gigatons of CO_2-eq/year (year 2005) representing 14.5% of all anthropogenic emissions (49 gigatons CO2-eq for the year 2004; IPCC, 2007). Furthermore, regarding the species, cattle are labelled as the main contributor to the sector's emissions with about 4.6 gigatons CO_2-eq, representing 65% of sector emissions, while small ruminants have much lower emission levels in the interval from 7-10% of sector emissions (0.47 gigatons CO_2-eq). The main source of GHG emissions in the ruminants' supply chain focused on milk or meat production is enteric fermentation and feed production. Meat production systems contribute more to the sector's emissions than the milk production system. Also, the grazing system, compared to the mixed one, produce more GHG. This holds for all studied ruminants, cattle, sheep and goats. Furthermore, in ruminant production systems, there is a strong negative relationship between productivity and emission intensity that is emission intensity decreases as yield increases. The reduction of sectors emission could be achieved by: reduction of production and consumption, lowering the emission intensity of production and by combination of mentioned above. The application of different mitigation techniques could result in large environmental benefits. The mitigation potential varies in intervals from 14 to 41% depending on the selected species, production system and world's region.

Considering that the livestock sector currently is in the challenging period when the necessity of sectors GHG emissions reduction and significant demand growth for animals' products (projected to be +70% between the year 2005 and 2050 (*FAO, 2011*) need to be harmonized, stimulation of farmers for higher production level could be one of the effective mitigation measures, and we as scientific community should give are share. Also, it is needed to emphasize that the agriculture will mitigate its environmental impact to a much greater extent compared to the steel, cement, iron, and oil industries, or transport and construction sector.

Furthermore, we need to ask ourselves, how did we come from the producer of the most basic needs of all living beings, food, to the label of the destroyer of the planet? And more importantly, how to change the consciousness of the population for which we produce and which in the end without us really cannot, just obviously not aware of it?

Cultured Meat – Meat without Harming a Single Animal

Cultured meat, or lab-grown meat, is meat produced by in vitro cell cultures of animal cells (as opposed to meat obtained by slaughtering animals).



Picture 3. Expected increase of human and domestic animals' population (*Flynn, 2019*)

'Cultured meat, produced in bioreactors without the slaughter of an animal, has been approved for sale by a regulatory authority for the first time. The development has been hailed as a landmark moment across the meat industry' (Guardian, 2020).

Lab-grown meat appears in many additional names; cultured meat, in vitro meat, synthetic meat, ... This 'meat' is made by growing muscle cells in a nutrient serum and assisting them into muscle-like fibers. Simpler 'animal' products, such as artificial milk or egg whites, can be produced by yeast that has been genetically altered to produce the proteins found in milk or eggs. Then those proteins are extracted and blended in the adequate volumes. Cultured meat includes applying the practices of tissue engineering (cellular agriculture) to the creation of muscle for consumption as food.

Cellular agriculture includes a set of technologies to create products typically obtained from livestock farming, using culturing techniques to create the individual product. Cellular agriculture could be divided into two types: tissue engineering-based and fermentation-based cellular agriculture, depending on the production method used. *Tissue engineering-based cellular agriculture* includes cultured meat and leather systems in which cells or cell lines are taken from living animals and are tissue-engineered to produce useable tissue. Starting material, i.e. the cells, can be obtained from an animal using a biopsy procedure (*Post, 2014*), or a genetically modified cell line could be created that only requires animals from which to source the original cells (*Genovese et al., 2017*). *Fermentation-based cellular agriculture* opposes tissue engineering-based systems in that it does not use any tissue from a living animal. Alternately, products are created by fermentation using bacteria, algae or yeast that have typically been genetically modified, by adding recombinant DNA, so they produce organic molecules. These molecules can be used to biofabricate familiar animal products (e.g., gelatin, casein (used for milk), and collagen (used for leather)).

Cultured meat could be defined as an early-stage technology with potential benefits and challenges, so... Furthermore, when it comes to cultivated meat, there is of course the question of the impact on the environment, what is the emission, water consumption, footprint ... The premise that artificial meat will save the planet from GHG emission is not based on scientific evidence, nor are there scientific estimates of the extent to which meat production will contribute to the reduction of GHG, and will it at all? The only estimates are regarding the financial turnover on the artificial meat market in amount of 140 billion euros in the next few years.

Role of the Animal Production Sector in European Bioeconomy

The animal production sector with an amount of 168 billion euros annually contributes significantly to the European economy (*Eurostat, 2014*), moreover, it supports food safety, rural development and ecosystem services. The animal production sector represents 45% of the total agricultural activity and provides jobs for almost 30 million people (*ATF, 2019*). In addition, the European animal production sector generates additional export possibilities for Europe both in terms of product and expertise. Finally, the European animal production sector plays a central role in obtaining food safety and nutrition worldwide.

Food production represents a major part of the bio-economic system. In many parts of Europe, the animal production sector is inseparably linked to the vitality of rural social-economic infrastructure. Furthermore, animal production may pose a challenge to the environment and some aspects of human health. Although the animal production sector offers multiple possibilities for contribution to climate-smart, sustainable and competitive Europe, it needs a creative and innovative systematic approach and strong, flexible to do research and study in order to implement the knowledge, technology and skills that will prepare the sector for European challenges in the future.

Research, development and innovation have made the animal production sector in Europe more competitive and effective today. Creating a stimulating environment for research and innovation in the livestock sector can lead to a production system that ensures safe and healthy food supply, reduces environmental impact, improves resource utilization, provides ecosystem services, satisfies consumer needs in a way that society values, and contributes to a sustainable economy. Given the above, it is extremely important to ensure investment in research, development and innovation and application of the same at the local, national, and global level.

How to Ensure Sustainability of Animal Production Systems—Case Study

‘Danish Crown will no longer be hunting for growth by slaughtering more animals. In the Group’s new Feeding the Future strategy, focus will be on reducing the carbon footprint of meat and use this as a foundation for increasing earnings on the Group’s products’ (*Danish Crown, 2021*).

Danish Crown (2021) stated that Danish farmers were a key driver in global developments for a set of generations, with high investments in knowledge, facilities, people, and high-quality genetics that ensured the top position of Danish farmers in European farming.

Furthermore, they (*Danish Crown, 2021*) continue ‘*if meat is to lose its reputation as a climate culprit, fields must be grown intelligently, slurry handled critically, and animals tended to carefully’*.

Danish Crown is planning to invest billion-kroner in farm-to-fork innovation to create a position where sustainability will be making the incremental value of the Group’s products. Jais Valeur, CEO of Danish Crown stated (*Danish Crown, 2021*): *'Our goal is not to produce more pigs, but instead to create more value from the raw materials supplied by our owners'*.

Since the market is constantly changing and plant-based products currently became popular, the animal production sector needs to invest in innovation and production of offering sustainable foods meaning the reduction of the carbon footprint of farms as well as education of consumers regarding the animal products and their real impact on the environment as well as on human health. As Jais Valeur said (*Danish Crown, 2021*): *'we need to create a positive, upward-moving spiral'*.

Instead of a Conclusion

About 400,000 years ago, Homo Sapiens appeared on the evolutionary horizon, not for nothing a special animal somewhere in the middle of the food chain. It survived for thousands of years as a collector and hunter, living in smaller (up to 100 individuals) mostly related groups and spreading to a lesser extent across the planet. Furthermore, approximately 12,000 years ago, the process of domestication of plant and animal species began, i.e. the agrarian revolution, enabling a sudden jump of Homo Sapiens in the food chain, rapid population growth, creation of civilizations and rapid expansion to all parts of the planet. Homo Sapiens becomes the ultimate predator.

Today, agriculture is facing a new task; how to produce enough food for a growing human population, in conditions of limited resources and increasingly frequent labelling as pollutants by those who clearly do not understand the basic nature of agriculture for human survival, we are producers of food!!! The human population currently lives in a world of a large number of imposed needs (consumer society) but the basic need of all living beings are water and food!!!

Research, development and innovation have made the agricultural sector in Europe competitive and effective in producing enough quantity of quality food for the citizens of Europe. Now, this sector needs to operate in the production system that ensures safe and healthy food supply, reduces environmental impact, improves resource utilization, provides ecosystem services, contributes to a sustainable economy and satisfies consumer needs in a way that society values. And finally, we need to educate the population we are feeding; we are not Farming for Failure, we are **Farming to Sustain!**

Poljoprivreda i stočarska proizvodnja - od osnivača civilizacije do neuspeha ili održivosti

Vesna Gantner, Denis Kučević, Muhamed Brka

Rezime

Poljoprivredna revolucija učinila nas je onim što jesmo, najvećim predatorima na Zemlji. Ovaj napredak u evoluciji omogućio je nagli skok Homo Sapiensa u lancu ishrane, brzi rast stanovništva, stvaranje civilizacija i brzo širenje na sve delove planete. Ovih dana pred nama, kao poljoprivrednim stručnjacima, stoji novi zadatak: kako proizvesti dovoljno hrane za rastuću ljudsku populaciju, u uslovima ograničenih resursa i sve češćeg označavanja zagađivača od strane onih koji jasno

ne razumeju osnovnu ulogu poljoprivrede za opstanak ljudi, poljoprivreda proizvodi hranu !!!
Istraživanje, razvoj i inovacije učinili su poljoprivredni sektor u Evropi konkurentnim i efikasnim u proizvodnji dovoljne količine kvalitetne hrane za građane Evrope. Sada ovaj sektor treba da radi u proizvodnom sistemu koji osigurava sigurno i zdravo snabdevanje hranom, smanjuje uticaj na životnu sredinu, poboljšava korišćenje resursa, pruža usluge ekosistema, doprinosi održivoj ekonomiji i zadovoljava potrebe potrošača na način koji društvo ceni. I na kraju, moramo obrazovati stanovništvo koje hranimo: mi se ne bavimo poljoprivredom za neuspeh, mi se bavimo poljoprivredom kako bi osigurali održivost!

Ključne reči: poljoprivreda, stočarska proizvodnja, održivost

References

ATF, Animal Task Force (2019): Vision Paper towards European Research and Innovation for a sustainable and competitive livestock production sector in Europe. A framework for suggested priorities for R&I within Horizon Europe, February 2019.
DANISH CROWN (2021): Sustainability to push up the price of Danish meat. FEEDING THE FUTURE - STRATEGY 2021-2026. https://www.danishcrown.com/news/sustainability-to-push-up-the-price-of-danish-meat/
ECOCLIMAX (2015): The origin of crops and domestic animals. Environmental notes. http://www.ecoclimax.com/2015/07/the-origin-of-crops-and-domestic-animals.html
FAO. Food and Agriculture Organization of the United Nations (2011): World Livestock 2011 – Livestock in food security. Rome.
FAO. Food and Agriculture Organization of the United Nations (2013): Greenhouse gas emissions from pig and chicken supply chains – A global life cycle assessment, by MacLeod, M., Gerber, P., MOTTET A., TEMPIO G., FALCUCCI A., OPIO C., VELLINGA T., HENDERSON B., STEINFELD H. ROME (2019): Farm health online. https://www.farmhealthonline.com/US/health-welfare/cattle/environment/
FLYNN D. (2019): The ban against lab-grown food using “meat’ on the label grows to 7 states. Food Safety News, FSN.
GENOVESE N.J., DOMEIER T., PRAKASH B., TELUGU V.L., ROBERTS R.M. (2017): Enhanced development of skeletal myotubes from porcine induced pluripotent stem cells. Scientific Reports. 7:41833.

GLOSTER EDU (2019): Cattle Zoology Project. https://edu.glogster.com/glog/cattle-zoology-project/307vwbiu45o

GREENPEACE (2020): Farming for Failure. Greenpeace European Unit vzw-asbl Belliardstraat 199 Rue Belliard 1040 Brussels, Belgium.

GUARDIAN (2020): No-kill, lab-grown meat to go on sale for first time. Meat industry. https://www.theguardian.com/environment/2020/dec/02/no-kill-lab-grown-meat-to-go-on-sale-for-first-time

IPCC, INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE (2007): Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. B. Metz, O.R. Davidson, P.R. Bosch, R. Dave & L.A. Meyer, eds. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

POST M. (2014): Cultured beef: Medical technology to produce food. Journal of the Science of Food and Agriculture 94:1039–1041.

US CENSUS BUREAU (2016): https://www.census.gov/library/publications.html.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

IMPACT OF NUTRIENT SELF-SUPPLY THROUGH CHOICE FEEDING ON GROWTH PERFORMANCE, FEEDING BEHAVIOUR AND PROTEIN EFFICIENCY IN GROWING FINISHING PIGS

Giuseppe Bee, Catherine Ollagnier

Swine Research Unit Agroscope Posieux, Posieux, Switzerland
Corresponding author: Giuseppe Bee, giuseppe.bee@agroscope.admin.ch
Invited paper

Abstract: This pilot study aimed to compare the growth performance, nutrient deposition efficiency and feeding behaviour of growing pigs fed on a standard two-phase feeding or a choice feeding regime. The experiment was performed with 12 Swiss Large White barrows between 23.2 and 108.0 kg body weight (**BW**). Six pigs assigned to the standard (**ST**) treatment were offered *ad libitum* access to a grower (**ST-G**) and finisher (**ST-F**) diet from 23.2 to 63.4 kg and from 63.4 to 108.0 kg, respectively. The ST-G and ST-F diets were formulated based on the Swiss feeding recommendation for swine for an average BW of 40 and 80 kg, respectively. The other six pigs assigned to the choice (**CH**) treatment had constant *ad libitum* access to both a grower (**CH-G**) and a finisher (**CH-F**) diet formulated for a reference BW of 20 and 100 kg. All diets were isocaloric and differed only in the crude protein and essential amino acid content according to the reference BW used for feed formulation. To determine the empty body nutrient deposition rate, pigs were scanned using dual-energy x-ray absorptiometry at 25.8 and 103.8 kg BW. Individual feed intake and feeding behaviour were monitored with automatic feeders. Changes in BW were determined weekly. Compared to ST pigs, CH pigs ingested more feed daily ($P = 0.05$) and grew faster ($P = 0.02$). Total crude protein consumption tended to be greater ($P = 0.08$) in CH than ST pigs due to a numerically greater intake of the protein-rich CH-G diet during the finisher period. A greater crude protein intake in CH than ST pigs was accompanied by a greater ($P = 0.04$) daily protein deposition rate but a similar protein efficiency. Regarding feeding behaviour, CH pigs went more often to the feeder, spent less time at the feeder, ate less feed per visit and had shorter intervals between two meals than ST pigs ($P < 0.01$ for each) in the grower but not in the finisher period. Considering the feeding behaviour traits, the CH pigs with a greater protein deposition potential preferred the protein-rich CH-G over the CH-F diet. In conclusion, these results show that, like the wild pigs, the domesticated modern pigs maintained the ability for an adequate nutrient self-supply according to their nutritional requirements.

Key words: dual-energy x-ray absorptiometry, empty body composition, nutrient deposition, pig

Introduction

Regarding the increasingly limited feed resources, there is a clear incentive to further improve the nutrient or, more specifically, the crude protein (**CP**), essential amino acids (**EAA**) and phosphorus efficiency in pig production. To achieve this objective, nutrient supply in a specific feeding scheme should cover as closely as possible the nutrient requirements of growing pigs at a given body weight (**BW**). The widely used phase-feeding strategies have precisely this goal in mind *(Han et al., 2000)*. The core element of developing phase-feeding strategies leverages the principle that pigs' ability to ingest feed surpasses its capacity to deposit protein *(NRC, 2012)*, and the latter is closely linked to the dietary supply of EAA *(van Milgen and Dourmad, 2015; Ruiz-Ascacibar et al., 2019)*. In most swine diets, lysine is the first limiting EAA, and the requirements for the other EAAs are, expressed in percentage of lysine, relatively constant *(van Milgen and Dourmad, 2015)*. As the protein and lysine requirements per kg of diet decrease from birth to slaughter *(NRC, 2012)*, the key factor of phase-feeding strategies lies in fixing the dietary CP and lysine requirements for optimal growth and lean tissue deposition. Subdividing the growth period into small BW ranges to increase the number of phase-feeding periods limits the time span when CP and lysine supplies are below or above requirements. Interestingly, *Menegat et al. (2020)* showed that increasing the number of dietary phases from 2 to 3 or 4 did not significantly improve the overall growth performance and carcass characteristics of pigs. One explanation could be that variation in BW, growth rate, and feed intake within a pen prevents, on an individual basis, the accurate delivery of an optimal CP and lysine level for growth and, ultimately, protein deposition *(Pomar and Remus, 2019)*.

To overcome or limit these variabilities in group-housed pigs, there are two possibilities: either the feed composition is adjusted daily according to their BW and thus their nutrient requirements or pigs could be offered a choice of diets differing in their CP and lysine contents, thereby allowing them to compose their diet. Hence, pigs could adjust their nutrient supply according to the requirements of their actual growth phase. This ability for adequate nutrient self-supply, which is a common trait in the wild pig *(Henry, 1985)*, seems to have also been preserved in the domestic pig despite the strong genetic selection for feed efficiency and lean tissue deposition. The older publications of *Kyriazakis et al. (1991)* and *Ettle and*

Roth (2009) and also the more recent one of *Pichler et al. (2020)* revealed that the nutrient self-supply characteristic of modern pig lines has been preserved. Specifically, the aforementioned experiments examined the impact of choice versus conventional phase-feeding strategies on growth performance, feeding behaviour, and carcass or meat quality. This pilot study aimed to extend this knowledge and determine how choice versus phase feeding impacts nutrient and energy deposition rates alongside protein and energy deposition efficiency. To address these questions, the feed intake and the body composition of choice- and phase-fed pigs were monitored using automatic feeders and dual-energy x-ray absorptiometry (DXA).

Materials and Methods

The Swiss cantonal Committee for Animal Care and Use approved all procedures involving animals (Animal welfare permit number 32382).

Animals, diets and housing conditions

For this pilot study, 12 Swiss Large White barrows originating from five litters of the Agroscope sow herd were used. From weaning to the start of the experiment, the pigs were reared in the same pen and offered *ad libitum* access to the same standard starter diet (weaning to 25 kg BW). At an average BW of 23.2 ± 1.39 kg (mean ± standard deviation), the pigs were randomly allotted to the standard (**ST**) and the choice (**CH**) treatment. Pigs in the ST group were offered *ad libitum* access to a grower (**ST-G**) and finisher (**ST-F**) diet from 23.2 to 63.4 (± 3.01) kg and from 63.4 to 108.0 (± 3.01) kg, respectively. The ST-G and ST-F diets were formulated according to the Swiss feeding recommendations for pigs *(Agroscope, 2017)* for an average BW of 40 and 80 kg, respectively. During the whole experimental period, pigs in the CH group had constant *ad libitum* access to both a grower (**CH-G**) and a finisher (**CH-F**) diet formulated for a reference BW of 20 and 100 kg according to the Swiss feeding recommendations for pigs *(Agroscope, 2017).*

To monitor individual feed intake, each pen was equipped with two automatic feeders and an individual pig recognition system (Schauer Maschinenfabrik GmbH & Co. KG, Prambachkirchen, Austria), as previously described *(Bee et al., 2008)*. The pigs were raised in two pens of equal size (17.35 m^2) connected by an alleyway and therefore accessible to the 12 pigs. Water was offered *ad libitum* through nipple drinker. Pigs were weighed weekly. When they reached a BW ≥ 98 kg, they were DXA-scanned two days later and slaughtered four days after the DXA measurements.

Feeding behaviour

Feeding behaviour data were collected by single-space automatic feeders (MLP, Agrotronic Schauer, Austria). The feeders are 0.6 m wide and 2.2 m long. The feeding system recorded all daily visits at the feeder, feed intake (**FI**) per visit and time spent at the feeder. Data evaluations considered only feeder visits that coincided with the intake of feed (but not sham visits). Between-visit intervals shorter than 5 min (which was used as the meal criterion) were regarded as within-meal feeder visits (**WMFV**), and these visits were grouped into meals *(De Haer and Merks, 1992)*. As proposed by *Carcò et al. (2018)*, the day, rather than the single meal, was considered the temporal basis for describing the feeding behaviour of the pigs in the experimental period. The total FI, total feeder visits and total feeding time per day per pig were therefore calculated. Those data were then used to calculate the average total time spent feeding per day (**TTF** expressed in min), the average frequency of feeder visits (**FFV**), the average time per visit (**TV** = TTF/FFV, expressed in min), the average daily feed intake (**ADFI**), the mean feed intake per visit (**FIV** = ADFI/FFV, expressed in g), the mean rate of feed intake (**RFI** = ADFI/TTF expressed in g/min) and the interval between two meals (**FI** expressed in min).

Dual-energy x-ray absorptiometry (DXA) measurements

The DXA scans of the live animals were performed at an average BW of 25.8 (± 2.1) and 103.8 (± 3.4) kg BW using a GE Lunar DXA (i-DXA, GE Healthcare Switzerland, Glattbrugg, Switzerland) with a narrow-angle fan beam (Collimator Model 42129), applying the protocol described by *Kasper et al. (2020)*. Briefly, the day prior to the DXA scan, the pigs were fasted for at least 16 h and then sedated using isoflurane (Attane, Piramal Critical Care, Inc., Bethlehem, PA, USA). The scans were conducted using the 'total body thick' mode with the enCORE software (version 16). Subsequently, the scan images were pre-processed to remove artefacts and to position the regions of interest. The variables 'total mass', 'BMC' (bone mineral content), 'lean' and 'fat' of the live empty body were obtained from the software. The water, ash, protein, fat and energy contents of the empty body of the animals were calculated using the DXA values and the regression equations published by *Kasper et al. (2020)*. Pigs were weighed after the DXA exam.

Chemical analysis of the feed

The diet analyses were conducted in duplicate except when the results differed by more than 5%, then up to four replicates were obtained. The dry matter and ash content were determined by gravimetry after drying at 105°C for 3 h and after 3 h at 550°C, respectively. In the same samples, the CP (CP = total nitrogen ×

6.25) content was analysed with a LECO FP-2000 analyser (Leco, Mönchengladbach, Germany) (International Organization for Standardization (ISO), 2008). The amino acid composition of the diets was determined after 24 h of acid hydrolysis (48 h for leucine, isoleucine and valine). Methionine and cystine were hydrolysed after peroxidation with formic acid. The amino acid profile was determined by HPLC coupled with a fluorescence detector (Alliance 2695; Waters, Milford, MA, USA), as described in the manual (Waters AccQ Tag Chemistry Package 052874 TP, rev. 1). To determine the crude fat content, samples were hydrolysed in 10% HCl (v/v) for 1 h. The hydrolysate was dried and subsequently extracted with petrol ether using the Büchi SpeedExtractor E 916 (Büchi Labortechnik AG, Flawil, Switzerland). The dry residual of fat was determined by gravimetry. The fat content in the freeze-dried samples was determined using the Avanti Soxtec System (2050 Extraction Unit; Foss Tecator, Hillerød, Denmark).

Calculations and statistical analysis

Using the calculated nutrient and energy content of the empty body at the start and the end of the experiment, the daily nutrient and gross energy deposition rates were calculated as the amount of deposited nutrients and energy from the start to the end of the experiment divided by the days on feed. The CP and energy deposition efficiency were calculated as the total CP and gross energy deposition divided by the amount of ingested CP and digestible energy.

The data were analysed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with the individual pig as the experimental unit. The model used for the data analyses included the feeding regime as the fixed effect and the litter of origin as the random effect. Least square means and the pooled standard error of the mean were calculated and reported in tables. Treatment differences were considered to be significant at $P \leq 0.05$ and tendency at $P > 0.05$ and $P \leq 0.10$.

Results

Diets

The CH-G diet contained 12 g/kg more CP content than the ST-G diet, and the CH-F diet contained 13 g/kg less CP content than the ST-G diet (Table 1). The differences in the CP contents can be explained by the different reference BWs that were used to formulate the two grower and two finisher diets *(Agroscope, 2017)*. In accordance with the CP levels, the digestible EAA content was, on average, 14% greater [range: +6% (histidine) to +21% (lysine, methionine, threonine)] in the CH-G than the ST-G diet and 17% lower [range −7% (lysine, cystine, threonine) to −17% (tyrosine, isoleucine)] in the CH-F than the ST-F diet. The digestible methionine content was twice greater in the CH-F than in the ST-F diet. The crude fibre and crude fat content were similar between the CH-G and ST-G diets and between the CH-F and ST-F diets.

Table 1. Feed ingredients (%), nutrient composition and energy content of the experimental diets[1]

Item[2]	CH-G	CH-F	ST-G	ST-F
Barley	30.00	30.00	30.00	30.00
Oat	3.50	3.50	3.50	3.50
Corn	0.60	10.49	3.62	7.53
Wheat	40.00	40.00	40.00	40.00
Blended fat	1.10	0.22	0.71	0.43
Potato protein	5.84	1.80	3.85	2.83
Soy bean meal extract	3.39		3.90	2.04
Rapeseed press cake	3.50	3.50	3.50	3.50
Dried sugar bett pulp	5.00	5.00	5.00	5.00
Apple pommace	0.53	0.46	0.40	0.29
L-Lysin-HCl	0.441	0.255	0.323	0.181
DL-Methionin	0.039	0.246	0.012	
L-Threonin	0.088	0.036	0.044	
L-Tryptophan	0.008			
Monocalcium phosphate	1.007	0.111	0.476	0.209
Limestone	1.199	0.845	1.003	0.896
NaCl	0.423	0.198	0.311	0.256
Pellan	0.30	0.30	0.30	0.30
Celite 545	2.00	2.00	2.00	2.00
Vitamin-mineral mix	0.40	0.40	0.40	0.40
Sweetener Sucram	0.02	0.02	0.02	0.02
Flavour Pigortek	0.02	0.02	0.02	0.02
Natuphos 5000 G	0.01	0.01	0.01	0.01
Mikrogrit	0.60	0.60	0.60	0.60
Nutrient content, g or MJ/kg				
Crude ash	75	58	67	61

[1] The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a BW of 20 and 100 kg, respectively. The ST-G and ST-F diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively.

[2] The digestible and net energy coefficients from each feed ingredient were obtained from the Swiss (Agroscope, 2017) and French (Noblet et al., 2003) databases, respectively. Considering the relative amount of each feed ingredient in the diet, digestible and net energy content were calculated.

Crude protein	176	135	164	148
Crude fat	37	30	34	32
Crude fibre	40	40	40	40
Sugar	30	29	31	30
Starch	484	554	506×	534
Digestible energy	13.70	13.70	13.70	13.70
Net energy	10.10	10.26	10.15	10.21
Digestible amino acids[3]				
Lysine	10.03	5.82	8.30	6.25
Methionine	2.92	4.22	2.41	2.07
Cystine	2.72	2.29	2.62	2.47
Threonine	5.76	3.47	4.77	3.74
Tryptophan	1.64	1.10	1.44	1.28
Phenylalanine	7.11	4.85	6.42	5.66
Tyrosine	5.06	3.19	4.44	3.84
Valine	6.79	4.64	6.11	5.4
Leucine	10.79	7.62	9.81	8.77
Isoleucine	5.52	3.57	4.93	4.28
Histidine	3.08	2.31	2.91	2.63

Growth performance

Pigs were weighed weekly and slaughtered six days after weighing ≥ 98 kg according to protocol. Despite this defined time table, pigs in the CH group were heavier ($P < 0.01$) the day of slaughter than the ST pigs (Tabela 2). The difference in slaughter weight can be explained by the greater ADG and greater ADFI in the finisher ($P = 0.03$) and overall period ($P \leq 0.05$) in the CH group. Pigs in the CH group tended ($P = 0.08$) to ingest more CP over the whole grower-finisher period than the ST pigs. The CP efficiency, expressed as kg daily ingested CP/kg ADG, was greater ($P < 0.01$) in the grower but not in the finisher and overall period.

[3] The content of digestible essential amino acids was based on nutritional characteristics of ingredients estimated from their chemical analyses with Allix.

Table 2. Growth performance of pigs either offered constant free access to the CH-G and CH-F diets (Choice) or offered ad libitum access to the ST-G from 20 to 60 kg BW and to the ST-F diet from 60 kg BW to slaughter (Standard) [4]

	Treatment			
Item[5]	Choice	Standard	SEM	P-value[6]
BW, kg at				
Start	23.20	23.15	0.633	0.95
Start of finisher period	65.53	63.38	1.015	0.17
Slaughter	110.89	107.39	1.534	< 0.01
ADG, kg/d				
Grower	0.908	0.862	0.0272	0.23
Finisher	1.217	1.108	0.0512	0.03
Overall	1.040	0.971	0.0354	0.02
ADFI, kg/d				
Grower	1.886	1.783	0.0382	0.07
Finisher	3.271	3.042	0.0615	0.03
Overall	2.492	2.357	0.0455	0.05
Total feed intake, kg				
Grower	89.538	84.678	3.4011	0.08
Finisher	121.234	120.088	3.7638	0.83
Overall	210.573	204.566	4.0769	0.14
Total CP intake, kg				
Grower	13.923	13.858	0.3414	0.85
Finisher	19.175	17.753	0.6132	0.14
Overall	32.871	31.384	0.5270	0.08
Gain-to-feed, kg/kg				
Grower	0.479	0.481	0.0118	0.79
Finisher	0.373	0.365	0.0134	0.47
Overall	0.417	0.413	0.0111	0.58
CP intake/ADG, kg/kg				
Grower	0.326	0.340	0.0060	< 0.01
Finisher	0.422	0.404	0.0126	0.26

[4] The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight (BW) of 20 and 100 kg, respectively. The ST-G and ST-F diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively.

[5] ADG = average daily gain; ADFI = average daily feed intake; CP = crude protein

[6] P-values of the main factor experimental treatment.

Overall	0.375	0.373	0.0080	0.78
Days on feed, d				
Grower	47.5	47.5	2.02	1.00
Finisher	37.2	39.5	1.38	0.26
Overall	84.6	86.9	2.20	0.33
Age at slaughter, d	145.8	149.5	2.65	0.22

Feeding behaviour

The CH pigs went more often to the feeder (FFV), spent less time at the feeder (TV), ate less feed per visit (FIV) and had shorter intervals between two meals (FI) than ST pigs ($P < 0.01$ for each; (Tabela 2.). Overall, the CH pigs spent more time per day in total ($P = 0.04$) at the feeder (TTF) because the 2.8 min shorter feeder visits were compensated for by up to 11.4 greater number of feeder visits. The CH pigs appeared to be nibbler, as they had a larger ($P < 0.01$) number of feeder visits (WMFV) within a meal than the ST pigs. The feeding behaviour of the two control groups did not differ significantly during the finisher period, even though similar tendencies were noted. Interestingly, the CH pigs changed their feeding behaviour more drastically from the grower to the finisher period. Compared to ST pigs, pigs in the CH group reduced the frequency of feeder visits (dFFV, $P < 0.01$), stayed for a longer time at the feeder (dTV, $P = 0.03$) and increased the between meal interval (dFI, $P < 0.01$).

As per design, the CH pigs had constant access to the CH-G and CH-F diets that differed mainly in the CP and digestible EAA level. The current data suggest that neither in the grower nor the finisher period did the CH pigs prefer one diet over the other, as none of the feeding behaviour traits differed (Tabela 4).

Table 3. Feeding behaviour in the grower and finisher period f pigs offered constant free access to the CH-G and CH-F diets (Choice) or offered ad libitum access to the ST-G from 20 to 60 kg BW and to the ST-F diet from 60 kg BW to slaughter (Standard)[7]

	Treatment			
Item[8]	Choice	Standard	SEM	P-value[9]
Grower period				
TTF, min	*78.5*	63.7	4.41	*0.04*
FFV, n	*20.8*	9.4	1.73	*< 0.01*
WMFV, n	*24.1*	11.1	2.04	*< 0.01*
TV, min	4.0	*6.8*	0.33	*< 0.01*
FIV, g	99	*193*	12.2	*< 0.01*
RFI, g/min	25	28	1.6	0.12
FI, min	33.5	*78.8*	48.2	*< 0.01*
Finisher period				
TTF, min	76.1	65.6	5.61	0.21
FFV, n	15.1	9.8	2.07	0.11
WMFV, n	17.0	12.3	2.52	0.24
TV, min	5.6	6.8	0.65	0.15
FIV, g	254	329	37.9	0.19
RFI, g/min	44	48	2.8	0.35
FI, min	*45.6*	63.7	5.65	0.08
Difference between the grower and finisher period[10]				
dTTF, min	2.9	−1.4	4.08	0.42
dFFV, n	*5.9*	−0.2	*0.81*	*< 0.01*
dWMFV, n	*7.3*	−1.0	1.17	*< 0.01*
dTV, min	*−1.7*	0	0.46	*0.03*

[7] The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight (BW) of 20 and 100 kg, respectively. The ST-G and ST-F diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively.

[8] TTF = total time feeding per day; FFV = frequency of feeder visits; WMFV = within meal feeder visits; TV = time per visit; FIV = feed intake per visit; RFI = rate of feed intake; FI = interval between two meals.

[9] P-values of the main factor experimental treatment.

[10] dTTF = difference between TTF in the grower and finisher period; dFFV = difference between FFV in the grower and finisher period; dWMFV = difference between WMFV in the grower and finisher period; dTV = difference between TV in the grower and finisher period; dFIV = difference between FIV in the grower and finisher period; dRFI = difference between RFI in the grower and finisher period; dFI = difference between FI in the grower and finisher period.

dFIV, g	−157	−136	27.4	0.61
dRFI, g/min	−19	−19	2.0	0.99
dFI, min	−11.5	15.8	4.88	*< 0.01*

Table 4. Feeding behaviour in the grower (20 – 60 kg BW) and finisher period (60 – to slaughter) of pigs in the choice-feeding group with constant access to the CH-G and CH-F diets[11]

	Diets			
Item[12]	CH-G	CH-F	SEM	P-value[13]
Grower period				
DFI, g/d	949	934	121.9	0.93
TTF, min	36.5	42.0	5.7	0.51
FFV, n	10.0	11.6	1.63	0.43
WMFV, n	11.3	13.5	1.92	0.35
TV, min	3.9	3.7	0.42	0.53
FIV, g	101	81	12.9	0.12
RFI, g/min	15.6	22.7	1.60	0.12
FI, min	33.5	31.8	4.45	0.75
Finisher period				
DFI, g/d	1878	1394	291.9	0.27
TTF, min	41.3	34.9	6.85	0.52
FFV, n	8.2	7.8	1.64	0.75
WMFV, n	9.2	8.9	1.91	0.84
TV, min	4.6	4.8	0.81	0.40
FIV, g	259	196	43.35	0.22
RFI, g/min	44.8	41.4	2.80	0.07
FI, min	49.7	39.6	8.57	0.40

[11] The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight of 20 and 100 kg, respectively.

[12] TTF = total time feeding per day; FFV = frequency of feeder visits; WMFV = within meal feeder visits; TV = time per visit; FIV = feed intake per visit; RFI = rate of feed intake; FI = interval between two meals.

[13] P-values of the main factor experimental treatment

Nutrient and energy content of the empty body

At the start of the experiment, the water, nutrient and gross energy content of the empty body of CH and ST pigs did not differ (Table 5). Four days before slaughter, the ash content was greater (P = 0.02) and the water and protein contents tended (P = 0.09) to be greater in the CH than the ST pigs, whereas fat and energy contents of the empty body did not differ. The differences in ash, water and protein contents can be explained by the greater (P = 0.04) empty body weight of the CH pigs because when ash, water, protein and fat contents were expressed as a percentage of the empty body weight, no treatment differences were observed.

Table 5. Nutrient and energy content of the empty body determined by dual-energy x-ray absorptiometry (DXA) at the start of the experiment and prior to slaughter of pigs offered constant free access to the CH-G and CH-F diets (Choice) or offered ad libitum access to the ST-G from 20 to 60 kg BW and to the ST-F diet from 60 – to slaughter (Standard) [14]

	Treatment			
Item[15]	Choice	Standard	SEM	P-value[16]
Empty body nutrient composition				
At the start of the experiment				
Empty body, g	25865	25316	1015.9	0.55
Water, g	17867	17510	689.8	0.57
Ash, g	693	687	19.8	0.82
Protein, g	4087	3982	202.3	0.57
Fat, g	2754	2679	119.3	0.52
Gross energy, MJ	214	208	9.5	0.49
At the end of the experiment				
Empty body, g	105533	101652	1294.2	0.04
Water, g	63478	60506	1283.4	0.09
%	60.09	59.49	0.740	0.48
Ash, g	3011	2834	66.7	0.02
%	2.86	2.79	0.007	0.19
Protein, g	17463	16591	376.4	0.09
%	16.53	16.31	2.222	0.40

[14] The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight (BW) of 20 and 100 kg, respectively. The ST-G and ST-F diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively.

[15] The water, ash, protein, fat and energy contents of the live animals were calculated using the DXA values and the regressions published by Kasper et al. (2020). The %-values were calculated as the ratio between the water, ash, protein, and fat content and the empty body weight.

[16] P-values of the main factor experimental treatment.

Fat, g	20212	20399	977.4	0.85
%	19.19	20.06	0.992	0.43
Gross energy, MJ	1246	1230	32.4	0.60

Water, nutrient and energy deposition rate and protein and energy deposition efficiency

The greater (P = 0.02) daily empty body weight gain was due to the greater (P ≤ 0.04) protein, ash and water deposition rate in the CH pigs than in the ST pigs (Table 6). In contrast, fat and energy deposition rates were similar among treatments. Protein and energy deposition efficiencies were similar in the CH and ST pigs.

Table 6. Daily empty body, nutrient and energy deposition rate and protein and energy deposition efficiency of pigs offered constant free access to the CH-G and CH-F diets (Choice) or offered ad libitum access to the ST-G from 20 to 60 kg BW and to the ST-F diet from 60 – to slaughter (Standard) [17]

	Treatment			
Item	Choice	Standard	SEM	P-value[18]
Daily weight gain[19]				
Empty body, g/d	1051	963	30.9	0.02
Water, g/d	602	541	24.6	0.04
Ash, g/d	31	27	1.0	< 0.01
Protein, g/d	177	159	7.2	0.04
Fat, g/d	229	225	12.2	0.74
Gross energy, MJ	13.6	13.0	0.45	0.13
Nutrient deposition efficiency[20], %				
Protein	44.55	43.73	1.603	0.69
Digestible energy	39.29	39.73	0.798	0.52

[17] The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight (BW) of 20 and 100 kg, respectively. The ST-G and ST-F diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively.

[18] P-values of the main factor experimental treatment.

[19] Daily weight gain = gain of weight determined between the two dual-energy x-ray absorptiometry (DXA) measurements/number of days between the two DXA measurements.

[20] Protein deposition efficiency = empty body protein content at slaughter – empty body protein content at 20 kg body weight/total protein intake between the two DXA measurements; Digestible energy deposition efficiency = gross energy content of the empty body at slaughter – gross energy content of the empty body at 20 kg body weight/total digestible energy intake between the two DXA measurements

Discussion

It is expected that at the beginning of the growing period, the amount of CP and lysine per kg of diet required by the growing pig is high and then gradually declines with increasing BW and age *(NRC, 2012; Agroscope, 2017)*. If the feed intake was driven by the CP and lysine requirements, pigs given the choice between the high (CH-G) and the low CP diet (CH-F) would select a greater amount of the protein-rich CH-G diet at the beginning. With increasing BW and age, pigs would then gradually increase the CH-F diet intake at the expense of the CH-G diet. This seems not to be the case in this experiment. Out of the six pigs, four pigs (animal ID: 7583, 7549, 7586, 7594) seemed to prefer the CH-G over the CH-F diet (Figure 1), as the cumulative differences between the CH-G and CH-F diets favoured the CH-G diet. In contrast, two pigs (animal ID: 7518 and 7613) consumed slightly more of the CH-F diet. The overall greater preference for the protein-rich diet ultimately resulted in a greater overall CP intake of pigs in the CH group compared to the ST group (Figure 2). One could hypothesise that this preference was due to differences in the appetence between the CH-G and the CH-F diets. There are three reasons that suggest that this might have not been the case. First, during feed formulation, strict care was taken to ensure that, if avoidable, the same amounts of the same feed ingredients were used in both diets. Second, a flavour and high intensity sweetener was included in the diet to limit possible differences in palatability between the diets *(Sterk et al., 2008)*. Third, the lack of significant difference in feeding behaviour parameters did not suggest that the CH-G diet was preferred to the CH-F diet. These findings partly contradict the results of earlier studies *(Kyriazakis et al., 1990; Bradford and Gous, 1991b, a)* that showed that the ability for an adequate nutrient self-supply is preserved in domesticated pigs. However, *Bradford and Gous (1991b)* found that when the CP levels of the two diets from which the pig can choose are close together, the physiological effect of each of the diets on the pig is similar. Ultimately, this makes it difficult for the pig to differentiate the diets.

The aforementioned greater CP intake of CH compared to ST pigs was converted into a greater daily protein deposition rate. However, substantial differences were observed between the six CH pigs in the deposition rate of protein (varying from 152 g/d to 208 g/d) and of fat (varying from 216 g/d to 270 g/d) (Figure 3). The variance between the six ST pigs was much smaller, at least for the protein deposition rate. The differences in protein and fat deposition rates in CH pigs appeared to be linked to the preferential selection of the CH-G or CH-F diet. For instance, the pigs that primarily selected the protein-rich CH-G diet (Figure 1; animal ID: 7518 and 7586) displayed the greatest protein deposition rate. In contrast, the pig with the lowest protein deposition rate selected primarily the low-protein CH-F diet. In a study comparing an improved (Large White × Landrace) to an unimproved (Meishan) line, *Kyriazakis et al. (1993)* established that based on their greater protein deposition potential, pigs of the improved line preferred the diet with the greater CP level than the pigs of the unimproved line. Similarly, *Meers et al. (2010)* found in pigs of similar genetic backgrounds that their nutrient intake was influenced by their body composition or composition of BW gain. In their study, they observed that female pigs with a greater amount of lean mass preferentially selected the diet with a greater CP content, while those with a greater fat mass preferentially selected the low CP diet. However, the diet selection effect was less evident in castrated male pigs. They explained this sex difference with the fact that body composition was more variable in female pigs than castrates. In addition, they hypothesised that the difference in the dietary CP level of the two diets was not sufficiently different for barrows to sense the physiological effect of each of the two diets. When considering the present findings, the ST pigs deposited a similar amount of protein, whereas when given the choice to nutrient self-supply, there seems to be a different potential in protein deposition within pigs of the same genetic background. As reviewed by *Roura and Fu (2017)* dietary EAAs such as lysine, methionine, threonine and tryptophan are important drivers of feed selection and intake in pigs. However, the exact mechanism(s) by which the body composition and the composition of BW gain drive the feeding behaviour remain partially not understood.

Feeding behaviour patterns of the ST pigs in the grower period were very similar to the feeding patterns observed in a recent study from our research group *(Bee et al., 2021)*. In contrast, in the finisher period, the ST pigs went more often to the feeder, stayed for a shorter time at the feeder and ingested less feed, and the between-meal interval was shorter compared to the pigs in the standard group of the aforementioned study. One can only speculate about the reasons for these differences. Environmental factors or genetics can be excluded as both studies were conducted in the same experimental facilities, and the pigs were of the same genetic background. However, there were large differences in stocking density, with six pigs assigned to one single-space automatic feeder in this study, whereas in the study of *Bee et al. (2021)*, there were 12 pigs per feeder. In addition, differences existed in the nutrient density of the diets, which might have contributed also to these different eating patterns. Unclear is why these differences between studies were limited to the finisher period.

When comparing the feeding behaviour traits of CH and ST pigs, they differed only in the grower period but not in the finisher period. One could speculate that the shorter but more frequent visits of the two feeders in the grower period occurred because the CH pigs needed to get accustomed to accessing two feeders, and perhaps, this spurred on the spirit of exploration. The exploratory activity faded in the finisher period as they markedly visited less the feeder and stayed for a longer period at the feeder and eating more feed per visit. In contrast, *Pichler et al. (2020)* did not find marked differences in feeding behaviour traits between the grower and finisher period of pigs given the choice to eat from two different diets. When comparing the present study with that of *Pichler et al. (2020),* it was noticeable that the pigs went on average less to the feeder and spent less time eating daily in both the grower and finisher periods in the present study.

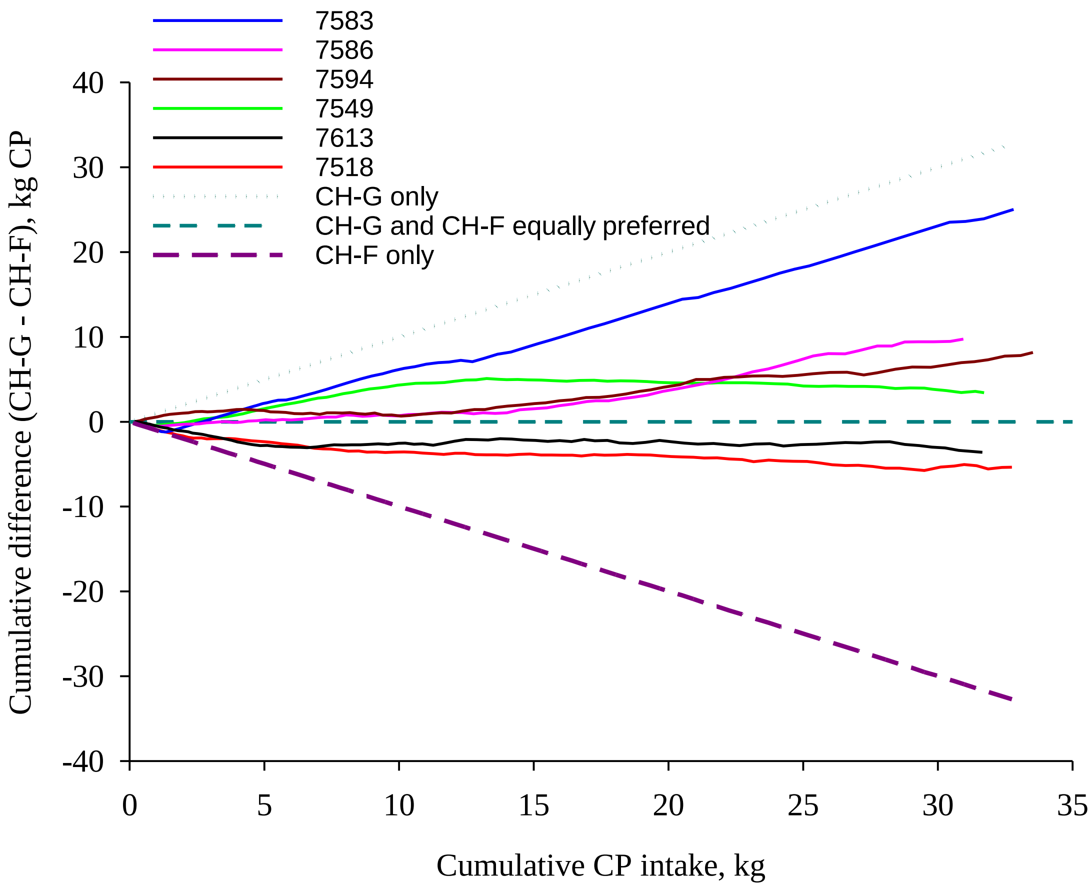


Figure 1. The paths of diet selection of the six pigs given a choice between the CH-G and CH-F diet. Each line refers to an individual pig. The dotted lines refer to a pig choosing only diet CH-G or CH-F diet. The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a BW of 20 and 100 kg, respectively.

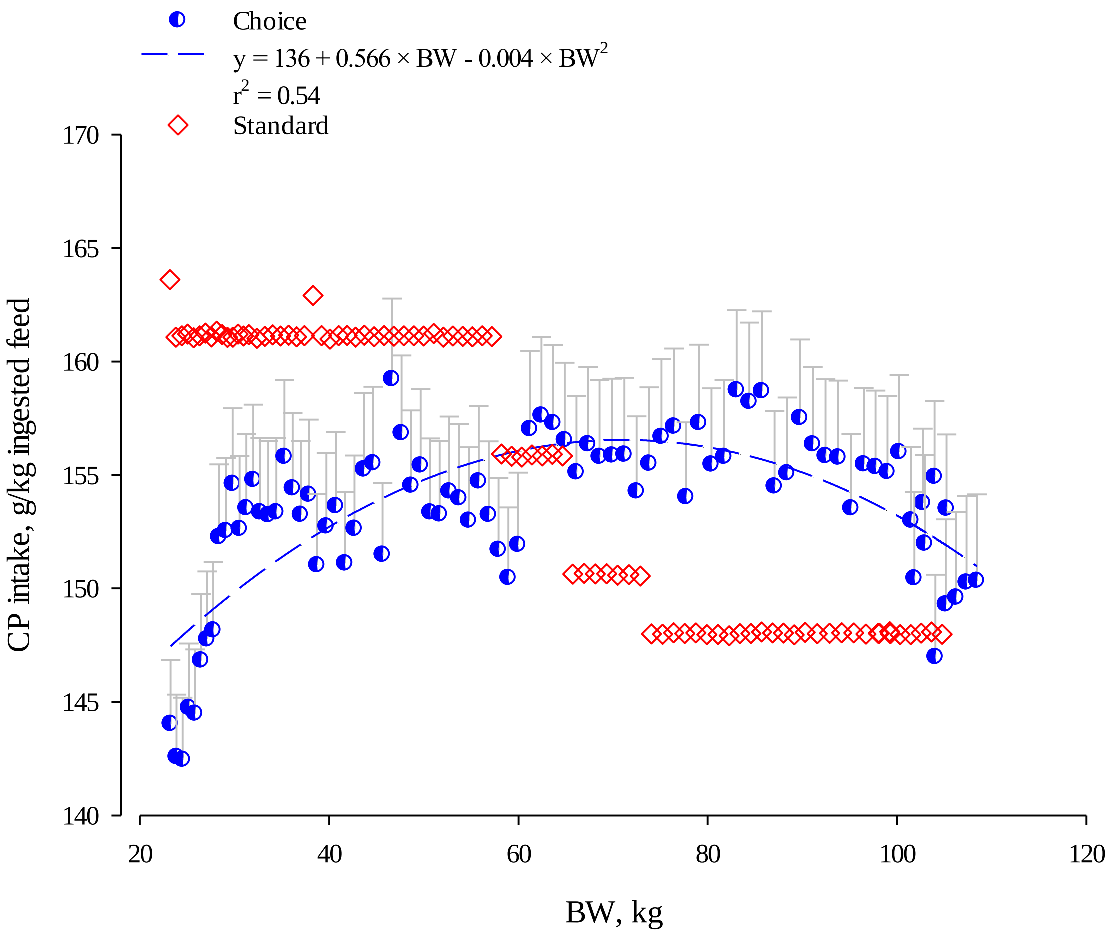
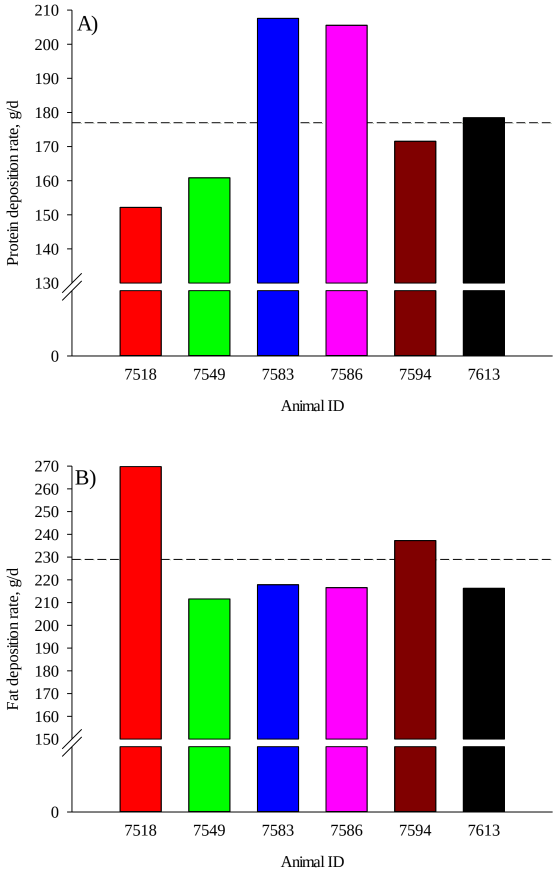


Figure 2. The average daily crude protein intake (CP, expressed as g CP/kg ingested food) over the grower and finisher period of pigs given a choice between the CH-G and CH-F diets (Choice, blue circle) and pigs having access to the ST-G and ST-F diets (Standard, red diamond). The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight (BW) of 20 and 100 kg, respectively. The ST-G and ST-F diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively. The regression line fits the data points of the CP intake of the Choice group.


A)
Protein deposition rate, g/d
210
200
190
180
170
160
150
140
130
0
7518
7549
7583
7586
7594
7613
Animal ID
B)
Fat deposition rate, g/d
270
260
250
240
230
220
210
200
190
180
170
160
150
0
7518
7549
7583
7586
7594
7613
Animal ID

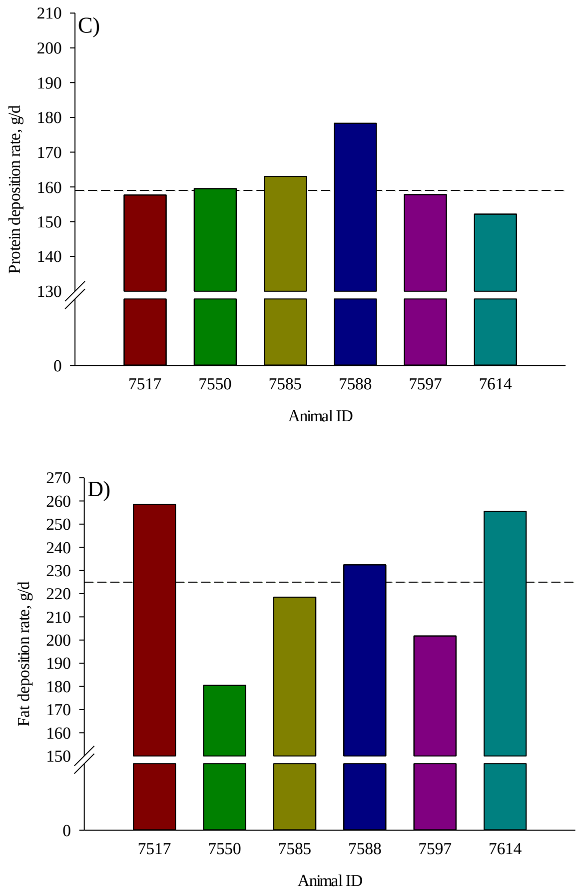


Figure 3. Individual protein and fat deposition rate (expressed as g deposition/d) of the six pigs given a choice between the CH-G and CH-F diets (panels A and B, respectively) and the six pigs fed the standard grower-finisher diet (panels C and D, respectively). The CH-G and CH-F diets were formulated based on the Swiss feeding recommendations for swine (Agroscope, 2017) and optimised for a body weight (BW) of 20 and 100 kg, respectively. The standard grower and finisher diets were formulated based on the same feeding recommendations but optimised for a BW of 40 and 80 kg, respectively. The dotted lines designate the average value for each group, as presented in Table 6.

Conclusion

The current results agree with previous studies *(Kyriazakis et al., 1990; Bradford and Gous, 1991b)*, revealing that modern pig breeds can compose their diet according to their requirements and convert it efficiently into greater performance. This implies that, regarding protein supply, it is not necessary to offer a diet formulated for a given BW range to group penned pigs like it is proposed in the feeding recommendations *(NRC, 2012; Agroscope, 2017)* and implemented in phase-feeding regimes. Nevertheless, a better understanding of the mechanism(s) regulating the interaction between the growth of periphery tissues and the central feeding centres is needed, which then might help in designing the required choice of feeds.

Uticaj samostojeće ishrane kroz izbor na performanse rasta, ponašanje tokom hranjenja i efikasnosti proteina u uzgoju tovnih svinja

Giuseppe Bee, Catherine Ollagnier

Rezime

Ova pilot studija je imala za cilj da uporedi performanse rasta, efikasnost deponovanja hranljivih materija i ponašanje pri hranjenju svinja hranjenih standardnim dvofaznim hranjenjem ili režimom hranjenja po izboru. Eksperiment je izveden sa 12 švajcarskih svinja rase jorkšir između 23,2 i 108,0 kg telesne težine (BW). Šest svinja dodeljenih standardnom (ST) tretmanu su imale ad libitum pristup grover (ST-G) i finišer (ST-F) obroku od 23,2 do 63,4 kg i od 63,4 do 108,0 kg, respektivno. Obroci ST-G i ST-F formulisani su na osnovu švajcarskih preporuke za ishranu svinja prosečne težine od 40, odnosno 80 kg. Ostalih šest svinja dodeljenih tretmanu (CH) imalo je stalan *ad libitum* pristup i grover (CH-G) i finišer obroku (CH-F) formulisanom za referentnu težinu od 20 i 100 kg. Svi obroci su bili izokalorični i razlikovali su se samo po sadržaju sirovog proteina i esencijalnih aminokiselina prema referentnoj BW koja se koristi za formulaciju hrane. Da bi se odredila brzina deponovanja hranljivih materija, svinje su skenirane korišćenjem rendgenske apsorpciometrije sa dvostrukom energijom pri 25,8 i 103,8 kg BW. Pojedinačni unos hrane i ponašanje pri hranjenju praćeni su automatskim hranilicama. Promene u BW utvrđivane su nedeljno. U poređenju sa ST svinjama, CH svinje su unosile više hrane dnevno (P = 0,05) i brže rasle (P = 0,02). Ukupna potrošnja sirovih proteina imala je tendenciju da bude veća (P = 0,08) u CH u odnosu na svinje ST zbog brojčano većeg unosa CH-G ishrane bogate proteinima tokom završnog perioda. Veći unos sirovih proteina u CH nego kod ST svinja praćen je većom (P = 0,04) dnevnom stopom deponovanja proteina, ali sličnom efikasnošću proteina. Što se tiče ponašanja pri hranjenju, CH svinje su češće odlazile do hranilice, provodile manje vremena kod hranilice, jele manje hrane po poseti i imale kraće intervale između dva obroka u odnosu na ST svinje (P <0,01 za svakoga) u period rasta, ali ne i u finalnom periodu. S obzirom na osobine ponašanja pri hranjenju, CH svinje sa većim potencijalom deponovanja proteina preferirale su CH-G obrok bogat proteinima u odnosu na CH-F obrok. Zaključno, ovi rezultati pokazuju da su, poput divljih svinja, pripitomljene savremene svinje zadržale sposobnost za odgovarajuće snabdevanje hranljivim materijama u skladu sa svojim nutritivnim potrebama

Ključne reči: rendgenska apsorpciometrija sa dvostrukom energijom, sastav praznog tela, deponovanje nutrijenata, svinja

Acknowledgment

The authors thank G. Maïkoff and Marion Girard for their support and technical assistance.

References

Agroscope (2017): Fütterungsempfehlungen und Nährwerttabellen für Schweine (Feeding recommendations and nutrient tables for pigs). Agroscope, Posieux, Switzerland. Retrieved on 07.07.2020 from https://www.agroscope.admin.ch/agroscope/de/home/services/dienste/futtermittel/fuetterungsempfehlungen-schweine.html.

BEE G., JACOT S., GUEX G., BIOLLEY C. (2008): Effects of two supplementation levels of linseed combined with CLA or tallow on meat quality traits and fatty acid profile of adipose and different muscle tissues in slaughter pigs. Animal 2, 800-811.

BEE G., KRAGTEN S.A., FRÜH B., GIRARD M. (2021): Impact of 100% organic diets on pig performance, carcass composition and carcass nutrient deposition efficiency. Organic Agriculture, 1-13.

BRADFORD M.M.V., GOUS R.M. (1991a): A comparison of phase feeding and choice feeding as methods of meeting the amino acid requirements of growing pigs. Anim. Sci. 52, 323-330.

BRADFORD M.M.V., GOUS R.M. (1991b): The response of growing pigs to a choice of diets differing in protein content. Anim. Sci. 52, 185-192.

CARCÒ G., GALLO L., DALLA BONA M., LATORRE M.A., FONDEVILA M., SCHIAVON S. (2018): The influence of feeding behaviour on growth performance, carcass and meat characteristics of growing pigs. PLOS ONE 13, e0205572.

DE HAER L., MERKS J. (1992): Patterns of daily food intake in growing pigs. Anim. Sci. 54, 95-104.
ETTLE T., ROTH F. (2009): Dietary selection for lysine by piglets at differing feeding regimen. Livest. Sci. 122, 259-263.
HAN I.K., LEE J., KIM J., KIM Y., KIM J., PAIK I. (2000): Application of phase feeding in swine production. Journal of Applied Animal Research 17, 27-56.
HENRY Y. (1985): Dietary factors involved in feed intake regulation in growing pigs: A review. Livest. Prod. Sci. 12, 339-354.
KASPER C., SCHLEGEL P., RUIZ-ASCACIBAR I., STOLL P., BEE G. (2020): Accuracy of predicting chemical body composition of growing pigs using dual-energy X-ray absorptiometry. bioRxiv, 2020.2009.2015.286153.
KYRIAZAKIS I., EMMANS G., WHITTEMORE C. (1990): Diet selection in pigs: choices made by growing pigs given foods of different protein concentrations. Anim. Sci. 51, 189-199.
KYRIAZAKIS I., EMMANS G.C., WHITTEMORE C.T. (1991): The ability of pigs to control their protein intake when fed in three different ways. Physiology and Behavior 50, 1197-1203.
KYRIAZAKIS I., LEUS K., EMMANS G.C., HALEY C.S., OLDHAM J.D. (1993): The Effect of Breed (Large White X Landrace V Purebred Meishan) on the Diets Selected by Pigs Given a Choice between 2 Foods That Differ in Their Crude Protein Contents. Anim. Prod. 56, 121-128.
MEERS S., PRINGLE T., JONES R., AZAIN M. (2010): Effect of body composition on diet selection in finishing pigs. J. Anim. Sci. 88, 1733-1740.
MENEGAT M.B., DRITZ S.S., TOKACH M.D., WOODWORTH J.C., DEROUCHEY J.M., GOODBAND R.D. (2020): Phase-feeding strategies based on lysine specifications for grow-finish pigs. J. Anim. Sci. 98, skz366.
NOBLET J., BONTEMS V., TRAN G. (2003): Estimation de la valeur énergétique des aliments pour le porc. Prod. Anim. 16, 197-210.
NRC (2012): Nutrient requirements of swine. National Academy Press, Washington, DC, USA.
PICHLER J., SCHWARZ C., GIERUS M., SCHEDLE K. (2020): Choice feeding in fattening pigs: Effect of diets differing in nutrient density on feeding behaviour and fattening performance. Czech J. Anim. Sci. 65, 247-257.
POMAR C., REMUS A. (2019): Precision pig feeding: a breakthrough toward sustainability. Animal Frontiers.
ROURA E., FU M. (2017): Taste, nutrient sensing and feed intake in pigs (130 years of research: then, now and future). Anim. Feed Sci. Technol. 233, 3-12.
RUIZ-ASCACIBAR I., STOLL P., KREUZER M., BEE G. (2019): Dietary crude protein and amino acid restriction has a different impact on the dynamic of protein,

amino acid and fat deposition in entire male, castrated and female pigs. Animal 13, 74-82.

STERK A., SCHLEGEL P., MUL A.J., UBBINK-BLANKSMA M., BRUININX E.M.A.M. (2008): Effects of sweeteners on individual feed intake characteristics and performance in group-housed weanling pigs1. J. Anim. Sci. 86, 2990-2997.

VAN MILGEN J., DOURMAD J.-Y. (2015): Concept and application of ideal protein for pigs. Journal of Animal Science and Biotechnology 6, 1-11.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

RESEARCH OF PRODUCTION RESULTS IN THE TRANSITION FROM CONVENTIONAL TO ROBOTIC MILKING OF COWS

Pero Mijić[1], Zdenko Ivkić[2], Tina Bobić[1]

[1]Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia
[2]Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, 31000 Osijek, Croatia
Corresponding author: Pero Mijić, pmijic@fazos.hr
Invited paper

Abstract: Innovations in animal husbandry are most often where production processes are most sensitive or where the largest workforce participates. Milking cows is one of such jobs where automation of jobs has been introduced more and more in recent years, ie where robots are increasingly used for milking. Today, there are about 50,000 robots of various designs and manufacturers in use in the world. Research shows that number will double in the coming years. The robotization of dairy farms has gained momentum with producers in the Republic of Croatia. However, in robotization of farms, farmers face new challenges and practices that they did not know before. That is why any professional and scientific help is welcome and worth studying. The results of this study were conducted on two dairy farms that underwent a transition from conventional to robotic milking. The results showed a significant ($p < 0.0001$) decrease in the number of older cows seen through the number of lactations after the transition from conventional to robotic milking (from 2.51 to 2.16 and 1.77 lactation, respectively). This indicates that it is necessary to do increased selection and selection of cows better adapted to robotic milking. The average production per cow increased significantly ($p < 0.0001$) (by about 500 kg milk in the second year after the introduction of robotic milking), while the percentage of milk fat and urea in milk decreased significantly ($p < 0.0001$). The number of somatic cells increased, but not significantly. It can be concluded that the first (transitional) year (the year of transition to robotic milking) is very important and technologically challenging. However, in the second year of production, robotic milking shows significant positive effects on production.

Key words: production results, conventional milking, robotic milking, cows

Introduction

Today's milk production has become a very intensive livestock industry with large technological investments. Due to their economic sensitivity and complexity, milk production is accompanied by various innovations. Milking cows is quite sensitive and demanding, furthermore about 50% of working time on the farm is spent on milking every day (*Havranek and Rupić, 2003*).

In order to facilitate milking, but also to increase production efficiency, automated milking systems (AMS), i.e. milking robots, are increasingly being introduced on dairy farms. The reasons are very clear and well-argued: higher labour efficiency, lower labour costs, lack of manpower interested in working on milking jobs, better farm management and more (*De Koning et al., 2002; Kuczaj et al., 2020*). Milking robots have proven to be particularly suitable on farms with a free way of keeping cows, but also on farms where cows are kept on pastures (*Woodford et al., 2015*). The use of milking robots with accompanying computer and software programs enables complete automation of the milking process. It has also been found that there is a decrease in the share of human labour in milking, and an increase in the share of labour in farm management (*Rodenburg, 2017*).

The first robots began to be used in the Netherlands in 1992 (*Bijl et al., 2007*), and since then their number has been steadily growing. It is not possible to say exactly what the current number of cow milking robots in the world is. Rough estimates say that around 50.000 milking robots are currently used in the world (*Mijić and Bobić, 2019*). The Netherlands is today a leader in robotic milking, where about 22% of farmers own a milking robot (*Huiden, 2018*).

The largest part of the world market (about 80%) is covered by three manufacturers of robotic equipment (Lely, DeLaval and GEA). The other three manufacturers (Fullwood, SAC and BouMatic) share the rest of the market. According to US projections (*Tranel, 2017*), the number of milking robots in the next five years in the world could be doubled. It is interesting to note that as much as 90% of the total number of milking robots in the world are located in cattle-developed countries in north-western Europe. These are most common family farms with one to three robots (*Huiden, 2018*).

Robotic milking of cows has opened up a number of possibilities for various researches. Thus, for example, *Brito et al. (2020)* state that some countries, when evaluating genetic and genomic tests of cattle, among other things, collect information about animals they take from automated systems on the farm (milking robots, feeding robots, robots for cleaning and care, videos, etc.). Some other research (*von Kuhlberg et al., 2020*) studies the use of phantom robots in heifers to adapt them to robotic milking when lactation begins. This type of training of heifers proved to be quite good, especially in the first five days of milking. In trained animals, stress levels were significantly lower, resulting in higher milk production at this initial stage of lactation.

Automation and robotization of dairy farms is increasingly present among producers in the Republic of Croatia (*Mijić and Bobić, 2019*). The first six milking robots were installed in 2008. The latest data show (*Mijić and Bobić, 2020*) that the number of cows for milking cows in Croatia is constantly increasing. Currently, 40 milking robots from four producers were installed on 27 farms, or 0.6% of the total number of farms in Croatia.

Following the above information, the hypothesis were that the introduction of automation on the farm is a technologically challenging and stressful period for both animals and farmers. However, we assume that automation is also economically justified. The aim of the study was to investigate and analyse the production results of dairy farms that have undergone a transition from conventional to robotic milking cows.

Materials and Methods

For the purposes of this research two dairy farms were selected, which switched from conventional to robotic milking of cows. On both observed farms, milking robots were installed in the same year (Table 1.), and had Holstein cows.

The research covered three production years:

- A - year before the introduction of robots (conventional milking),
- B - first year of robotic milking (transition year) and
- C - second year of robotic milking.

Production data from dairy cows were taken from the database of the Croatian Agency for Agriculture and Food, which performs official milk control. In order to determine the difference between conventional and robotic milking on some production traits, an analysis of variance (ANOVA) with the statistical program STATISTICA (2018), was performed.

Table 1. Basic data on the study farms

Farm	Robot manufacturer	Number of robots on the farm	Number of cows at milking	Year of transition to AMS
I	DeLaval	1	78	2019.
II	Lely	3	152	2019.

Results and Discussion

The results in Table 2. show that the average number of lactations of all cows on the observed farms was 2.24. The average lactation production corrected at 305 days was 7,923 kg of milk, and the average daily production was 25.35 kg of milk. Considering the protein content in milk (3.48%), the fat (4.00%) and the urea content in milk (24.93 mg / 100mL), it could be said that the farm ration for dairy cows was well balanced. Furthermore, the Somatic Cell Count (SCC) was within standard limits (average about 281,000). Slightly different values are reported by *Bach et al. (2009)*. The average daily milk production was higher and amounted to 29.8 kg, while the values of milk fat and protein were lower and amounted to 3.65% and 3.38%. However, the observed farms were designed and built from the beginning for robotic milking. This is an important fact, because the movements and feeding of cows on the farm can be better designed *(Rodenburg, 2017)*.

Table 2. Descriptive statistic of the production traits for all three investigated years corrected to 305 days

Trait	Average	Minimum	Maximum
Lactation	2.24	1.00	10.00
Milk (305 days, kg)	7.923	875	14.204
Daily Milk Yield (kg)	25.95	5.00	68.70
Fat (%)	4.00	2.46	6.68
Protein (%)	3.48	2.71	4.53
Somatic Cell Count (1.000/mL)	281.13	13.00	1.998
Urea (mg/dL)	24.93	12.00	65.00

During the analysis of production traits (Table 3), after transition from conventional to robotic milking (from 2.51 to 2.16 and 1.77, respectively) a significant ($p < 0.0001$) decrease in the average number of lactations was observed. These results indicated that with switching from conventional to the robotic milking, the culling cows become increased. The reasons are in the creation of a uniform herd regard to the requirements of the robot (correct position of the teats, uniform udder quarters, etc.). In addition, cows with mammary gland and hoofs health problems are not suitable animals for robotic milking.

According to *Pezzuolo et al. (2017)* when AMS is fully implemented on the farm, the duration of lactation of cows is longer and averages values are 3.47. The first (transition) year of robotic milking (B) is extremely important for the further successful farm business *(Tse et al., 2018).*

The results of this research has shown a decrease in production of standard lactation by 195.2 kg of milk in transition year compared to the previous year (A) when cows were milked in a conventional milking parlour. However, already in the following year (C) there was a significant ($p < 0.0001$) increase in lactation production for even 532.1 kg of milk compared to year A, or 727.3 kg compared to year B (Table 3). Furthermore, the significant ($p < 0.0001$) decrease for the milk fat and urea was determined. For these trends is very important a number of departures cows at milking (number of visits to the robot). According to a study of the *De Koning (2010)* at liest 76% of cows in the farm should be milked more than twice a day. Such a number of milking can lead to an increase in milk production and a positive economic effect due to the introduction of robotic milking.

Table 3. Production traits (305 days) during three investigated years (A, B, C)

Trait	Year			Significance
	A	**B**	**C**	
Lactation	2.51^{a}	2.16^{b}	1.77^{c}	$p<0.001$; $p<0.0001$
Milk (kg)	7.828,5^{a}	7.630,3^{ab}	8.357,6^{c}	$p<0.0001$
Fat (%)	4.06^{a}	4.04^{ab}	3.85^{c}	$p<0.0001$
Protein (%)	3.48^{a}	3.50^{a}	3.47^{a}	NS
Somatic Cell Count (1.000/mL)	266^{a}	310^{a}	283^{a}	NS
Urea (mg/dL)	25.34^{a}	25.16^{ab}	23.86^{c}	$p<0.0001$

* values within the same row marked with different letter differ statistically significant ($p<0.001$; $p<0.0001$;); NS – not significant

The effect of robotic milking had a positive trend of milk production for all observed lactations (Figure 1). So that, the milk production of primiparous cows from year A to year C increased by 551 kg, for the cows in the second lactation by 824 kg, and for the cows in the third lactation by 822 kg, respectively. The smallest increase in production was recorded in cows in the fourth and subsequent lactations, where the increase was only 25 kg of milk. It can be seen that older cows react less to the effect of robotic milking, and the reasons may be in the weaker adaptation to robotic milking, the higher incidence of health problems (mastitis, metabolic problems, etc.) and the fewer visits to the milking robot (*Jacobs and Siegford, 2012*). Also, for successful production it is very important to balance the farm ration well, as this will affect the amount of concentrate consumed. This component is the most expensive part of the meal and significantly affects the economic profitability of the farm *(Migliorati et al., 2005)*.

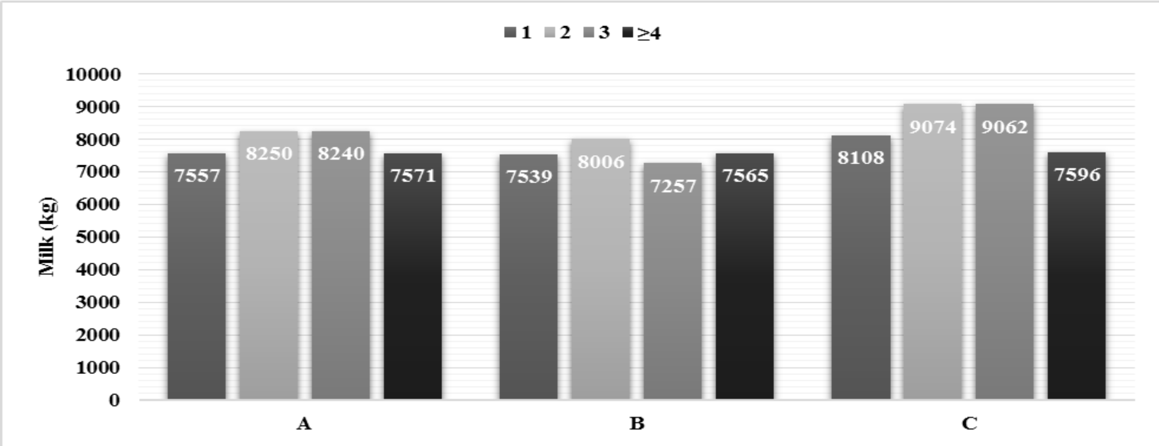


Figure 1. The amount of milk according to the ordinal number of lactation before (A) and after the introduction of AMS (B and C)

One of the frequently mentioned features cited as negative in robotic milking is a slightly higher number of somatic cells in milk than in conventional milking *(Svennersten-Sjaunj and Pettersson, 2008; De Koning, 2010)*. This is partially confirmed in this study (Figure 2). The primiparous cows had a smallest somatic cells count after the introduction of robotic milking (by 13.2%). Cows in other lactations had a slightly higher number of the SCC, but these values were still at an acceptable level. Thus, cows in the second lactation had an increase in SCC by 21.5%, cows in the third lactation by 11.7%, and cows in the fourth and subsequent lactations by 17.6%. However, raw milk was of standard quality and met the requirements prescribed by the Ordinance on determining the composition of raw milk in the Republic of Croatia *(N.N. 27/2017)*. Such a slight increase in SCC with increasing lactation is expected, according to *Schepers et al. (1997).* Generally, in older cows the status of mammary gland infection is slightly higher. One of the reasons is the demanding production of milk, both in quantity and quality, which has consequences for the health of the udder of cows (*Klungel et al., 2000*). However, after adapting cows to robotic milking, udder health and SCC can be even better than in conventional milking cows. Such a significant effect ($p < 0.05$) takes about 25 weeks *(Berglund et al., 2002)*.

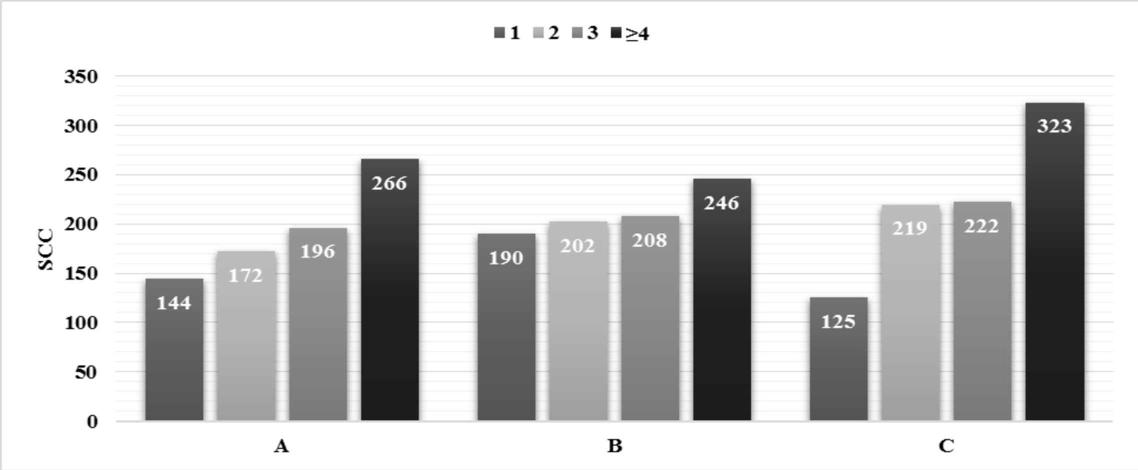


Figure 2. The median values of the somatic cell count (SCC) according to the ordinal number of lactation before (A) and after the introduction of AMS (B and C)

Conclusion

Based on the conducted research, it can be concluded that the effect of AMS, i.e. the introduction of a robotic milking on the researched example was very positive. The positive trends were especially visible in the amount of produced milk, both at the daily and lactation level. The critical period was in the first (transitional) year of introducing robots into the production process. Then there may be a certain stagnation of production parameters such as a drop in milk fat or a slight increase in the somatic cells count in milk. However, the results in the second year of using the robot show the justification for the introduction of a robotic milking. Almost all production traits had significantly better results. It is important to emphasize that the first (transitional) year (the year of transition to robotic milking) poses the biggest challenges for farmers in production: adaptation of the facility and installation of robots, habituation of cows to robotic milking, increased herd culling, mastering technical features of robotic milking systems and information, etc. Robotization of farms can facilitate and improve the daily demanding tasks of milk production, and increase the production and profitability of the farm.

Istraživanje proizvodnih rezultata pri prelasku sa konvencionalne na automatsku mužu krava

Pero Mijić, Zdenko Ivkić, Tina Bobić

Rezime

Inovacije u stočarstvu najčešće su tamo gde su proizvodni procesi najosetljiviji ili gde učestvuje najveća radna snaga. Muža krava jedan je od takvih poslova u kojima se poslednjih godina sve više uvodi automatizacija radnih mesta, odnosno gde se roboti sve više koriste za mužu. Danas se u svetu koristi oko 50.000 robota različitog dizajna i proizvođača. Istraživanja pokazuju da će se taj broj u narednim godinama udvostručiti. Robotizacija mlečnih farmi dobila je zamah kod proizvođača u Republici Hrvatskoj. Međutim, u robotizaciji farmi, poljoprivrednici se suočavaju sa novim izazovima i praksama koje ranije nisu poznavali. Zato je svaka stručna i naučna pomoć dobrodošla i vredna proučavanja. Rezultati ove studije sprovedeni su na dve mlečne farme koje su prošle tranziciju sa konvencionalne na robotsku mužu. Rezultati su pokazali značajno ($p<0,0001$) smanjenje broja starijih krava, posmatrano kroz broj laktacija nakon prelaska sa konvencionalne na robotsku mužu (sa 2,51 na 2,16 i 1,77 laktacije, respektivno). Ovo ukazuje na to da je potrebno izvršiti povećanu selekciju krava bolje prilagođenih robotskoj muži. Prosečna proizvodnja po kravi značajno se povećala ($p<0,0001$) (za oko 500 kg mleka u drugoj godini nakon uvođenja robotske muže), dok se procenat mlečne masti i uree u mleku značajno smanjio ($p<0,0001$). Broj somatskih ćelija se povećao, ali ne značajno. Može se zaključiti da je prva (prelazna) godina (godina prelaska na robotsku mužu) veoma važna i tehnološki izazovna. Međutim, u drugoj godini proizvodnje robotska muža pokazuje značajne pozitivne efekte na proizvodnju.

Ključne reči: proizvodni rezultati, konvencionalna muža, robotska muža, krave

Acknowledgement

The research was done within the framework Research project Fitness Potential of Animals in Economically Sustainable Agricultural Production, Faculty of Agrobiotechnical Sciences Osijek.

References

BACH A., DEVANT M., IGLEASIAS C. FERRERT A. (2009): Forced traffic in automatic milking systems effectively reduces the need to get cows, but alters eating behavior and does not improve milk yield of dairy cattle. Journal of Dairy Science, 92, 1272–1280.

BERGLUND I., PETTERSSON G., SVENNERSTEN-SJAUNJA K. (2002): Automatic milking: effects on somatic cell count and teat end-quality. Livestock Production Science, 78, 2, 115-124.

BIJL R., KOOISTRA S. R., HOGEVEEN H. (2007): The profitability of automatic milking on dutch dairy farms. Journal of Dairy Science, 90, 1, 239-248.

BRITO L. F., OLIVEIRA H. R., McCONIN B. R., SCHINCKEL A. P., ARRAZOLA A., MARCHANT-FORDE J. N., JOHNOSON J. S. (2020): Large-Scale Phenotyping of Livestock Welfare in Commercial Production Systems: A New Frontier in Animal Breeding. Frontis in Genetics, 11, 793. doi: 10.3389/fgene.2020.00793

DE KONING C.J.A.M., VAN DE VORST Y., MEIJERING A. (2002): Automatic milking experience and development in Europe. In Proceedings of the First North American Conference on Robotic Milking, Toronto, ON, Canada, 20–22 March 2002; pp. 1–11.

HAVRANEK J., RUPIĆ V. (2003): Mlijeko od farme do mljekare. Udžbenik Sveučilišta u Zagrebu, Hrvatska mljekarska udruga, Zagreb.

HUIDEN F. (2018): Milking robots are becoming smarter and cheaper. Dairy Global. https://www.dairyglobal.net/Milking/Articles/2018/4/Milkinig-robots-are-becoming-smarter-and-cheaper-271457E/

KUCZAJ M., MUCHA A., KOWALCZYK A., MORDAK R., CZERNIAWSKA-PIATKOWSKA E. (2020): Relationships between selected physiological factors and milking parameters for cows using a milking robot. Animal, 10, 2063. doi: 10.3390/ani10112063

MIGLIORATI L. SPERONI M., LOLLI S., CALZA F. (2005): Effect of concentrate feeding on milking frequency and milk yield in an automaticmilking system. Italian Journal of Animal Science, 4, 2, 221-223.

MIJIIĆ P., BOBIĆ T. (2019): Automatizirani muzni sustavi ili robotizirana mužnja krava: prednosti i nedostatci. Zbornik predavanja, str. 68-77. XIV savjetovanje uzgajivača goveda u Republici Hrvatskoj. / Zdenko Ivkić (ur.). Plitvička Jezera, Hrvatska, 30-31. 01. 2019. Hrvatska agencija za poljoprivredu i hranu.

MIJIĆ P., BOBIĆ T. (2020): Procjena uspješnosti prelaska s konvencionalne na robotiziranu mužnju krava na primjeru hrvatskih farmi. Zbornik predavanja, str. 99-111. XV Savjetovanje uzgajivača goveda u Republici Hrvatskoj. / Krunoslav

Dugalić (gl. i odg. ur.). Tuheljske toplice, Hrvatska, 29-30. 01. 2020. Hrvatska agencija za poljoprivredu i hranu.
RODENBURG J. (2017): Robotic milking: Technology, farm design, and effects on work flow. Journal of Dairy Science, 100: 7729–7738. https://doi.org/10.3168/jds.2016-11715
STATISTICA (2018). StatSoft Staistica.
VON KUHLBERG M. K., WWNSCH-DORENDORF M., GOTTSCHALK J., WANGER T., HERRMANN N., EINSPANIER A. (2021): The effects of a training program using a phantom to accustom heifers to the automatic milking system. Journal of Dairy Science, 104, 1, 928-936. https://doi.org/10.3168/jds.2020-18715
TRANEL L. (2017): Economics of Robotic Milking Systems. https://www.usda.gov/ oce/forum/past_speeches/2017/2017_Speeches/Larry_Tranel.pdf
WOODFORD K. B., BRAKENRIG M. H., PANGBORN M. C. (2015): New Zealand case studies of automatic-milking-systems adoption. Proceedings of the New Zealand Society of Animal Production, 75, 127-131.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

ASSESSMENT OF BIOSECURITY AND WELFARE OF CALVES REARED IN INTENSIVE HOUSING SYSTEM

Ljiljana Samolovac[1], Slavča Hristov[2], Dušica Ostojić Andrić[1], Vlada Pantelić[1], Dragan Nikšić[1], Dragan Stanojević[2], Tamara Stamenić[2]

[1] Institute for animal husbandry, Belgrade, Serbia
[2] University of Belgrade, Faculty of agriculture, Belgrade-Zemun
Corresponding author: Ljiljana Samolovac, ljiljanasamolovac@gmail.com
Invited paper

Abstract: The quality of farm animal welfare largely depends on a number of measures and procedures carried out on farms, which are defined by one name as biosecurity. The application of certain management practices differs from farm to farm, and accordingly their impact on the quality of animal welfare differs. The quality of biosecurity, welfare and the presence of factors that threaten them depend on the technology of production on the farm, animal husbandry systems, microclimatic and hygienic conditions, management, procedures performed on animals and the way they are performed, the attitude of employees towards animals, their training and competence, etc.The aim of this study was to determine the impact of intensive calf rearing on differences in biosecurity and welfare quality assessment on two dairy farms. The technological process of production on both farms is similar, since both farms operate within the same production system. On both farms, there is a nursery in a separate facility, but without individual calving boxes. Calves are separated from their mothers immediately after birth. One of the significant differences between the farms was the way the calves were kept in the first 7 days of life. On one of the farms (A) the calves were kept tied in the nursery, while on the other farm (B) they were housed in individual boxes, also located within the nursery. At 8 days of age, calves were placed in group boxes, in a special facility, rearing stable.

The greatest weaknesses and threats to biosecurity and welfare on both farms were manifested at the earliest age of calves, and they relate to the accommodation and feeding of newborn calves with colostrum. Determined that newborn calves were kept tie-stall housing system or in dimly lit individual boxes of inadequate size and design, housed in the nursery together with the cows. This increased the calf's exposure to a number of pathogens. The risk to biosecurity and welfare is higher when inadequate colostrum consumption is taken into account in terms of quantity, manner and time of feeding. When it comes to calves of older categories, the situation was significantly more favourable in terms of nutrition and housing conditions, as well as health surveillance and provided great opportunities in terms of further improvement.

Key words: biosecurity, welfare, risk factors, intensive production system

Introduction

The precondition for achieving high quality of farm animal welfare is the implementation of certain, precisely determined, measures that are defined as biosecurity. The most common biosecurity measures are a system of management practices used to protect animals from pathogenic agents and to prevent the spread of harmful agents from the farm to the environment. (*Linch, 2012; Damiaans et al., 2018*). Biosecurity can be divided into external and internal. External biosecurity refers to measures aimed at preventing the introduction of the disease into the herd (keeping the animal in quarantine, the principle of "all in – all out", control of the movement of visitors, disinfection barrier). Internal biosecurity is a set of measures taken to prevent the spread of disease within the herd (separation of different categories of animals, isolation of sick animals, control of the movements of the staff, control of the presence of birds, rodents and other animals on the farm, safe removal of corpses, etc.). The result of consistent implementation of the mentioned measures is a high quality of welfare of farm animals.

The definition of animal welfare most often refers to the general condition of individuals (*Huges et al., 1976; Broom et al., 1986*) observed in a particular environment. This means that animals (in this case calves) should be in an environment that will meet their basic needs in a satisfactory way: nutrition, housing, expression of physiological behaviors, interaction with individuals of the same species, absence of pain, injury and negative emotions, etc. (*Rollin et al., 1993*). The quality of welfare of calves in intensive agricultural production systems depends on a number of factors with different significance and intensity of impact, and one of the most important is biosecurity.

Given the pronounced interrelationship between biosecurity and welfare, it is clear that the presence of certain shortcomings, so-called risk factors, has negative impact on their quality. The quality of biosecurity, welfare and the presence of factors that endanger them depend on the technology of production on the farm, animal husbandry systems, microclimatic and hygienic conditions, management, procedures performed on animals and the way they are performed, the attitude of employees towards animals and their training and competence, etc. Different methods of production imply different degrees of biosecurity, and thus different quality of welfare, because the specifics of each farm depend on risk factors that threaten biosecurity and welfare.

Numerous authors have addressed biosecurity issues from a variety of perspectives (*Beggs et al., 2015; Renaud et al., 2018; Emanuelson et al., 2018; Stanković et al., 2011 and 2014; Dammianis et al., 2019 and 2020; Robichaud et al., 2019; Boersema et al., 2013; Shortatall, 2017; Ježek et al., 2019; Winder et al., 2016; Richens et al., 2018; Stanković and Hristov, 2009; Ferit Can, 2018; Anderson, 1998; Nitovski et al., 2013; Pedersen et al., 2009; Bojkovski et al., 2012)* and welfare (*Hristov et al., 2011 and 2012; Samolovac et al., 2019 and 2020; Relić et al., 2014; Vasseur et al., 2009 and 2010; Weawer et al., 2000; Osaka et al. 2014; Hristov et al., 2015; Vasseur et al., 2010; Kieland et al., 2010; Gottardo et al., 2011; Wikman et al., 2013; Winder et al., 2016; Gottardo et al., 2011; Ostojić-Andrić et al., 2015; Relić and Bojkovski, 2010; Bojkovski et al., 2012; Stanković et al., 2011; de Vries et al., 2013; Lundvall and Saras- Johansson, 2011; Schütz et al., 2012; Burton et al., 2012; Elingsen et al., 2014; Winder et al., 2016;Robichaud et al., 2019).*

The basis for the study of the welfare of calves in the intensive housing system were two hypotheses: the first - biosecurity measures, welfare quality and risk factors on farms are interrelated and intertwined, and differ on individual farms, depending on the applied production technology and housing system, and the second - in different housing systems, there are different threats and weaknesses that affect the quality of biosecurity and welfare.

The aim of this study was to determine the impact of intensive calf rearing on differences in biosecurity and welfare quality assessment on two farms.

Material and Method

Assessment of biosecurity, risk factors and welfare of calves up to 30 days of age was performed on 2 dairy farms with intensive rearing. The technological process of production on both farms was similar, since both farms operated within the same production system. Both farms had a nursery in a separate facility, but without individual calving boxes. Calves were separated from their mothers immediately after birth. One of the significant differences between the farms was the way the calves were kept in the first 7 days of life. On one of the farms (A) the calves were kept tied in the nursery, while on the other farm (B) they were housed in individual boxes, also located within the nursery. At the age of 8 days, calves were placed in group boxes, in a special facility, rearing stable.

The assessment of biosecurity and risk factors was performed on the basis of data collected by the method of a structured questionnaire derived from the Project

"Development and implementation of welfare and biosecurity standards in order to improve the technology of cattle and pig production". Good and bad characteristics, threats and opportunities on farms, based on the data from the questionnaire were determined by SWOT analysis (analysis of strengths, weaknesses, opportunities and threats of the farm as a production unit) which included: biosecurity plan, isolation, health status, movement and traffic control, attitude towards other persons, control of nutrition and water supply, removal of dead calves, presence of other animal species on the farm, control of rodent populations, control of insect population, control of bird population, sanitation and farm attitude towards the environment (*Anon, 2011*).
The Welfare Quality® Assessment Protocol (*WQAP, 2009*), a scientific method for assessing the welfare of farm animals, was used to assess the welfare of the animals. The overall assessment of welfare protection on farms A and B was obtained on the basis of assessment of welfare criteria, which included a number of indicators: assessment of planning, organization and implementation of welfare protection, assessment of the staff regarding welfare protection, competencies of the staff regarding welfare protection, breeders' attitude towards animal needs, assessment of monitoring and inspection of animals and equipment, animal treatment; nutrition and watering of animals, housing conditions, microclimatic conditions, hygienic conditions in the facility, hygiene and care of the animal's body; reproduction, productivity, behaviour and health.
Risk factors for the welfare of calves on farms A and B were divided into 3 groups, namely: risk factors related to nutrition, housing conditions and management, i.e. production technology on the farm. According to the strength and character of the impact, they were classified from low to very strong (low, moderate, medium strong, strong and very strong impact). Exposure of calves to the impact was defined as: rare, very rare, moderate and very common. Based on the characterization and duration of action, and according to EFSA methodologies from 2006 and 2009, risk factors were classified into four categories: high, low, negligible and risk-free.

Results and Discussion

The obtained overall estimates for biosecurity on the observed farms are shown in the following table (Table 1).

Table 1. Assessment of biosecurity indicators on farms A and B

Indicators	Score	
	Farm A	Farm B
Biosafety plan	1.67	1.67
Insulation	2.67	2.67
Health status	3.40	3.60
Movement and traffic control	2.75	3.00
Relation to other persons	2.37	2.37
Nutrition and water supply control	3.50	3.50
Removal of dead calves	2.80	2.80
The presence of other species of animals on the farm	1.00	1.00
Rodent population control	2.80	2.80
Insect population control	2.00	2.00
Bird population control	1.33	1.33
Sanitation	2.92	2.92
The relation of the farm and environment	3.50	3.50
Average rating	**2.52**	**2.55**

The SWOT analysis shows that the most pronounced weaknesses on both farms are the lack of implementation of defined procedures related to biosecurity plans, control of visitor movements and control of the population of insects, rodents, birds and other animals. In contrast, the strongest points are the controlled quality of water (city water supply) and food (regular laboratory analyzes); health status on farms under the permanent supervision of the veterinary service and the socially responsible relation of the farm towards the environment. However, in these segments there is a need for further improvement. Great opportunities for improving the existing situation are provided in the field of isolation of the farm, increasing the control of the movement of visitors and workers, removal of corpses and sanitation, in order to prevent the occurrence and spread of infectious diseases.
The total welfare assessment on farms A and B based on the indicator assessment is shown in the following table (Table 2).

Table 2. Assessment of welfare indicators on farms A and B

Inidicators	**Score**	
	Farm A	**Farm B**
Assessment of welfare plans, organization and implementation	1.00 - 1	1.00- 1
Assessment of staff regarding welfare	2.75 - 3	3.00- 3
Competences of staff regarding welfare protection	2.78 - 3	3.22 - 3
The relation of breeders towards needs of animals	2.67 - 3	3.00 - 3
Assessment of monitoring and inspection of animals and equipment	4.62 - 5	4.62 - 5
Treatment of animals	2.67 - 3	2.67 - 3
Nutrition and watering of animals	3.73 - 4	3.73 - 4
Housing conditions	2.70 - 3	3.00- 3
Microclimatic conditions	2.25 - 2	2.12 - 2
Hygienic conditions in the facility	2.67 - 3	2.55 - 3
Hygiene and body care of animals	3.00 - 3	3.00 - 3
Reproduction	3.00 - 3	3.00 - 3
Productivity	3.33 - 3	3.22 - 3
Behaviour	3.45 - 4	3.18 - 3
Health condition	3.33 - 3	3.33 - 3
Average rating	**2.93 - 3**	**2.98 - 3**

Given that the quality of welfare directly depends on the degree of implementation of defined biosecurity measures, it is not surprising that the obtained results of the assessment of welfare indicators on the observed farms are in accordance with the assessment of biosecurity measures. The most favourable situation was in terms of monitoring of animals and equipment thanks to the daily multi-hour presence of staff in the facilities, and in terms of food and water quality due to regular laboratory analyzes. The greatest weakness was manifested in the plans and implementation of welfare protection (lack of procedures, lack of clearly written instructions, lack of organized training of workers) and in terms of microclimatic conditions which were very often unfavourable (high temperature, high humidity). The analysis of the largest number of observed indicators related to the quality of welfare indicates the fact that there are great opportunities for their improvement.

Risk factors that negatively affected biosecurity and quality of welfare differed in part depending on the farm and category of calves, because they showed different intensity and duration of action, and are shown in the following table (Table 3).

Table 3. Assessment of risk factors on farms A and B (age categories are given in brackets)

Risk category	Farm A	Farm B
High	Calves tied to the bed (0-7) Colostrum amount (0-7) Insufficient water supply (0-7) Continuous arriving of animals to the facilities and departing (0-7) Allergens, hemoglobin, Fe not controlled (0-30)	Insufficient floor area (0-30) Insufficient light (0-7) Insufficient water supply (0-7) Continuous arriving of animals to the facilities and departing (0-30) Allergens, hemoglobin, Fe not controlled (0-30)
Low	Colostrum quality (0-7) Lack of maternal care (0-7) Insufficient light (0-30) Exposure to pathogenic agents (0-7)	Colostrum quality and consumption time (0-7) Lack of maternal care (0-7)
Negligible	Colostrum consumption time (0-7) Microclimatic conditions (0-7) Exposure to pathogens (8-30) Disinfection without analgesia (8-30)	Colostrum quantity (0-7) Microclimatic conditions (8-30) Light (8-30) Exposure to pathogens (8-30) Surface, floor quality and bedding hygiene (8-30)
Risk-free	Nutrition and water supply (8-30) Microclimatic conditions (8-30) Floor area and quality (8-30) Bedding hygiene (8-30) Mixing animals from different sources (0-30) Health monitoring (0-30)	Nutrition and water supply (8-30) Microclimatic conditions (8-30) Mixing animals from different sources (0-30) Health monitoring (0-30) No dehorning (0-30)

It is obvious that the greatest weaknesses and threats to biosecurity and welfare on both farms were manifested at the earliest age of calves. According to the SWOT analysis, the biggest weaknesses are the housing and feeding of newborn calves with colostrum. It is absolutely unacceptable to keep newborn calves tied to beds or in dimly lit individual boxes of inadequate size and design. Housing of calves in the nursery together with cows increases their exposure to numerous pathogenic agents. The risk to biosecurity and welfare is higher when inadequate colostrum consumption is taken into account in terms of quantity, manner and time of feeding. When it comes to calves of older categories, the situation is significantly more favourable in terms of nutrition and housing conditions, as well as health monitoring and provides great opportunities in terms of further improvement.

The improvement of biosecurity measures has been implemented continuously for many years, but there are still some dilemmas and doubts. There are still significant differences of opinion between veterinarians and animal breeders regarding the importance of implementing biosecurity measures and procedures, as stated by *Boersema et al. (2013), Shortatall (2017)*, but breeders themselves attach insufficient importance to certain measures that should be implemented in order to protect biosecurity on their own farms (*Ježek et al., 2019; Winder et al., 2016; Richens et al., 2018*). The overall assessment of the implemented biosecurity measures on the observed farms was similar for both farms and very close to the average. Low overall biosecurity scores on farms were recorded by *Dammians et al. (2020),* 48.6 out of 100 index points in the questionnaire, and *Stanković and Hristov (2009),* report the following results on two farms: 3.81 and 2.31. Different production systems imply different degrees of biosecurity on farms, and thus the quality of animal welfare, but it cannot be a priori claimed that one system is better than the other. This is indicated by a series of researches. According to *Beggs et al. (2015),* the size of the herd is to some extent a limiting factor in terms of biosecurity and welfare because it implies a higher population density, more difficult organization of storage and distribution of food, easier spread of the disease. However, larger farms hire workers with a higher level of education, conduct better veterinary supervision, have better records of activities, which is in line with the results obtained in our research. Also, *Renaud et al. (2018)*, have determined that the farms with the lowest risk were those with veterinary supervision in the nursery every 3 hours. In Sweden, organic farms were compared with farms with conventional production methods. In the first system, antibiotics were less used, which is according to the standards and market demand when it comes to organic production. However, veterinarians are often to late for animal treatment, which had a bad impact on biosecurity and animal welfare *(Emanuelson et al., 2018*). *Stanković et al. (2014)*, also state the more frequent occurrence of infectious diseases in the tie system. There was no established plan for the implementation of biosecurity measures on the observed farms and no training was provided to staff in that regard. The health condition of the herd was regularly monitored by the veterinary service, so that the treatment of the animals was performed regularly and on time, as soon as the occurence of a disease was noticed. The basic principles of farm construction and site selection were also respected. Namely, the facilities for housing animals were at the proper distance from the main road. The principle that was not respected was that the facilities inside the farm were insufficiently isolated from each other and insufficiently protected from the presence of other animals, birds, rodents and insects, although disinsection and deratization were carried out regularly. These characteristics are similar to the data provided by *Stanković et al. (2011)*. A large number of diseased animals, in addition to constant veterinary supervision, indicate a serious danger and biosecurity risk. The biosecurity risk was represented by the movement of staff on the farm between different facilities, the absence of clearly stated instructions that regulate the movement, etc. The visitors did not undergo a more detailed check related to their recent activities and contacts with the animals, although they received protective clothing and footwear, and hand disinfection was mandatory. Also, there were disinfection barriers at the entrance to each facility, but they were not always operational. In the case of the dead calves, the corpses were usually, but not always, removed in a very short time, and after that the location was sanitized. The facilities were mechanically cleaned daily, and detailed washing and disinfection were performed after emptying the box or the entire facility. Similar problems in biosecurity protection were observed by *Dammianis et al. (2019)* and *Robichaud et al. (2019)*, and relate to poor isolation of sick animals, mixing of animals from different sources without quarantine, non-existent or non-functional disinfection barriers on the farm, cleaning and disinfection of facilities that are not performed after each production cycle, poor hygiene of facilities and animals, lack of protective wardrobe for employees, movement of visitors, etc. The observed farms did not respect the principle of "all in – all out", which often appears as a problem on farms (*Damiaans et al., 2019; Pedersen et al., 2009; Bojkovski et al., 2012).*

According to *Ferit Can (2018)*, the main difficulties in implementing a biosecurity plan on farms are: educational level, sociological and cultural characteristics (habits, tradition), costs and finances (profit should be greater than investment), farm size, geographical and climatic conditions, epidemiological situation and regulations. There are a number of suggestions for better biosecurity on farms. Some of them relate to the use of vaccines in order to prevent the occurrence of infectious diseases, as well as construction solutions that will contribute to better microclimatic conditions, the use of individual calving boxes, the use of individual "small houses" for calves (*Anderson, 1998*), keeping a closed herd, better veterinary supervision, adoption of a plan for the implementation of biosecurity measures (*Nitovski et al., 2013; Shortall et al., 2017*), control of the movement of staff and visitors, control of the population of rodents, birds and insects, prevention of contact with other animals *(Stanković et al., 2011.*). Considering the location and quality of facilities, there is a basis for improving all biosecurity measures on the observed farms such as: isolation of facilities on the farm, disinfection, disinsection and rodent and pest control, prevention of other animals and birds in facilities, control of movement of staff and visitors, improvement of microclimatic conditions, improvement of the hygiene of facilities and animals, strict respect for the principle of "all in-all out", education and training of staff on various bases (biosecurity measures, treatment of animals, technological procedures, etc.). The established practice of daily supervision of animals, equipment and production technology by highly professional and competent staff, chemical analysis of food and responsible behaviour towards the environment should be continued and improved over time. The application of these measures would greatly contribute to the general welfare of animals.

Like in the case of biosecurity, there was no established welfare protection plan on the observed farms nor were workers referred to any training in the area. They relied more on experience in day-to-day work or instructions from immediate supervisors. There was often a shortage of manpower, so workers were forced to move from one facility to another. Staff in charge of nutrition, treatment, implementation of technological procedures and organization of work on farms had adequate higher and higher education. Special training was attended by staff who had specific responsibilities such as hoof treatment, but not those who were in charge of feeding calves with colostrum. It was the colostrum diet that was the most sensitive part. The quality of colostrum is controlled only organoleptically, which is one of the most significant welfare problems in calves, as reported by *Hristov et al. (2011)* agree. Also, the level of iron and the presence of allergens in food have not been controlled. The amount of colostrum consumed, especially on farm A, and the method of administration (from a bucket) were not adequate for the age and consumption of calves, so colostrum intake was insufficient, and consequently the creation of passive immunity was highly debatable. The technology of feeding calves with colostrum of undetermined quality from buckets at will does not give good results in terms of acquiring passive immunity (*Samolovac et al., 2020; Relić et al., 2014; Vasseur et al., 2009 and 2010,). Weawer et al. (2000)* recommended an intake of 4 l of colostrum to reduce the risk of calf death, and *Osaka et al. (2014)* recommend 3.6 l. However, different results have been reported in practice. As stated by *Relić et al. (2014),* the intake of the first quantities of colostrum in calves on three farms with intensive rearing conditions was less than 1 l. One of the biggest threats to the quality of welfare was the limited movement of animals, i.e. calves tied to the bed or housing them in individual boxes of inadequate surface, which does not allow to fully realize some physiological behaviours such as explorative and maternal behaviour, less social contacts. (*Hristov et al., 2015*). There are rare cases in the world where calves are kept in individual "boxes" or tied (*Vasseur et al., 2010*).

According to a number of researchers *(Kieland et al., 2010; Gottardo et al., 2011; Wikman et al., 2013),* workers are generally aware that animals feel pain, fear, and express certain emotions. However, interventions such as dehorning or animal identification were performed without the use of local anesthetics, which is, unfortunately, a common practice on a large number of farms, as noticed by the results of research by *Winder et al. (2016)*, *Gottardo et al. (2011)*. Positive relation towards animals should be one of the goals of improving the quality of welfare in order to make sure that “animals have a life worth living” (*Hristov et al. 2012*). In the conducted research, the treatment of animals was very often rough, impatient, noisy, with the exception of milking. Microclimatic conditions on farms A and B were often unfavourable during the cold and warm periods of the year.

Temperatures exceeded 30^0 C in summer, while humidity was high in the buildings during the entire observation period. According to *Samolovac et al. (2019)*, unfavorable microclimatic conditions affect the increased morbidity and mortality of calves. The hygiene of animals and facilities can and must be much better, especially when it comes to nursery and calf breeding. Poor housing conditions represent one of the biggest threats to the quality of welfare (*Ostojić-Andrić et al., 2015*). During the examination, there was no protocol or plan related to the protection of the animal welfare on the farms, nor the training for staff in order to implement the protection of the quality of welfare. The overall welfare assessment of calves on the examined farms A and B was similar to the evaluations obtained in the research of *Vasseur et al. (2010), Relić and Bojkovski (2010), Hristov et al. (2011), Bojkovski et al. (2012), Stanković et al. (2011)*, and better than the estimates given by *de Vries et al. (2013)* on a larger number of herds. However, this situation provides only a solid basis for further improvement of the quality of welfare with existing production technology. First of all, the staff should be trained in terms of the importance of all aspects of animal welfare and biosecurity on farms, and certain written procedures and protocols should be adopted accordingly. The results of numerous researches show that the relations of breeders towards animals depend on their personal attitude, ethical principles, cultural and socio-demographic conditions, levels of education, etc. (*Ferit Can, 2018; Lundvall and Saras-Johansson, 2011; Schütz et al., 2012*). The way humans behave towards animals should be one of the goals of improving welfare as a whole, and not a characteristic of an individual or a small group of people, as stated by *Burton et al. (2012)* and *Elingsen et al. (2014)*. Raising the awareness of breeders about the importance of respecting the principle of welfare would also improve their treatment of animals, care for hygienic conditions in facilities and hygiene of animal. To improve housing and microclimatic conditions, it is necessary to change the technology of keeping and equipment in stables in terms of changing the way calves are kept (calves should be placed in boxes of appropriate size, with a quality surface and clean and dry bedding, outside the nursery), improving hygiene levels, by providing quality ventilation in facilities, providing outlets for the movement of calves and staying outside the stables, which would positively affect the overall health, behaviour of animals, or reduce the risks of compromising overall biosecurity and welfare in the herd, as stated by *Winder et al. (2016)* and *Robichaud et al. (2019).*

Based on the risk assessment on farms A and B, some recommendations can be made that would reduce the risk and improve the overall welfare of the calves. As already mentioned, it is necessary to adopt a clear and precise plan for the implementation of biosecurity measures and improvement of the quality of welfare on farms, the implementation of which would be familiar to all employees

(*Nitovski et al., 2013; Shortall et al., 2017*). Newborn calves should be placed in clean, disinfected individual boxes with adequate nutrition and water supply, especially in the part related to colostrum nutrition. In that sense, an alternative way of feeding, graduated bottles, buckets with artificial breast or esophageal probe can be introduced, and the quality of colostrum can be controlled by laboratory analyzes, at least occasionally by the method of random sampling. The same control principle can be introduced for the level of hemoglobin in the blood of calves, as well as the content of iron and allergens in food. Make microclimatic and hygienic conditions optimal or at least strive for it, and maintain good practice of constant control and supervision of the situation on farms by professional staff.

Conclusion

Based on the presented results on two farms with intensive production, it can be concluded that:

- Preservation of biosecurity on farms depends to a large extent on the applied production technology and rearing system
- Deficiencies in the implementation of all biosecurity procedures and measures jeopardize the quality of animal welfare, calves in this case, because biosecuritty and welfare are inextricably linked and interdependent
- Protocols on biosecurity and welfare protection were not adopted on the observed farms
- The greatest threat to biosecurity and welfare was found to be the conditions of feeding and housing calves in the first seven days of life
- Accordingly, the biggest changes in order to improve conditions relate to the introduction of procedures for the protection of biosecurity and welfare; introduction of individual housing for newborn calves outside the nursery; supply of high-quality colostrum from graduated bottles (for this purpose a colostrum bank should be formed, “milktaxi” should be put into use)
- Regularly train staff in the field of animal welfare and implementation of biosecurity measures
- Strengthen control over the movement of staff and visitors within the farm
- Regularly disinfect, disinsect and deratize farms

- Continuously work on improving accommodation, microclimatic and hygienic conditions in calf housing facilities
- Continue with daily zootechnical and veterinary supervision of animals and equipment

Procena biosigurnosti i dobrobiti teladi u intenzivnom sistemu držanja

Ljiljana Samolovac, Slavča Hristov, Dušica Ostojić-Andrić, Vlada Pantelić, Dragan Nikšić, Dragan Stanojević, Tamara Stanojević

Rezime

Kvalitet dobrobiti farmskih životinja u velikoj meri zavisi od niza mera i postupaka koji se sprovode na farmama a koje se jednim imenom definišu kao biosigurnost. Primena određenih upravljačkih praksi se razlikuje od farme do farme, pa shodno tome se razlikuje njihov uticaj na kvalitet dobrobiti životinja. Kvalitet biosigurnosti, dobrobiti i prisustvo faktora koji ih ugrožavaju zavise od tehnologije proizvodnje na farmi, sistema držanja životinja, mikroklimatskih i higijenskih uslova, menadžmenta, postupaka koji se sprovode na životinjama i načina na koji se sprovode, odnosa zaposlenih prema životinjama, njihovoj obučenosti i kompetentnosti itd. Cilj rada je bio da se utvrdi uticaj intenzivnog načina gajenja teladi na razlike u proceni biosigurnosti i kvaliteta dobrobiti na dve farme mlečnih krava sa intenzivnim načinom držanja. Tehnološki proces proizvodnje na obe farme je sličan, obzirom da su obe farme poslovale u okviru istog proizvodnog sistema. Na obe farme je porodilište u odvojenom objektu, ali bez individualnih bokseva za teljenje. Telad se odvajaju od majki odmah nakon rođenja. Jedna od značajnih razlika između farmi bio je način držanja teladi u prvih 7 dana života. Na jednoj od farmi (A) telad su držana vezana na ležištu u porodilištu, dok su na drugoj farmi (B) bila smeštena u individualne bokseve, takođe locirane u okviru porodilišta. Sa 8 dana starosti telad su smeštana u grupne bokseve, u posebnom objektu, odgajivalištu. Najveće slabosti i pretnje za biosigurnost i dobrobit na obe farme ispoljene su u najranijem uzrastu teladi, a odnose se na smeštaj i napajanje novorođene teladi kolostrumom. Utvrđeno je da se novorođena telad drže vezana na ležištima ili u slabo osvetljenim individualnim boksevima neadekvatne veličine i dizajna, smeštena u porodilištu zajedno sa kravama. Time se povećava izloženost

teladi brojnim patogenim agensima. Rizik po biosigurnost i dobrobit je veći kad se uzme u obzir i neadekvatno konzumiranje kolostruma u pogledu količine, načina i vremena napajanja. Kada su u pitanju telad starijih kategorija situacija je značajno povoljnija u pogledu uslova ishrane i držanja, kao i zdravstvenog nadzora i pruža velike mogućnosti u smislu daljeg unapređenja.

Ključne reči: biosigurnost, dobrobit, faktori rizika, intenzivni sistem proizvodnje

Acknowledgment

Research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, Agreement on the realization and financing of scientific research work of SRO no. 451-03-9/2021-14/200022.

References

ANON. (2009): Welfare Quality: Assesment Protocol for Cattle.

ANON. (2011): Projekat "Razvoj i implementacija standarda dobrobiti i biosigurnosti u cilju unapređenja tehnologije proizvodnje goveda i svinja". Evidencioni broj projekta 20110. Rukovodilac projekta dr Slavča Hristov. Projekat je finansiran od strane Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije.

ANDERSON J.F. (1998): Biosecurity - A New Term for an Old Concept - How To Appl. The bovine practitioner. 32.2, 61-70.

BEGGS D. S., FISHER A. D., JONGMAN E. C., HEMSWORTH P. H. (2015): A survey of Australian dairy farmers to investigate animal welfare risks associated with increasing scale of production. J. Dairy Sci., 98, 5330–5338.

BOERSEMA J. S. C. , NOORDHUIZEN J. P. T. M., LIEVAART J. J. (2013): Hazard perception of Dutch farmers and veterinarians related to dairy young stock rearing. J. Dairy Sci., 96, 5027–5034.

BOJKOVSKI J., PAVLOVIĆ I., RELIĆ R., BUGARSKI D., SAVIĆ B., PANOUSIS N., GIADINIS N., STANKOVIĆ B., PETRUJKIĆ T. (2012): Zdravstveni problemi i dobrobit teladi u intenzivnoj proizvodnji. Radovi sa XXVI savetovanja agronoma, veterinara, tehnologa i agroekonomista, 18 (3-4), 85-91.

BURTON R.J.F., PEOPLES S., COOPER M.H. (2012): Building ‘cowshed cultures’: A cultural perspective on the promotion of stockmanship and animal welfare on dairy farms. Applied Animal Behaviour Science, 28, 174-187.

DAMIAANS B., RENAULT V., SARRAZIN S., BERGE A. C., PARDON B., RIBBENS S., SAEGERMAN C., DEWULF J. (2020): A risk-based scoring system to quantify biosecurity in cattle production. Preventive Veterinary Medicine**,** 179. https://doi.org/10.1016/j.prevetmed.2020.104992

DAMIAANS B., RENAULT V., SARRAZIN S., BERGE A. C., PARDON B., RIBBENS S., SAEGERMAN C., DEWULF J. (2019): Biosecurity practices in Belgian veal calf farming: Level of implementation, attitudes, strengths, weaknesses and constraints. Preventive Veterinary Medicine**,** 172. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7127683/

DENIS-ROBICHAUD J., KELTON D. F., BAUMAN C. A., BARKEMA H. W., KEEFE G. P., DUBUC J. (2019): Biosecurity and herd health management practices on Canadian dairy farms. J. Dairy Sci., 102, 9536–9547.

DE VRIES M., BOKKERS E.A.M., VAN SCHAIK G., BOTREAU R., ENGEL B., DIJKSTRA T, DE BOER I.J.M. (2013): Evaluating results of the Welfare Quality multi-criteria evaluation model for classification of dairy cattle welfare at the herd level. J. Dairy Sci., 96 (10), 6264-6273.

EMANUELSON U., SJÖSTRÖM K., FAL N. (2018): Biosecurity and animal disease management in organic and conventional Swedish dairy herds: a questionnaire study. Acta Vet Scand., 60:23.
https://actavetscand.biomedcentral.com/articles/10.1186/s13028-018-0376-6

ELLINGSEN K., COLEMAN G.J., LUND V., MEJDELL C.M. (2014): Using qualitative behaviour assessment to explore the link between stockperson behaviour and dairy calf behaviour. Applied Animal Behaviour Science, 153, 10-17.

FERIT CAN M. (2018): Farm Level Biosecurity: Challenges and Suggestions. Dairy and Vet Sci J., 1-3, 7(5): JDVS.MS.ID.555722

GOTTARDO F., NALON E., CONTIERO B., NORMANDO S., DALVIT P., COZZI G. (2011): The dehorning of dairy calves: Practices and opinions of 639 farmers. J. Dairy Science, 94 (11), 5724-5734.

HRISTOV S., STANKOVIĆ B, MAKSIMOVIĆ N. (2012): Welfare of dairy cattle – today and tomorrow. Third International Scientific Symposium "Agrosym Jahorina 2012", 55-62.

HRISTOV S., STANKOVIĆ B., TODOROVIĆ-JOKSIMOVIĆ M., MEKIĆ C., ZLATANOVIĆ Z., OSTOJIĆ-ANDRIĆ D., MAKSIMOVIĆ N. (2011): Welfare problems in dairy calves. Biotechnology in animal husbandry, 27 (4), 1417-1424.

HRISTOV S., STANKOVIĆ B. (2009): Welfare and biosecurity indicators evaluation in dairy production. Biotechnology in Animal Husbandry, 25 (5-6), 623-630.

JEŽEK J., GRABNAR P., BECI B., KLINKON M., NEMEC M., HODNIK J.J., STARIČ J. (2019): Management practices affecting calves welfare on farms in Slovenia. Acta universitatis agriculturae et silviculturae mendelianae brunensis, 67, 1147-1152.

KIELLAND C., SKJERVE E., ØSTREAS O., ZANELLA A.J. (2010): Dairy farmer attitudes and empathy toward animals are associated with animal welfare indicators. J. Dairy Science, 93 (7), 2998-3006.

LUNDVALL J., SARAS – JOHANSSON M. (2011): Human-animal interactions in dairy production. Literature review, Swedish University of Agricultural Sciences, The Faculty of Veterinary Medicine and Animal Science, Department of Animal Nutrition and Management Uppsala.

NITOVSKI A., MILENKOVIĆ M., RADOVIĆ B., MILANOVIĆ V., GRČAK D., GRČAK M. (2013): Makeing a plan of biosecurity on a cattle farm. Macedonian Journal of Animal Science, 3 (1), 9–15.

OSTOJIĆ ANDRIĆ D., HRISTOV S., PETROVIĆ M. M., PANTELIĆ V., BOJKOVSKI J., NOVAKOVIĆ Ž., LAZAREVIĆ M., NIKŠIĆ D. (2015): Housing conditions and welfare of dairy cows in Serbia. Proceedings of the 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production, 62-73, 2015.

OSAKA I., MATSU Y., TERADA F. (2014): Effect of the mass of immunoglobulin (Ig)G intake and age at first colostrum feeding on serum IgG concentration in Holstein calves. J. Dairy Science, 97 (10), 6608-6612.

PEDERSEN R.E., SØRENSEN J.T., SKJØTH F., HINDHEDE J., NIELSEN T.R. (2009): How milk-fed dairy calves perform in stable versus dynamic groups. Livestock Science 121, 215–218.

RELIĆ R., HRISTOV S., SAMOLOVAC LJ., BOJKOVSKI J., ROGOŽARSKI D. (2014): Colostrum management in calves' welfare risk assessment. Bulletin UASVM Veterinary Medicine, 71 (1), 187-192.

RELIĆ R., BOJKOVSKI J. (2010): Housing conditions in calves welfare risk assessment. Journal of Agricultural Sciences, 55(3), 283-292.

RENAUD D. L., KELTON D. F., LEBLANC S. J., HALEY D. B., DUFFIELD T. F. (2018): Calf management risk factors on dairy farms associated with male calf mortality on veal farms. J. Dairy Sci., 101, 1785–1794.

RICHENS I.F., HOUDMONT J., WAPENAAR W., SHORTALL O., KALER J., O'CONNOR H., BRENNAN M.L. (2018): Application of multiple behaviour change models to identify determinants of farmers' biosecurity attitudes and behaviours, Preventive Veterinary Medicine**,** 155, 61-74.

SAMOLOVAC LJ., MARINKOVIĆ M., PETRIČEVIĆ M., STAMENIĆ T., ĆOSIĆ I., BESKOROVAJNI R., STANKOVIĆ B. (2020): Effect of farm and birth season on calf body weight in the first week of life. Biotechnology in Animal Husbandry, 36 (3), 297-307.

SAMOLOVAC LJ., HRISTOV S., STANKOVIĆ B., MALETIĆ R., RELIĆ R., ZLATANOVIĆ Z. (2019): Influence of rearing conditions and birth season on calf welfare in the first month of life. Turkish Journal of Veterinary and Animal Sciences, 43: 102-109.

SCHÜTZ K.E., HAWKE M., WAAS J.R., MCLEAY L.M., BOKKERS E.A.M.,

VAN REENEN C.G., WEBSTER J.R., STEWART M. (2012): Effects of human handling during early rearing on the behaviour of dairy calves. Animal welfare, 21, 19-26.

STANKOVIĆ B., HRISTOV S., ZLATANOVIĆ Z., BOJKOVSKI J., MILOŠEVIĆ STANKOVIĆ I., MAKSIMOVIĆ N. (2015): Respiratory disorders and biosecurity on dairy farms. Proceedings of the 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production, 415-427.

STANKOVIĆ B., HRISTOV S., OSTOJIĆ-ANDRIĆ D., ZLATANOVIĆ Z., SAMOLOVAC LJ., MAKSIMOVIĆ N. (2014): The most common health disorders and welfare of dairy cows and calves. Biotechnology in Animal Husbandry, 30 (4), 549-560.

STANKOVIĆ B., HRISTOV S., BOJKOVSKI J., ZLATANOVIĆ Z., MAKSIMOVIĆ N., TODOROVIĆ-JOKSIMOVIĆ M., DAVIDOVIĆ V. (2011): The possibility of dairy farms isolation assessment - biosecurity aspect. Biotechnology in Animal Husbandry, 27 (4), 1425-1431.

SHORTALL O., GREEN M., BRENNAN M., WAPENAAR W., KALER J. (2017): Exploring expert opinion on the practicality and effectiveness of biosecurity measures on dairy farms in the United Kingdom using choice modeling, J. Dairy Sci., 100:2225–2239.

VASSEUR E., RUSHEN J., DE PASSILLÉ A.M., LEFEBVRE D., PELLERIN D. (2010): An advisory tool to improve management practices affecting calf and heifer welfare on dairy farms. J. Dairy Sci., 92, 4414–4426.

VASSEUR E., RUSHEN J., DE PASSILLÉ A.M. (2009): Does a calf's motivation to ingest colostrum depend on time since birth, calf vigor, or provision of heat? J. Dairy Science 9 (8), 3915-3921.

WEAVER D.M., TYLER J.W., VANMETRE D.C., HOSTETLE, D.E., BARRINGTON G.M. (2000): Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med., 14:569– 577.

WIKMAN I., HOKKANEN A-H., PASTELL M., KAUPPINEN T., VALORS A., HÄNNINEN L. (2013): Dairy producer attitudes to pain in cattle in relation to disbudding calves. J. Dairy Science, 96 (11), 6894-6903.

B. WINDER C.B., LE BLANC S.J., HALEY D.B., LISSEMORE K.D., GODKIN M.A., DUFFIELD T.F. (2016): Practices for the disbudding and dehorning of dairy calves by veterinarians and dairy producers in Ontario – Canada. J. Dairy Sci., 99,10161–10173.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

THE ASSESSMENT OF BREEDING VALUE OF FIRST CALVING HOLSTEIN - FRISIAN HEIFERS BY APPLYING SELECTION INDEX METHODOLOGY

Marina Lazarević[1], Nevena Maksimović[1], Nenad Mićić[1], Miloš Marinković[1], Vlada Pantelić[1], Dragan Nikšić[1], Dragan Stanojević[2]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade, Serbia
[2] Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade-Zemun, Serbia
Corresponding author: Marina Lazarević, marinaplazarevic@gmail.com
Invited paper

Abstract: The research was performed on Holstein-Friesian and Black and White cattle, reared on farms of Agricultural Corporation of Belgrade - PKB. The study included 5238 first calving heifers reared on 6 farms in the period from 2006 - 2014 and represent progeny of 61 bulls. The following dairy traits were analysed in a standard lactation (305 days): milk yield (kg) **- MY**, milk fat content (%) **- % MF**, milk fat yield (kg) **- MFY**, protein content (%) **- % PC** and protein yield (kg) **- PY**. The relative economic weight of milk traits included in selection index is expressed through single standard deviation. The first calving Holstein - Friesian heifers, in standard lactation produced on average 7765.35 ± 1401.80 kg of milk, with a milk fat content of 3.49 ± 0.22 and protein content of 3.23 ± 0.13. The impact of bull, farm, year and season of calving on the variability of milk traits was present at the level of very high statistical significance, while the genetic group did not influence the variability of yield and protein content, however it influenced other milk traits at different levels of statistical significance. Heritability of observed milk traits was medium to low. The content of milk fat and protein had the lowest values of heritability, 0.052, and 0.042, respectively. The heritability of milk yield was 0.305. Negative genetic correlation were determined between milk yield and milk fat content (-0.380), also between milk yield and protein content (-0.500). Coefficient of correlation of selection index and aggregate genotype was 0.3829.

Key words: Holstein – Frisian breed, milk traits, selection index

Introduction

The principal goal of dairy cattle breeding is improvement of economic efficiency of production. The economic merit of an individual animal is a function of the traits identified in the breeding objective weighted by their economic value. Selection indices provide an estimate of the individuals' economic merit (based on the breeding objective) and simplify selection. Animals are ranked on the basis of the selection index and the best individuals selected. Globalization of breeding programs is reflected by similarity in breeding objectives. The selection index provides a simplified interpretation of where a country is headed in terms of breeding philosophy (*Leitch, 1994*).

Selection indexes have been extensively applied in the estimation of breeding value of dairy cattle for single traits as well as for combinations of traits for selection purposes. Milestones in methodology, such as multiple-trait evaluation procedures by BLUP, (co)variance component estimation, nonlinear models, discounted gene flow, dynamic programming, and international sire evaluations, together with increased computing power and the development of integrated AI and recording schemes, have contributed to efficient implementation of selection indexes (*Philipsson et al., 1994*).

Breeding for dairy cattle worldwide has primarily focused on improving production traits (*Nielsen et al., 2005). Miglior et al. (2005)* surveyed the selection indexes of 15 countries from different geographical regions and showed that the average relative emphasis for production across all countries was 59.5%. This finding indicates that production is still the most important component of selection indexes used in dairy cattle.

Dekkers (2007) reported that the selection of production (protein yield, protein %) and functional traits (longevity, milkability, and somatic cell score) increased the selection index efficiency to 58%.

Komlósi et al. (2010) used a bio-economic model to estimate economic values of 15 milk production, functional, growth and carcass traits for Hungarian Holstein-Friesian cattle. The highest relative economic importance was obtained for milk yield (25%), followed by productive lifetime of cows (23%), protein yield and the direct component of a cow's total conception rate.

Sorensen et al. (2010) found that the selection of milk yield, somatic cell score, udder depth, teat placement, and foot angle improved efficiency of response in the aggregate genotype by 1% to 4% over selection for milk yield alone. *Sun et al. (2010)* reported that for improving milk yield, selection indices comprising milk, fat, or protein yields were 98%–100% as efficient as an index comprising all three traits. Selection on milk yield alone was 5% less efficient in improving milk yield compared with the selection using an index of all three traits.

Selection indices are dynamic and will change as economic conditions change, and as improved parameter estimates become available. Future modifications may also combine quantitative traits with marker gene information (*Leitch, 1994).*

The objectives of this study were to develop a multitrait selection index for first calving Holstein-Friesian heifers and to test the accuracy of the index. The index would give farmers an option of selecting traits depending on the breeder's selection goals.

Material and Methods

The research was performed on Holstein-Friesian and Black and White cattle, reared on farms of Agricultural Corporation of Belgrade - PKB. The study included 5238 first calving heifers. All cows were reared on 6 farms of Agricultural Corporation Belgrade in the period from 2006 - 2014 and represent progeny of 61 bulls. The study included the bulls with a minimum of 5 daughters.

The following dairy traits were analysed in a standard lactation (305 days): milk yield (kg) – **MY**, milk fat content (%) **- % MF**, milk fat yield (kg) – **MFY**, protein content (%) **- % PC** and protein yield (kg) – **PY**.

The variability of milk traits was assessed by the method of least squares *LSMLMW*. The mixed model with random influence of bull – sire, fixed influence of genetic group, farm, year and season of calving was used in the analysis of the impact of genetic and non-genetic sources of variability.

The Mixed model used in the analysis of the impact of genetic and non-genetic sources of variability on milk traits:

$Y_{ijklmn} = \mu + O_i + F_j + G_k + S_l + H_m + e_{ijklmn}$

where:

- Y_{ijklmn}: studied trait,
- μ: population average for the said trait,
- O_i: random effect of i^{th} sire (i=1,…,61),
- F_j: fixed effect of j^{th} farm (j=1,…,6),
- G_k: fixed effect of k^{th} calving year (k=1,…,9),
- S_l: fixed effect of l^{th} – calving season (l=1,2,3,4),
- H_m: fixed effect of n^{th} – genetic group (n=1,...,5),
- e_{ijklmn}: random error with characteristics N $(0,\sigma^2)$.

Within each year, four calving seasons were observed: winter (December, January and February), spring (March, April and May), summer (June, July and August) and autumn (September, October and November).

Depending on the share of Holstein genes, all cows were divided into 5 groups: I - below 50% of Holstein genes, II - from 50% to 75% Holstein genes, III - from 75% to 87.5% Holstein genes, IV - from 87.5% to 93.75% Holstein genes and V - over 93.75% Holstein genes.

The heritability of the investigated traits was calculated by the method of interclass correlation between the half-siblings, where the progeny of one sire have 25% of common genes, based on the formula:

$$h^2 = \frac{4\sigma_a^2}{\sigma_a^2+\sigma_e^2} \; \frac{4\sigma_a^2}{\sigma_a^2+\sigma_e^2}$$

where: h^2 – hereditary coefficient (heritability), $\sigma_a^2 \sigma_a^2$ – variance between sires (additive genetic variance), $\sigma\sigma_e^2$ – variance errors.

The heritability coefficients of studied traits, i.e. values of genetic and phenotypic variances and co-variances, necessary for the construction of selection indexes, were calculated by the method of least squares. Data were analysed using the statistical software package SAS (*SAS Institute Inc. 9.3, 2012*).

For the construction of the selection index equation, three most important milk traits were included: milk yield, milk fat content and protein content.
Breeding value estimated according the method of selection index is shown in the following selection index equation:

$$I = b_1 (X_1 - \overline{XX}_1) + b_2 (X_2 - \overline{XX}_2) + ... + b_n (X_n - \overline{XX}_n)$$

where: I – relative breeding value of animal estimated using the selection index, b_i – coefficient of multiple regression for each trait included in the selection index; $(X_i - \overline{XX}_i)$ – difference between the phenotypic value of the trait included in the selection index for given individual animal and population average for given trait.

The relative economic weight of milk traits included in selection index is expressed through single standard deviation as proposed and described by *Falconer and Mackay (1996):*

$$REW = \sigma_p / \sigma_p^2$$

where: REW – relative economic weight of a trait; σ_p – phenotypic standard deviation; σ_p^2 – phenotypic variance of each trait.

Table 1. The relative economic weight (REW) for milk traits studied by using one standard deviation

Trait	σ_p	σ_p^2	REW according to MY
MY	1312.893	1723687.426	1
%FC	0.178	0.032	-7377.46
%PC	0.108	0.012	-12127.22

The total breeding value expressed through the aggregate genotype has the following form:

$G=v_1G_1+v_2G_2+...+v_nG_n$, where:

G – aggregate genotype, $v_{1...n}$ – economic value of a trait, $G_{1...n}$ – genotypes of traits included in the aggregate genotype.

Assuming that the correlation between the genetic value of the animal (G) and the value of the selection index (I) are at a maximum, then the following equation is obtained:

$P*b=G*v$, i.e.,

$b=G*v*P^{-1}$, where:

P – matrix of phenotypic variances and covariances, G – matrix of genetic variances and covariances, v – vector of economic values of traits included in the selection index, b – vector of multiple regression coefficients.

In matrix form indicated equation has the following form:

$$\begin{bmatrix} VarP(X1) & CovP(X1X2) & CovP(X1X3) \\ CovP(X1X2) & VarP(X2) & CovP(X2X3) \\ CovP(X1X3) & CovP(X2X3) & VarP(X3) \end{bmatrix}$$

$$\begin{bmatrix} VarP(X1) & CovP(X1X2) & CovP(X1X3) \\ CovP(X1X2) & VarP(X2) & CovP(X2X3) \\ CovP(X1X3) & CovP(X2X3) & VarP(X3) \end{bmatrix} \begin{bmatrix} b1 \\ b2 \\ b3 \end{bmatrix} \begin{bmatrix} b1 \\ b2 \\ b3 \end{bmatrix} =$$

$$\begin{bmatrix} VarG(X1) & CovG(X1X2) & CovG(X1X3) \\ CovG(X1X2) & VarG(X2) & CovP(X2X3) \\ CovG(X1X3) & CovG(X2X3) & VarG(X3) \end{bmatrix}$$

$$\begin{bmatrix} VarG(X1) & CovG(X1X2) & CovG(X1X3) \\ CovG(X1X2) & VarG(X2) & CovP(X2X3) \\ CovG(X1X3) & CovG(X2X3) & VarG(X3) \end{bmatrix} \begin{bmatrix} v1 \\ v2 \\ v3 \end{bmatrix} \begin{bmatrix} v1 \\ v2 \\ v3 \end{bmatrix}$$

The coefficient of correlation of the selection index and aggregate genotype was calculated by using the formula:

$r_{IAG} = \sigma_I \,/\, \sigma_{AG}$

where: r_{IAG} – the coefficient of correlation of the selection index and aggregate genotype, σ_I – standard deviation of the selection index, σ_{AG} – standard deviation of the aggregate genotype.

Results and Discussion

The table 2 shows the descriptive statistical indicators and variability of milk traits in standard lactation.

Table 2. Descriptive statistical indicators for milk traits in standard lactation (305 days)

Trait	N	$\overline{XX}$	Min	Max	Sd	Cv %
MY		7765.35	2628.00	13087.00	1401.80	18.05
%MF		3.49	2.60	4.49	0.22	6.29
MFY	5238	270.03	102.05	438.41	47.31	17.52
%PC		3.23	2.40	3.68	0.13	4.06
PY		250.28	85.94	409.62	44.22	17.67

The first calving Holstein-Friesian heifers, in standard lactation produced on average 7765.35 ± 1401.80 kg of milk, with a milk fat content of 3.49 ± 0.22 and protein content of 3.23 ± 0.13. Milk yield was characterized by the highest variability. Slightly lower milk yield in standard lactation of first calving Holstein - Friesian heifers is stated by *Stanojević et al. (2012).*

Atil (2006) reports the milk yield of 4030 ± 1112 kg in the standard lactation, achieved by Holstein - Friesian breed in Turkey. *Beskorovajni (2014)* has determined the average milk yield of cows of Holstein - Friesian breed in the first standard lactation of 6478.82 kg with 3.55% milk fat. In Egypt, the average milk yield achieved by cows of Holstein - Friesian breed according to a study of El-*Awady and Oudah (2011)* is 3936 kg with 121 kg of milk fat and 90 kg of protein, while *Eman et al. (2016)* state the average milk yield for first calving heifers in Egypt, in the lactation of 305 days, of even 8801 kg with 268 kg of milk fat and 219 kg of protein.

As reported by *Hoekstra et al. (1994),* Holstein Friesian bulls have been used intensively in the Dutch-Friesian population. As a consequence, and also because of improved management, milk production has increased considerably. The population mean for 305 days milk yield for Black and White cows was 6214 kg in 1987 and 7001 kg in 1991.

The influence of factors on the variability of milk traits of first calving heifers of Holstein - Friesian breed in standard lactation is presented in the table 3. The impact of bull, farm, year and season of calving on the variability of milk traits was present at the level of very high statistical significance, while the genetic group did not influence the variability of yield and protein content, however it influenced other milk traits at different levels of statistical significance. *Stanojević et al. (2016)* reported that an increase in the share of Holstein-Friesian genes does not have such a drastic impact on milk yield, as opposed to the length of productive life.

Table 3. F – values for genetic and non genetic factors affecting milk traits in standard lactation (305 days)

Trait	F – values				
	Sire	Genetic group	Farm	Calving year	Calving season
	$df_1=60$	$df_1=4$	$df_1=5$	$df_1=8$	$df_1=3$
	$df_2=5157$	$df_2=5157$	$df_2=5157$	$df_2=5157$	$df_2=5157$
MY	6.64^{***}	2.62^{*}	27.14^{***}	4.55^{***}	18.90^{***}
%MF	1.90^{***}	2.43^{*}	160.54^{***}	29.20^{***}	7.64^{***}
MFY	6.43^{***}	4.22^{**}	23.95^{***}	10.25^{***}	22.19^{***}
%PC	1.72^{***}	$0.47^{n.s.}$	156.64^{***}	36.28^{***}	10.65^{***}
PY	6.40^{***}	$2.35^{n.s.}$	21.83^{***}	5.11^{***}	14.00^{***}

$p>0{,}05^{nz}$, $p<0{,}05^{*}$, $p<0{,}01^{**}$, $p<0{,}001^{***}$

Varying of milk traits, influenced by season and year of calving, ocurred due to the difference in temperature and humidity, as well as the quality and quantity of available food. Different housing conditions, nutrition and care, designated as the farm management, contributed to the differences in the manifestation of milk traits.

Stanojević et al. (2012) in their study of the impact of bull, farm and calving season on the phenotypic manifestation and variability of milk yield, milk fat yield and protein yield in standard lactation, have established high statistical significance (p<0.01) of said factors. Statistically significant effects (p<0.01) of the farm, bull, year and month of calving are reported by *Atil (2006).* By examining the impacts of the bull, farm, year, season and lactation order on variability of milk yield, fat content, milk fat yield and 4% fat corrected milk, *Beskorovajni (2014)* has found a significant effect of those factors on the variability of traits in the whole and standard lactation.

The table 4 shows the values of the coefficient of heritability.

Table 4. Estimates of heritability (h^2) and error of heritability (Sh^2) for milk traits in standard lactation (305 days)

Trait	h^2	Sh^2
MY	0.305	0.054
%MF	0.052	0.018
MFY	0.294	0.053
%PC	0.042	0.016
PY	0.292	0.053

Heritability of observed milk traits was medium to low. The content of milk fat and protein had the lowest values of heritability, 0.052, and 0.042, respectively. The heritability of milk yield was 0.305 ± 0.054, which is consistent with the results of *Radwan et al. (2015), Ghiasi et al. (2013)* and *El-Awady and Oudah (2011)*, while slightly higher values (0.47 and 0.48) are reported by *Atil (2006)* and *Hoekstra et al. (1994)*. Lower value of heritability for milk yield of 0.115 is stated by *Stanojević et al. (2012).* The heritability values of the milk fat and protein yield were 0.294 and 0.292, respectively. Higher values for heritability of milk yield and protein are stated by *Hoekstra et al. (1994). Eman et al. (2016)* used three models in estimating genetic parameters, variance and covariance components for some productive traits in first three lactations in Holstein-Friesian cattle based on inclusion and/or exclusion of direct maternal effect. Depending on the applied model, the heritability of milk fat yield ranged from 0.198 to 0.361, and heritability of protein yield from 0.181 to 0.295.

The table 5 shows the values of genetic and phenotypic correlations of milk traits in standard lactation.

Table 5. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for milk traits in standard lactation (305 days)

Trait	MY	%MF	MFY	%PC	PY
MY	–	–0.380	0.993	–0.500	0.998
%MF	–0.253	–	–0.274	0.243	–0.378
MFY	0.956	0.035	–	–0.489	0.992
%PC	–0.186	0.184	–0.140	–	–0.446
PY	0.982	–0.221	0.947	0.000	–

Negative to highly positive phenotypic and genetic correlations were determined between studied milk traits. Between the milk yield and yield of milk fat, and milk yield and yield of protein, there was positive and complete phenotypic and genetic correlation, 0.956, 0.982, 0.993 and 0.998, respectively.

High correlation enables the improvement of two or more traits simultaneously, while a negative genetic correlation between milk yield and milk fat content (-0380), also between milk yield and protein content (-0.500) indicates that selection for high milk yields leads to a decrease in milk fat content and protein content.

El-Awady and Oudah (2011) obtained lower value of genetic and phenotypic correlation among monitored milk traits. The genetic correlation between 305-day milk yield and each of 305-day fat yield and 305-day protein yield were positive (0.57). 305-day milk yield positively and highly genetic correlated with each of 305- day fat yield (0.66±0.06) and 305-day protein yield (0.77±0.07), and also between 305- day fat yield and 305- day protein yield (0.61±0.05).

As stated by *Strucken et al. (2012)* yield traits were positively correlated with each other (0.3 to 0.8) and whilst milk yield was negatively correlated with the content traits (−0.1 to −0.3), fat and protein yield were positively correlated with protein content (0.03 to 0.3).

The table 6 shows the selection index equation and coefficient of correlation of selection index and aggregate genotype.

Table 6: Selection indices equation and coefficient of correlation – r_{IAG} of selection indices and aggregate genotype

Selection indices	r_{IAG}
$I = 0.1738^a (X_1 – 7765.35) – 899.2083^b (X_2 – 3.49) – 310.3007^c (X_3 – 3.23)$	0.3829

^{a} – value of partial regression coefficient for MY, ^{b} – value of partial regression coefficient for %MF, ^{c} – value of partial regression coefficient for %PC, X_i – traits phenotypic values of each individual

Correlation coefficient of selection index and aggregate genotype was 0.3829. The obtained results are lower than those reported by other authors. The reason for this could be that selection indices they used were various combinations of fertility and production traits.

Ghiasi et al. (2013) used 4 different selection strategies to improve reproductive performance in the Iranian Holstein dairy cow. Selection indices in these strategies were various combinations of fertility traits and milk production. In all strategies, those selection indices that contained milk production, days from calving to the first service and number of inseminations to conception were the best selection indices.

Missanjo et al. (2013) developed selection index equation that included production and functional traits. The production component of the index included milk yield, fat yield, protein yield, fat percent, and protein percent while the functional component included the somatic cell count. The accuracy of the index was 0.911, and the correlation with the breeding objective was 0.954.

Atil et al. (2006) constructed four selection indices by using one standard deviation as a relative economic weight. The original index that included all three traits (305 day milk yield, lactation period and age at first calving) showed the highest accuracy (0.77) while reduced indices that included only two traits decreased accuracy of selection.

Conclusion

From the present results, the phenotypic manifestation and variability of milk traits are under influence of bull, farm, year and calving season. Different housing conditions, nutrition and care contributed to the differences in the manifestation of milk traits. Heritability of observed milk traits was medium to low. Negative to highly positive phenotypic and genetic correlations were determined between studied milk traits. Coefficient of correlation of selection index and aggregate genotype was 0.3829. In the further research reproductive and functional traits should be included in selection index which would lead to more genetic improvement.

Procena priplodne vrednosti prvotelki holštajn frizijske rase metodom selekcijskog indeksa

Marina Lazarević, Nevena Maksimović, Nenad Mićić, Miloš Marinković, Vlada Pantelić, Dragan Nikšić, Dragan Stanojević

Rezime

Istraživanje je sprovedeno na grlima holštajn - frizijske i crno - bele rase koja su uzgajana na farmama Poljoprivredne korporacije Beograd. Istaživanjem je obuhvaćeno 5238 prvotelki koje su uzgajane na 6 farmi u periodu od 2006. do 2014. godine i predstavljaju potomke 61 bika. Analizirane su sledeće osobine mlečnosti u standardnoj laktaciji (305 dana): prinos mleka (kg) - **MY**, sadržaj mlečne masti (%) -% **MF**, prinos mlečne masti (kg) - **MFY**, sadržaj proteina (%) -

% **PC** i prinos proteina (kg) - **PY**. Relativna ekonomska vrednost osobina mlečnosti uključenih u selekcijski indeks izražena je preko jedne standardne devijacije. U standardnoj laktaciji prvotelke holštajn - frizijske rase proizvele su prosečno 7765,35 ± 1401,80 kg mleka sa sadržajem mlečne masti 3,49 ± 0,22 i sadržajem proteina 3,23 ± 0,13. Uticaj bika, farme, godine i sezona teljenja u varijabilnosti osobina mlečnosti prisutan je na vrlo visokom nivou statističke značajnosti dok genetska grupa nije uticala na varijabilnost prinosa i sadržaja proteina a na ostale osobine mlečnosti je ispoljila uticaj na različitim nivoima statističke značajnosti. Heritabilitet posmatranih osobina mlečnosti je bio srednji do nizak. Najniže vrednosti heritabiliteta imaju sadržaj mlečne mast i proteina, 0,052 i 0,042. Heritabilitet prinosa mleka iznosio je 0,305. Negativna genetska korelacija utvrđena je između prinosa mleka i sadržaja mlečne masti (-0,380), zatim prinosa mleka i sadržaja proteina (-0,500). Koeficijent korelacije između selekcijskog indeksa i agregatnog genotipa je 0,3829.

Ključne reči: Holštajn - frizijska rasa, osobine mlečnosti, selekcijski indeks

Acknowledgment

Research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia**,** Agreement on the realization and financing of scientific research work of SRO no. 451-03-9/2021-14/200022.

References

ATIL H. (2006): A comparison of different selection indices for genetic improvement for milk traits in Holstein Friesian cattle in Turkey by using one standard deviation as relative economic weight. Pakistan Journal of Biological Science 9 (2): 285-288.

BESKOROVAJNI R. (2014): Genetski trend osobina mlečnosti praćenih u progenom testu bikova crno-bele i holštajn frizijske rase, Doctoral thesis, Faculty of Agriculture, Belgrade.

DEKKERS J. C. M. (2007): Prediction of response to marker-assisted and genomic selection using selection index theory. Journal of Animal Breeding and Genetics, 124, (6): 331-341.

EL-AWADY G.H., OUDAH E. Z. M. (2011): Genetic and Economic Analysis for the Relationship between Udder Health and Milk Production Traits in Friesian Cows. Asian-Aust. J. Anim. Sci. 24 (11): 1514-1524.

EMAN A., ABO ELFADL, RADWAN A. H. (2016): Model Comparisons and Genetic Parameters Estimates of Productive Traits in Holstein Cows. J. Applied Sci. 16 (8): 380-387.

FALCONER D.S., MACKAY (1996): Introduction to quantitative genetic 4th edition. Oliver and Boyd, Edinburgh,UK.

GHIASI H., NEJATI-JAVAREMI A., PAKDEL A., GONZALEZ-RECIO O. (2013): Selection strategies for fertility traits of Holstein cows in Iran. Livestock Science 152: 11–15

HOEKSTRA J., A.W. VAN DER LUGT, J.H.J. VAN DER WERF, OUWELTJES, W (1994): Genetic and phenotypic parameters for milk production and fertility traits in upgraded dairy cattle. Livestock Production Science 40: 225-232.

KOMLÓSI I., WOLFOVÁ M., WOLF J., FARKAS B., SZENDREI Z., BÉRI B. (2010): Economic weights of production and functional traits for Holstein-Friesian cattle in Hungary. J Anim Breed Genet. 127 (2):143-53.

LEITCH H. W. (1994): Comparison of international selection indices for dairy cattle breeding. Proceedings of Interbull Annual Meeting, 6 August, Ottawa, Canada, 1-3

MIGLIOR F., MUIR B. L.,VAN DOORMAAL B. J. (2005): Selection Indices in Holstein Cattle of Various Countries. J. Dairy Sci. 88: 1255-1263.

MISSANJO E., IMBAYARWO-CHIKOSI V., HALIMANI T. (2013): A Proposed Selection Index for Jersey Cattle in Zimbabwe. ISRN Veterinary Science, vol. 2013, Article ID 148030, 3 pages, http://dx.doi.org/10.1155/2013/148030

NIELSEN H. M., CHRISTENSEN L. G., GROEN A. F. (2005): Derivation of Sustainable Breeding Goals for Dairy Cattle Using Selection Index Theory. J. Dairy Sci 88 (5), 1882-1890

PHILIPSSON J., BANOS G., ARNASON T. (1994): Present and future uses of selection index methodology in dairy cattle. J. Dairy Sci. 77 (10): 3252-61.

RADWAN A.A.H., ABO ELFADL A.E., FARDOS M.A. (2015): Estimates of Population Parameters of Some Economic Traits in Holstein Friesian Cows by Using Statistical Program. Global Veterinaria 14 (1): 129-135.

SORENSEN L. P., MARK T., SORENSEN M. K., OSTERGAARD S. (2010): Economic values and expected effect of selection index for pathogen-specific mastitis under Danish conditions. Journal of Dairy Science *93* (1): 358-369.

STANOJEVIĆ D., ĐEDOVIĆ R., BOGDANOVIĆ,V., POPOVAC M., PERIŠIĆ P., BESKOROVAJNI R. (2012): Fenotipska i genotipska varijabilnost i povezanost osobina mlečnosti prvotelki crno-bele rase. Zbornik radova sa XXVI savetovanja agronoma, veterinara, tehnologa i agroekonomista. Padinska Skela. 15-22.

STANOJEVIĆ D., ĐEDOVIĆ R., BOGDANOVIĆ V., RAGUŽ N., POPOVAC M., JANKOVIĆ D., ŠTRBAC LJ. (2016): Evaluation of the heritability coefficients of longevity in the population of Black and White cows in Serbia. Mljekarstvo 66 (4), 322-329

STRUCKEN E. M., BORTFELDT R. H., TETENS J., THALLER G., BROCKMANN G. A. (2012): Genetic effects and correlations between production and fertility traits and their dependency on the lactation-stage in Holstein Friesians. BMC Genetics *13*:108

SUN C., MADSEN P., LUND M. S., ZHANG Y., NIELSEN U. S., SU G. (2010): Improvement in genetic evaluation of female fertility in dairy cattle using multiple-trait models including milk production traits. Journal of Animal Science. 88 (3): 871-878.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

PARITY EFFECT ON LAMBING RATE AND LAMBS BIRTH WEIGHT AT CROSSBRED OF MIS X ILE DE FRANCE BREEDS OF SHEEP

Violeta Caro Petrovic[1], Milan P. Petrovic[1,2], Dragana Ruzic-Muslic[1], Nevena Maksimovic[1], Marina I.Selionova[3], Bogdan Cekic[1], Ivan Cosic[1]

[1]Institute for Animal Husbandry 11080 Belgrade, Serbia
[2]Russian Academy of Natural Science-Balkan Scientific Center, Belgrade, Serbia
[3]Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, 127550, Russia
Corresponding author: Violeta Caro Petrović, violycaro@yahoo.com
Invited paper

Abstract: Success in lamb production depends on their body weight, and in recent years there had been a growing interest in understanding the impact of some factors such as parity on sheep productivity. Knowing the factors that affect the development and growth of lambs can help make changes in breeding plans. This paper was to detect important factors affecting lamb's birth weight which usually are not taken to consideration and have substantial part planning improvement in sheep production. Miss breed sheep were cross or pair with Ile de France rams three times, with the following characteristics observed; Body weights of lambs at birth, type of birth, number of lambs born per sheep per parity in the periods from 2018 to 2020. The result of each lambing per parity of each ewe was the basis of the study. The ANOVA, general linear model, univariate analysis of variance, and descriptive statistics derived to assess the following effects: Effect of parity on lambs' birth weight; Effect of parity on birth type, and lambs birth weight; Interaction effect of different subject effects on birth weight; The total marginal means of lambing rate per dam in three parities.
The result obtained showed a very significant influence of the indicated factors on tested traits ($P<0.01$). Based on the results of our research, we can conclude that parities have a very significant impact, so they need more attention in the future.

Keywords: Lamb, birth weight, lambing rate, parity

Introduction

Lamb viability dependent on their birth weight, and in recent years, there has been an increasing interest in the understanding of the influence of some factors such as parity on sheep productivity *(Macedo and Hummel 2006).* The lambs' body weight at birth has an essential role in achieving good sheep production while sheep productivity is affected by many factors, like breed improvement programs based on maximum utilization of genetic variation, but these features varied due to certain environmental factors *(Petrovic et al., 2011).* Increased percentage of lambing is the substantial contributor to get higher gains from sheep farms *(Mousa-Balabel, 2010).* Some important sources of variation of birth weight included breed, birth type, parity, breed by season, and season by year of lambing *(Ebangi et al., 1996).* The breed differences in lamb vigor at birth were report in pure and crossbred breeds (*Dwyer & Lawrence, 1999; Dwyer, 2003).* The lambs' weight at birth plays an imperative role in sheep's first production rate. Birth weight significantly varied due to years, season, sex, type of birth, and flock (*Farmanullah et al., 2020).* Although the year of lambing was a significant source of variation of birth weight, years are not repeatable, and their specific effects are of limited interest *(Ebangi et al., 1996).* Various influential factors affecting the birth weight of lambs' such as the maternal factor, maternal age, maternal nutrition, maternal body score, effect of ram, and environmental factors, have noted by various scholars *(Babar et al., 2004; Gardner et al., 2007; Petrovic et al., 2011; Kenyon et al., 2014; Petrovic et al., 2015; Caro Petrovic et al., 2018; Pesántez-Pacheco et al., 2019).*

Considering the non-genetic factors which influence the development and growth of lambs might help for changes in breeding plans and management practices to minimize the influential factors which reduce production performance (*Siddalingamurthy et al., 2017*).

This paper was to detect important factors affecting lamb's birth weight which usually are not taken to consideration and have substantial part planning improvement in sheep production.

Material and Methods

Mis breed sheep were cross or pair with Ile de France rams three times. The data of Mis breed ewes/dams that have lambed three times within the year of 2018 to 2020, the birth weight of their lambs, the lambs' birth type, and the number of lambs born per parity that obtained from the records kept in the mention years, was utilized.

The technology of sheep breeding was the same throughout the said years. The result of each lambing per parity of each ewe was the basis of the study.

The ANOVA, General Linear Model, Univariate Analysis of Variance, and Descriptive Statistics were performing using the SPSS software version 20. A total of 176 lambs, 37 ewes, 3 rams, and three times parity within the year of 2018 to 2020 have used for the estimation of the following effects:

Effect of parity (Par) on lambs' birth weight

Effect of parity on birth type (BT) and lambs birth weight (BWB)

Interaction effect of different subject effects on birth weight

The total marginal means of lambing rate per (LRP) dam in three (3) parities

Results and Discussion

The parity1 greatest lambs but got lowest average lambs birth weight. Parity 2 got the lowest number of lambs but the highest in lambs' average birth weight. Parity 3 was in between parities 1 and 2, holding the second placer both for average birth weight and the number of lambs (Table 1).

Table 1. Variation of lamb birth weight according to parity

Par	Mean	N	Std. Error of Mean	Variance	Minimum	Maximum	% of Total N
1.00	4.26	62	.13	1.03	2.10	6.70	35.2%
2.00	5.05	53	.12	.773	3.00	6.50	30.1%
3.00	4.59	61	.15	1.34	2.60	7.30	34.7%
Total	4.61	176	.08	1.15	2.10	7.30	100.0%

The test of between-subjects effects (Table 2) revealed that parity significantly affects lambs' birth weight (P<001).

Information on factors influencing the birth weight is of farmers' great interest, as well as the animal breeders, because birth weight is of great economic importance *(Bermejo et al., 2010).*

Table 2. Effect of parity on lambs' birth weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17.849[a]	2	8.925	8.426	.000
Intercept	3759.056	1	3759.056	3549.071	.000
par	17.849	2	8.925	8.426	.000
Error	183.236	173	1.059		
Total	3944.590	176			
Corrected Total	201.085	175			

Table 3. Average birth type and birth weight according to parity

Par		BT	BWB
1.00	Mean	2.18	4.26
	N	62	62
	Std. Deviation	.85	1.02
	Std. Error of Mean	.11	.13
2.00	Mean	1.68	5.04
	N	53	53
	Std. Deviation	.49	.88
	Std. Error of Mean	.07	.12
3.00	Mean	2.11	4.59
	N	61	61
	Std. Deviation	.88	1.15
	Std. Error of Mean	.11	.15
Total	Mean	1.99	4.61
	N	176	176
	Std. Deviation	.79	1.07
	Std. Error of Mean	.06	.08

Supreme in average birth type found in parity 1, (Table 3) however, showed the lowest, on the average birth weight. Parity 2 appeared the lowest in birth type but highest in lambs' birth weight (more lambs born single in this parity that justified the result). Parity 3 manifested as the second placer for both birth type and average lamb birth weight.

Table 4. Analysis of Variance of birth type*parity, birth weight*parity

			Sum of Squares	df	Mean Square	F	Sig.
BT * par	Between Groups	(Combined)	10.279	2	5.140	8.743	.000
	Within Groups		101.698	173	.588		
	Total		111.977	175			
BWB * par	Between Groups	(Combined)	17.849	2	8.925	8.426	.000
	Within Groups		183.236	173	1.059		
	Total		201.085	175			

Based on the results of analysis of variance (Table 4) showed that parities have a very significant ($P<0.01$) influence on lambs' birth type and birth weight. Considerable with our results was by Ebangi et al. (1996) note that birth types by parity were equally significant sources of variation. *Koycegiz et al. (2009)* state that parity was a significant source of variation ($p>0.05$) for litter size at birth, while differed with ours was that ewes in their 1st and 2nd parity had significantly ($p<0.05$) lower litter size at birth in comparison to ewes with greater parity.

A contradictory effect found by *Macedo and Hummel (2006)* birth weights were not affected by parity.

Partly justify our result were the comments of some authors. *Blickstein (2004)* noted a constraint on fetal growth and development is likely to be greater in twin than singleton pregnancies, each of which has a distinct fetal growth pattern. *Farmanullah et al. (2020)* noted that there are likely complex interactions between genetics and environmental factors of parental, placental, and fetal origin.

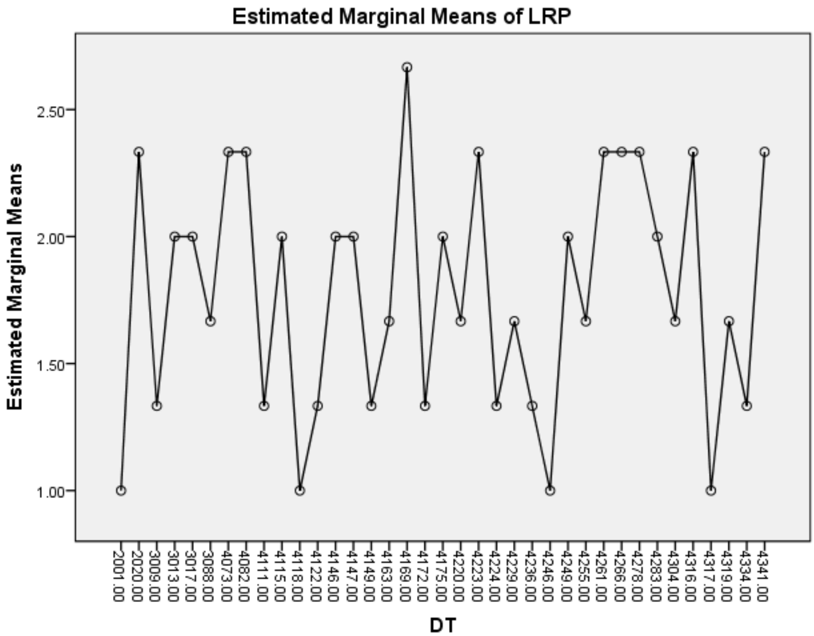


Figure 1. Total lambing rate per dam in 3 parities

The presented chart (Figure 1) showed the average lambing rate per dam in three parities. Dam tattoo (DT) 4169 was on top with regards to the average lamb rate of 2.67 for three parities. Second placer with an average lamb rate of 2.33. were dams numbers 2020, 4073, 4082, 4223, 4261, 4266, 4278, 4316, and 4341. Dams numbers 3013,3017,4115,4146,4147,4175,4249, and 4283 were the third placer with 2.00 an average lamb rate on three parities. The average lamb rate with 1.67 were dams numbers 3088, 4163,4220,4229, 4255,4304, and 4319 as fourth placers. Numbers 3009,4111,4122, 4149,4172, 4224, 4236, and 4334 were the 5th placers with 1.33 average lamb rate. The lowest on average lamb rate with 1.00 were numbers 2001, 4118, 4246, and 4317.

The parity of dam significantly affects the birth weight (*Siddalingamurthy et al., 2017*), was in accord with ours.

We do agree with *Mousa-Balabel (2010)* the number of born lambs per ewe is assuredly an economically important trait in a commercial sheep enterprise.

It's not included directly in our study but advocated ours were that as noted by the following authors: *Alexander (1974)*, the peculiarities of digestion and metabolism in ruminants make it difficult for the pregnant ewe to provide adequate glucose for fetal requirements; and it seems reasonable to explain all these influences on birth weight as acting, at least in part, through restriction of the nutrient supply to the fetus. *Pesántez-Pacheco et al. (2019),* fetal development is influenced by genetic and maternal factors which could interact with one another. Likewise, the mature ewes gave birth to heavier lambs than did maiden ewes, and ewes with single pregnancies gave birth to heavier lambs than did ewes with multiple pregnancies.

Conclusion

For successful and sustainable sheep breeding, the bodyweight of lambs at birth was especially essential. Many factors that affect birth weight are studied. However, on parity focused less. Based on the results obtain in our research, it can conclude that parities have a very significant influence on lambs' birth type and birth weight. The importance of the weight of lambs at birth should have paid more attention, and many contributing factors shall have studied in the future.

Acknowledgement

The paper was part of the research plan of Institute for Animal Husbandry, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022

Uticaj pariteta na stopu jagnjenja i masu tela jagnjadi pri rođenju

Violeta Caro Petrović, Milan P. Petrović, Dragana Ružić-Muslić, Nevena Maksimović, Marina I.Selionova, Bogdan Čekić, Ivan Ćosić

Rezime

Uspeh u proizvodnji jagnjadi zavisi od njihove mase tela, a poslednjih godina postoji sve veće interesovanje za razumevanje uticaja nekih faktora kao što je paritet na produktivnost ovaca.Telesna masa jagnjadi pri rođenju ima suštinsku ulogu u postizanju dobre proizvodnje ovaca, dok na produktivnost ovaca utiču mnogi faktori. Poznavanje faktora koji utiču na razvoj i rast jagnjadi mogu da

pomognu u promenama planova gajanja. Svrha ovog rada je bila da utvrdi važne faktore koji utiču na porođajnu masu jagnjadi, a koji se obično ne uzimaju u obzir i imaju značajan udeo u planiranju proizvodnje kod ovaca.
Ovce Mis rasa tri puta su ukrštane sa ovnovima rase Ile de France, pri čemu su posmatrane sledeće osobine: Masa tela jagnjadi pri rođenju, tip rođenja, broj rođenih jagnjadi po ovci po paritetu u periodu od 2018. do 2020. godine. Rezultat svakog janjenja po paritetu svake ovce bio je osnova ove studije. ANOVA, opšti linearni model, univarijantna analiza varijanse i deskriptivna statistika izvedeni su za procenu sledećih efekata: Efekat pariteta na porođajnu masu tela jagnjadi; uticaj pariteta na tip rođenja; efekat interakcije različitih subjekta na porođajnu masu; ukupna granična srednja stopa jagnjadi po ovci. Dobijeni rezulrtati su pokazali vrlo signifikantan uticaj posmatranih faktora na ispitivane osobine (P<0.01). Na osnovu rezultata našeg istraživanja, možemo zaključiti da pariteti imaju veoma značajan uticaj kod procene vrednosti priolodnih ovaca, pa im treba obratiti više pažnje u budućnosti.

Ključne reči: Jagnje, porođajna masa tela, stopa jagnjenja, paritet

References

ALEXANDER G. (1974): Birth Weight of Lambs: influences and consequences. In: Size at Birth, (eds. K Elliot and J. Knight). Elsevier, Amsterdam. pp.213-239.
BABAR M.E., AHMAD Z., NADEEM A., YAQOOB M. (2004): Environmental factors affecting birth weight in Lohi sheep. Pakistan Vet. J., 24, 1, 5-8.
BERMEJO L. A., MELLADO M., CAMACHO A., MATA J., ARÉVALO J. R., NASCIMENTO L. de (2010): Factors Influencing Birth and Weaning Weight in Canarian Hair Lambs, Journal of Applied Animal Research, 37, 2, 273-275.
BLICKSTEIN I. (2004): Is it normal for multiples to be smaller than singletons? Best Pract Res Clin Obstet Gynaecol 18, 613–623.
CARO PETROVIĆ V., PETROVIĆ P.M., RUŽIĆ MUSLIĆ D., MAKSIMOVIĆ N., DELIĆ N., CEKIĆ B., BJELIĆ Z. (2018): The effect of service ram on reproductive performances and birth weight of lambs. Biotechnology in Animal Husbandry 34, 3, 303-311.
DWYER C.M. (2003): ‘‘Behavioral development in the neonatal lamb: effect of maternal and birth-related factors,’’ Theriogen., 59, 1027-1050.
DWYER C.M., LAWRENCE A.B.(1999): ‘‘Does the behavior of the neonate influence the expression of maternal behavior in sheep? ,’’ Behav., 136, 367-389.
EBANGI A.L., NWAKALOR L.N. , MBAH D.A., ABBA D. (1996): Factors affecting the birth weight and neonatal mortality of Massa and Fulbe sheep breeds

in a hot and dry environment, Cameroon. Revue Élev. Méd. vét. Pays trop., 49, 4, 349-353.

FARMANULLAH, JAVED K, SALIM M, KHAN M, ALI S, ET AL. (2020): Consequence of birth year, type, sex, season and flock on birth weight trait of Kajli sheep. Insights Vet Sci.; 4, 035-041.

GARDNER D.S., BUTTERY P.J., DANIEL Z., SYMONDS M.E. (2007): Factors affecting birth weight in sheep: maternal environment. Reproduction, 133, 1, 297–307.

KENYON P.R., MALONEY S.K., BLACHE D. (2014): Review of sheep body condition score in relation to production characteristics, New Zealand Journal of Agricultural Research, 57, 1, 38-64.

KOYCEGIZ F., EMSEN E., DIAZ C.A.G., KUTLUCA M. (2009): Effects of Lambing Season, Lamb Breed and Ewe Parity on Production Traits of Fat Tailed Sheep and Their Lambs. J. Anim. Vet. Adv., 8, 195-198.

MACEDO R., HUMMEL J. D. (2006): Influence of parity on productive performance of Pelibuey ewes under intensive management in the Mexican dry tropics. *Livestock Research for Rural Development. Volume 18, Article #77.* Retrieved June 1, 2021, from http://www.lrrd.org/lrrd18/6/mace18077.htm

MOUSA-BALABEL T.M. (2010): The relationship between sheep management and lamb mortality. World Academy of Science, Engineering and Technology 4, 5, 372-377.

PESÁNTEZ-PACHECO JL, HERAS-MOLINA A, TORRES-ROVIRA L, SANZ-FERNÁNDEZ MV, GARCÍA-CONTRERAS C, VÁZQUEZ-GÓMEZ M, FEYJOO P, CÁCERES E, FRÍAS-MATEO M, HERNÁNDEZ F, MARTÍNEZ-ROS P, GONZÁLEZ-MARTIN JV, GONZÁLEZ-BULNES A, ASTIZ S. (2019): Influence of maternal factors (weight, body condition, parity, and pregnancy rank) on plasma metabolites of dairy ewes and their lambs. Animals, 9, 4,122. https://doi.org/10.3390/ani9040122.

PETROVIĆ P.M., CARO PETROVIĆ V., RUŽIC-MUSLIĆ D.,MAKSIMOVIĆ N, PETROVIĆ M.M., ILIĆ Z., STOJKOVIĆ J. (2015): Effect of genetic and environmental factors on the phenotype characteristics of lambs. Biotechnology in Animal Husbandry 31, 2, 223-233.

PETROVIC P.M., RUZIC MUSLIC D., CARO PETROVIC V. MAKSIMOVIC N. (2011): Influence of environmental factors on birth weight variability of indigenous Serbian breeds of sheep. African Journal of Biotechnology, 10, 22, 4673-4676.

SIDDALINGAMURTHY. H. K., MANJU, G.U., ROOPA DEVI. Y.S., MANJUNATHA. S. S., SREESUJATHA. R. M. (2017): Non-genetic factors affecting birth and weaning weight in Mandya sheep. Int. J. Adv. Res., 5, 4, 345-348.

SPSS (2011). Software Package for Social Sciences version 20.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

HEMATOLOGICAL STATUS OF ILE DE FRANCE SHEEP DEPENDING ON THEIR BODY CONDITION SCORE

Nikola Metodiev[1], PenkaMoneva[1], Ivan Yanchev[1], Kostadin Kanchev[2]

[1]Institute of Animal Science, 2232 Kostinbrod, Bulgaria
[2]Univeristy of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
Corresponding author: Nikola Metodiev, n_metodiev@abv.bg
Invited paper

Abstract: The aim of the present study was to evaluate the values of some blood parametres - red blood cell number (RBC), haemotcrit (Hct), haemoglubin (Hgb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of Ile de France sheep according their body condition score (BCS). The study was carried out in April, 2021, with 98 sheep, aged 1.5-7.5 years, raised in experimental base of IAS-Kostinbrod. Sheep were divided in two groups acording their BCS – I group (n=64) 3.0-3.5 and II group (n=34) 2.0-2.75. Blood analysis was performed with 5-Part-Diff Automated Hematology Analyzer. Sheep were categorized according to hematocrit level: low (LHct sheep) (hematocrit range 19.7-27.9%), high (HHct sheep) (hematocrit range 32.0-36.9%) and mean (MHct sheep) (hematocrit range 28.3-29.8%). The proportion of LHct, HHct and MHct sheep was also analyzed in both BCS groups of sheep. It was established that BCS affected significantly RBC ($P<0.01$), Hct ($P<0.05$) and Hgb ($P<0.05$), as sheep with higher BCS (I group) had higher values – 9.11 vs. 8.13 (x 10^{12}/ L, RBC) ; 27.99 vs. 25.32 (%, HCT); 94.02 vs. 85.14 (g/L,HGB). In both groups the proportion of sheep with LHct was the biggest – 48.44 % (Group I) and 64.71 (Group II). The proportion of sheep with higher BCS (3.0-3,5) was significantly ($P<0,05$) higher vs. sheep with lower (BCS2.0-2.7)5 only in MHct group of sheep. The data suggest that hematocrit levels are an important determinant, of achieving a more favorable proportion of animals with optimal BCS vs low BCS in the flock, especially in the presence of adverse factors such as parasite infestation and suspected malnutrition.

Key words: body condition score, sheep, haematological status, haemotcrit

Introduction

Body condition scoring is a practical and easily learned technique making it an ideal management tool and it has an advantage over live weight measurement in that it requires no specialised equipment *(Kenyon et al., 2014*). Some authors *(Morgan-Davies et al., 2008; Phythian et al., 2011),* suggested that BCS is good indicator of animal welfare as the Sheep Code of Welfare in New Zealand recommends that all adult sheep should be in the BCS range of 3.0–4.0 at all times *(Anon, 2010).* The BCS of an animal is assessed by the palpation of the lumbar region, specifically on and around the backbone (spinous and transverse processes) in the loin area, immediately behind the last rib and above the kidneys to examine the degree of sharpness or roundness *(Jefferies, 1961; Russel et al., 1969; Todorv et al., 1994).* The technique was first published by *Jefferies (1961*) and was based on a 0 to 5 scale, including only whole units, as 0 - extremely exhausted, 1 - weak, 2 - moderately fattened, 3 - advanced, 4 - fattened, 5 - very well fattened. *Russel et al., (1969)* adapted the system of Jefferies (1961), by introduced the term "subcutaneous fat" and the concept of 0.5 and 0.25 units. In Bulgaria, the BCS system was introduced in the 1990s by *Todorov et al. (1994)* and *Raicheva et al. (1995).*

It is widely accepted that BCS has many advantages over liveweight for determining the condition and wellbeing of an animal *(Kenyon et al., 2014).* Sheep that are in good physical condition breed more easily and twin to a greater extent than weak sheep (*Todorov et al., 1994).* The optimal pre-fertilization BCS should be 3.0-3.5. With obesity (BCS above 3.5) fertility deteriorates. Sheep with a relatively higher BCS, in the transition from seasonal anestrus to breeding season, show significantly higher sexual activity than other sheep *(Forcada and Abecia, 2006).*

The aim of the present study was to evaluate the values of some blood parameters of Ile de France sheep according their body condition score (BCS).

Material and Methods

The current study was conducted strictly in accordance with the guideline of the Institutional Animal Ethics Committee. The study was carried out in April, 2021, with 98 sheep, aged 1,5-7,5 years, raised in experimental base of IAS-Kostinbrod. The study was performed a month before starting the breeding campaign of the flock. Till the time of the experiment, ewes were raised indoors and were fed with meadow hay (1.0 kg. per head), corn silage (1.0 kg. per head) and concentrated mix with 15 % CP (0.4 kg. per head). All ewes were clinically healthy. At time of when BCS was done, fresh faeces were collected (aggregate sample) for parasitology tests(coproovoscopy and coprolarvoscopy). Sheep were infested only with Prostrongylus spp.(ifestatio rate low (+) to medium (++)). Sheep were divided in two groups acording their BCS – I group (n=64) 3.0-3.5 and II group (n=34) 2.0-2.75. BCS was performed by one person according to the adapted Jeffreies system by Todorov et al. (1994). Blood were collected with vacutainers from vena jugalaris after BCS exam. Blood analysis was performed with 5-Part-Diff Automated Hematology Analyzer (Urit- 5160 Vet, URIT Medical Electronic Co., Ltd.). The next parameters were analysed - red blood cell number (RBC), haemotcrit (Hct), haemoglubin (Hgb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean morpuscular volume (MCV). The proportion of sheep with low (LHct) (hematocrit range 19.7-27.9%), high (HHct) (hematocrit range 32.0-36.9%) and medium (MHct) (hematocrit range 28.3-29.8%) hematocrit levels was also analyzed in both groups. Significance of differences of the results of hematological parameters of the two groups were compared by t-Test: Two-Sample Assuming Unequal Variances (computer program EXCEL, Windows Office). The significance of difference about proportion of sheep with LHct, MHct and HHct was established by the Fisher's exact test (http://graphpad.com/quickcalcs/contingency1/.

Results and Discussion

The obtained results showed that the BCS singnificantly influced the parameters RBC, Hct and Hgb (Table 1) Sheep with higher BCS (3.0 – 3.5) had higher values of RBC, Hct and Hgb compared to sheep with lower BCS (2.0- 2.75) – 9.11 vs. 8.13 (x 10^{12}/ L, RBC); 27.99 vs. 25.32 (%, Hct); 94.02 vs. 85.14 (g/L, Hgb). The values of MCHC, MCH and MCV were not significanlty different. Higher values of Hct and Hgb were due to higher values of RBC number.

It is well known that the values of blood parametres vary according a lot of factors, such as more important – age, sex, breed, health status, physiological status, productive status, climate, season, altitude, etc. For sheep, the mean values of RBC, Hct, Hgb,MCHC, MCH, and MCV varied in next ranges *(*according *Radostits et al., 2000*) – 8.0-18.0 10^{12}/ L; 27.0 – 45,0 %; 90-150 g/ L; 310.0-340.0 g/ L; 8.0-1.02 pg; 28.0- 40.0 fL. From the obtained results we could conclude that sheep with higher BCS (3.0-3.5) had better haemopoetic function and produced more red blood cells. The same results were found from *Yilmaz et al. (2014*) at Saanen goats, as the higher BCS increased significantly the values of RBC, Hgb, Hct.

Table 1. Values of the blood parameters of the expeimetal groups

Parameters	I group x̄ ± SE	II group x̄ ± SE
RBC, 10^{12}/ L	9.11 ± 0.15	8.13± 0.29**
Hct, %	27.99 ± 0.49	25.32 ± 0.94*
Hgb, g/L	94.01 ± 1.49	85.15 ± 3.09*
MCHC, g/L	336.07± 1.19	336.26 ± 1.31
MCH, pg	10.28 ± 0.05	10.43 ± 0.09
MCV, fL	30.77 ± 0.17	31.22 ± 0.29

Note: Significance of the difference at ***P <0.05; ****P <0.01;

The proportion of sheep with LHct, MHct and HHct was presented in table 2. The proportion of sheep with HHct is equal – 29.69% vs 29.41% for Croup I and Group II respectively. In both groups the proportion of sheep with LHct was the biggest – 48.44 % (Group I) and 64.71 (Group II). There was a significant difference (P < 0.05) in proportion of sheep with MHct- 21.87% (Group I) vs. 5.88 % (Group II).

Table 2.The proportion of sheep from the experimetal groups with LHct, MHct and HHct

Group	LHct,n	LHct,%	MHct,n	MHct,%	HHct,n	HHct,%
I group	31	48.44	14*	21.87	19	29.69
II group	22	64.71	2	5.88	10	29.41

Note: Significance of the difference at ***P <0,05;

In our previous studies we found significant hematocrit-related difference in the magnitude of sheep adrenal response to shearing. Sheep with high hematocrit (HHct) level demonstrated more vigorous increase of plasma cortisol level in response to shearing than sheep with low hematocrit (LHct) level *(Moneva et al., 2017).* We did not find significant changes in GH levels throughout the entire experimental period except for significantly higher GH level in MHct ewes compared to LHct and HHct ewes at 3h following shearing *(Moneva et al., 2021).* The major findings of a recent review of the research on multiple effects of GH suggest a role of GH in the regulation of angiogenesis, immune function, hematopoietic system, normal differentiation and function of blood cells *(Devesa et al., 2016).* Therefore, increased GH level in MHct ewes can be associated with one or more of its many functions.GH stimulates the growth of small antral follicles to gonadotropin-dependent phases, as well as the maturation of oocytes. *(Silva et al., 2009).*

The breeding campaign is a kind of stress for the sheep, they are placed in daily contacts with rams. If rams have been isolated from females and/ or biotechnological methods are used for estrus synchronization, this leads to additional stress for female animals. As we pointed out in the introduction, sheep that are in good physical condition (BCS 3.-3.5) breed more easily, twin more and show higher sexual activity. We suggest that this may be due to the higher levels of Hct. On the other hand, the percentage of sheep with high hematocrit regardless of BCS is the same, but sheep with MHct were in a higher percentage in high BCS group. These data are in line with the theory of the existence of an optimal hematocrit level, which achieves the transfer of the maximum amount of oxygen to the tissues *(Schuler et al., 2010).*

Conclusion

It was established that BCS affected significantly RBC ($P<0.01$), Hct ($P<0.05$) and Hgb ($P<0.05$), as sheep with higher BCS (I group) had higher values – 9.11 vs. 8.13 (x 10^{12}/ L, RBC) ; 27.99 vs. 25.32 (%, HCT); 94.02 vs. 85.14 (g/L, HGB). There was a significant difference ($P < 0.05$) in proportion of MHct sheep - 21.87% (BCS 3.0-3.5) vs. 5.88 % (BCS 2.0-27.75).

The data suggest that hematocrit levels are an important determinant, of achieving a more favorable proportion of animals with optimal BCS vs low BCS in the flock, especially in the presence of adverse factors such as parasite infestation and suspected malnutrition.

Hematološki status il de frans ovaca u zavisnosti od njihovog telesnog stanja

Nikola Metodiev, Penka Moneva, Ivan Yanchev, Kostadin Kanchev

Rezime

Cilj ove studije bio je da se procene vrednosti nekih parametara krvi - broj crvenih krvnih zrnaca (RBC), hemotkrit (Hct), hemoglubin (Hgb), srednja koncentracija korpuskularnog hemoglobina (MCHC), srednji korpuskularni hemoglobin (MCH) i srednja vrednost korpuskularnog volumena (MCV) ovaca Il de frans rase, prema rezultatima/oceni njihove telesne kondicije (body condition score - BCS). Studija je sprovedena u aprilu 2021. godine sa 98 ovaca, starosti 1,5-7,5 godina, uzgajanih na eksperimentalnoj farmi IAS-Kostinbrod. Ovce su podeljene u dve grupe prema vrednosti BCS-a: I grupa (n = 64) 3,0-3,5 i II grupa (n = 34) 2,0-2,75. Analiza krvi je izvršena

sa 5-Part-Diff automatizovanim hematološkim analizatorom. Ovce su kategorisane prema nivou hematokrita: niski hematokrit (ovce LHct) (raspon hematokrita 19,7-27,9%), visoki hematokrit (ovce HHct) (raspon hematokrita 32,0-36,9%) i srednji hematokrit (ovce MHct) (raspon hematokrita 28,3-29,8%). Udeo LHct, HHct i MHct ovaca takođe je analiziran u obe BCS grupe ovaca.
Utvrđeno je da je BCS značajno uticao na RBC (P<0,01), Hct (P<0,05) i Hgb (P<0,05), jer su ovce sa većim BCS-om (I grupa) imale veće vrednosti - 9,11 naspram 8,13 (x 10^{12}/ L, RBC); 27,99 naspram 25,32 (%, HCT); 94,02 naspram 85,14 (g/L, HGB). U obe grupe, udeo ovaca sa LHct bio je najveći - 48,44 % (Grupa I) i 64,71 (Grupa II). Udeo ovaca sa većim BCS (3,0-3,5) bio je značajno (P <0,05) veći u odnosu na ovce sa nižim (BCS 2,0-2,7) samo 5 u MHct grupi ovaca. Podaci ukazuju na to da su nivoi hematokrita važna determinanta postizanja povoljnijeg udela životinja sa optimalnim BCS u odnosu na nizak BCS u stadu, posebno u prisustvu nepovoljnih faktora kao što su zaraza parazitima i sumnja na neuhranjenost.

Ključne reči: ocena telesne kondicije, ovce, hematološki status, hematokrit

Acknowledgement

This work was financially supported by National Science Fundat the Bulgarian Ministry of Education and Science(Grantno. KP-06-H26/2, 04.12.2018).

References

ANON (2010): Sheep and beef cattle, code of welfare 2010. National Animal Welfare Advisory Committee. Wellington, New Zealand, MAF Biosecurity New Zealand. Pp. 12–13.
DEVESA J., ALMENGLÓ C., DEVESA P. (2016): Multiple effects of growth hormone in the body: Is it really the hormone for growth? Clin Med Insights Endocrinol Diabetes, 9, 47-71.
FORCADA F., ABECIA J. A. (2006): The effect of nutrition on the seasonality of reproduction in ewes. Reproduction Nutrition Development, 46: 355-365.
JEFFERIES B. (1961): Body condition scoring and its use in management. Tasman. J. Agric. , 32, 19–21.

KENYON P.R., MALONEY S.K., BLACHE D. (2014): Review of sheep body condition score in relation to production characteristics. New Zealand Journal of Agricultural Research, 57, 1, 38–64

MONEVA P., YANCHEV I., DYAVOLOVA M., GUDEV D. (2017): Effect of altitude hypoxia on adrenal activity andneutrophil-to-lymphocyte ratio in low and highhematocrit sheep. Proceedingsof Scientific Conference with International Participation „Animal Science - Challenges and Innovations", Institute of Animal Science – Kostinbrod, pp. 39-44.

MONEVA P., TSANEVA M., YANCHEV I., METODIEV N., GUDEV D. (2021): Endocrine responses to shearing stress in pregnant Ile de France ewes with low and high basal hematocrit levels. Bulgarian Journal of Agricultural Science, 2021, 5 (accepted 2020)., in press.

MORGAN-DAVIES C., WATERHOUSE A., POLLOCK M.L., MILNER J.M. (2008): Body condition score as an indicator of ewe survival under extensive conditions. Animal Welfare 17: 71–77.

PHYTHIAN C.J., MICHALOPOULOU E., JONES P.H., WINTER A.C., CLARKSON M.J., STUBBINGS L.A., et al. (2011): Validating indicators of sheep welfare through a consensus of expert opinion. Animal 5: 943–952.

RAICHEVA E., TODOROVA L., SHINDARSKA Z. (1995): Investigation of the possibilitiesfor estimation of the body composition in vivo. Bul. J. Agric. Sci., 1: 45-47.

RUSSEL A.J.F., DONEY J.M., GUNN R.G. (1969): Subjective assessment of body fat in live sheep. J. Agric. Sci., 72,451–454.

SILVA J. R. V., FIGUEIREDO J. R., VAN DEN HURK R. (2009): Involvement of growth hormone (GH) and insulin-like growth factor (IGF) system in ovarian folliculogenesis. Theriogenelogy, 71: 1193-1208.

SCHULER B., ARRAS M., KELLER S., RETTICH A., LUNDBY C., VOGEL J., GASSMANN M. (2010): Optimal hematocrit for maximal exercise performance in acute and chronic erythropoietin-treated mice. Proc Natl Acad Sci USA.,107(1), 419-23.

TODOROV N., MITEV Y., OTUZBIROV R. (1994): Body condition score of sheep. ISBN 954-8180-12-X. (Bg).

YILMAZ M., TASKIN T., BARDAKCIOGLU H., LORIA A. (2014): Effect of body condition score on some blood parameters for anemia level in goats. Veterinarija ir Zootechnika (Vet Med Zoot). T. 67 (89), 41-46.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

CURRENT STATE OF GOAT BREEDING IN CENTRAL SERBIA

Nevena Maksimović, Dragana Ružić-Muslić, Violeta Caro Petrović, Bogdan Cekić, Ivan Ćosić, Nikola Delić, Marina Lazarević

Institute for Animal Husbandry, Belgrade, Republic of Serbia
Corresponding author: Nevena Maksimović, nevena_maksimovic@yahoo.com
Invited paper

Abstract: This paper describes the present state of goat production in Central Serbia, discussing the challenges and future prospects for this livestock system. Number of goats in the country is decreasing and it has been so throughout the years now. Production systems in goat sector in Serbia are predominately extensive or semi-intensive, especially in hilly-mountainous areas and goats are kept in small herds. The majority of goats raised in Central Serbia region are of French Alpine breed, which takes the most dominant place in breed structure. In addition to French Alpine breed, there are also Saanen, Balkan and Domestic white goats. Production of goats in Serbia is directed towards milk-meat, but primarily milk and there are no typical meat (fattening) breeds of goats in Serbia. In Central Serbia, herd books are kept for goats raised on this territory. Quality breeding animals are registered at Central herd book and are under productivity control. However, this is only a small portion of animals and the productivity of majority of goats is unknown. Productivity of high yielding breeds such as Alpine and Saanen goat is inconsistent and not high, especially milk production. Indigenous goat breeds are considered endangered and their breeding was almost completely abandoned. Goat sector in Central Serbia is almost completely dependent on government founding. The only sustainable prospects for preserving goat sector in Central Serbia for the future are clean organic production, reinforcement of markets for goat products and smart government founding.

Key words: dairy goats, Serbia, local breeds, production, herd book

Introduction

Despite resources and historical heritage, nowadays, goat breeding in Serbia is not developed. In fact, it is probably the least popular livestock production *(Maksimović et al., 2015)* which is almost completely dependent on government funding. Goat production in Serbia went through some difficult periods in the last few decades, especially in the second half of 20th century with the adoption of 1954's Law on the Prohibition of goat keeping, which resulted in decrease in number of these animals and consequently led to lack of their products on the market *(Maksimović et al., 2017)*. In recent years, the government has been trying to revitalize and prevent further deterioration of this type of farm animal production, mostly by funding breeders for production of quality breeding animals. One of the most important problems of goat production is the difficult adequate marketing of goat products, especially milk. The purchase price of milk is usually unrealistically low and it varies from year to year, which makes this production very uncertain. Also, consumers in Serbia prefer cow's milk and cheese over goat's or sheep's, because of the stronger flavour. This is also the case with goat meat. Statistical yearbook of Republic of Serbia shows no data referring to goat meat production, nor can number of heads of goats slaughtered in slaughterhouses, slaughtered for own consumption on farms, forced slaughter on farms and other slaughtering out of slaughterhouses be found. This alone tends to be very indicative of the state of goat production in the country. Recently, with the world wide promotion of healthy food and the rise of fitness industry, as well as the growing market of organic products, goat production is gaining increased attention in the country due to its nutritionally valuable and safe products. Organic production in the European dairy sector has grown considerably and goat producers are increasing their presence with products such as cheese, different types of milk and yoghourt *(Dubeuf, 2010)*. Based on the EU Regulation, organic goat milk production increased by 47.2% in the period from 2012 to 2017 (*Ruiz Morales et al., 2019* referring to *EUROSTAT* data). The total number of goats in organic production (including both meat and milk) was 833,087 heads with production of 49.4 million litres of milk. In 2019 that number was 1,015,399 heads of goats in European countries, with Serbia having 536 heads of goats in organic production *(EUROSTAT)*. However, number of heads of goats in total is not increasing in Serbia and farmers are still almost completely dependent upon government funding and therefore goat production in the country is still not sustainable.

Goat population and production in Serbia

According to the latest data published in the Statistical yearbook of the Republic of Serbia, 2020 there were 191,000 heads of goats in Serbia, of which 145,000 are bred in Central Serbia region, and the remaining 46,000 heads belonging to Vojvodina region, i.e. North Serbia.

Table 1. Number of goats raised in Serbia for period 2013 to 2019

Year	Heads of goats	
	Serbia	Central Serbia
2013	225,000	180,000
2014	219,000	165,000
2015	203,000	150,000
2016	200,000	155,000
2017	183,000	137,000
2018	196,000	148,000
2019	191,000	145,000

Source: *RZS, Statistical yearbook of the Republic of Serbia, 2020*

Table 2. Number of goats raised in Central Serbia in 2019 by category

Year	Category			
	Kids	Multiparous goats	Primiparous goats	Other goats
2019	28,000	99,000	11,000	7,000

Source: *RZS, Statistical yearbook of the Republic of Serbia, 2020*

According to the Agriculture Census, which was conducted in 2012, there were 231,837 heads of goats in Serbia, of which 171,774 were raised in Central Serbia region *(RZS, Statistical Office of the Republic of Serbia)*. If compared to the number stated in Statistical yearbook of the Republic of Serbia for 2019 (191,000) it is clear that the number of goats decreased. The number of goats has been declining since 2008 with an average decline of 8.7 thousand per year *(Sredojević et al., 2020)*.

Nastić and Potrebić (2015) report the number of holdings keeping goats, stating a total number of holdings to be 62,930, of which 54.21% are holdings with only 1-2 heads of goats, 40.55% are holdings with 3-9 heads, while the share of holdings in which 10-19 heads of goats are kept was 3.76%. Also, somewhat larger holdings with 20-49 heads of goats have a share of 1.20%, those with 50-99 heads have a share of 0.18% (115 of these holdings) and only 0.09% (56) of holdings have hundred or more heads of goats in the entire area of the Republic of Serbia. Although these data are not as fresh and perhaps this structure has changed a bit in the meantime, but it is clear that the majority of holdings keeps less than 10 heads of goats which is under any limit that allows sustainable production. In the Republic of Serbia, goat breeding takes place on 8% of agricultural farms (45.7 thousand).

Table 3. Number of goats in Serbia by regions in 2019

Year	Vojvodina region	Belgrade region	Šumadija and West Serbia region	East and South Serbia region
2019	46,000	8,000	68,000	70,000

Source: *RZS, Statistical yearbook of the Republic of Serbia, 2020*

As seen from perspective of the region, the majority of goats are raised south from Belgrade, i.e. in Šumadija (central part), West, East and South Serbia. Basically, Vojvodina and Belgrade constitute northern part and all the other regions constitute southern part of the country. The average herd size within the northern part of Serbia is 5.4 heads and 4.5 heads within the southern part. In both parts of Serbia, most represented are the farms with a herd consisting of up to 9 heads of goats *(Sredojević et al., 2020)*.

The majority of goats raised in Central Serbia region are of French Alpine breed, which is the most dominant breed. In addition to French Alpine breed, there are also Saanen, Balkan and Domestic white goats (also referred to as Serbian white goat), as well as different crosses between these breeds. The latter two are local breeds, both of which are of lower productivity, but well adapted to modest conditions of care, housing and nutrition, usually raised in high lands and are considered endangered, although the real number of these animals is unknown *(Maksimović et al., 2019)*.

Production systems in goat sector in Serbia are predominately extensive or semi-intensive, especially in hilly-mountainous areas. Just a small portion of farms implements confined systems with completely mechanized production process and this is usually based on keeping Alpine or Saanen goats.

Production of goats in Serbia is directed towards milk-meat, but predominantly milk *(Žujović et al., 2011)*. There are no typical meat (fattening) breeds of goats in Serbia. Despite the nutritional value, goat meat is still less appreciated due to the specific odour and flavour, especially if the animal is older *(Ivanovic et al., 2011)*. According to *FAOSTAT* in 2019 there was milk production of 32,046 tonnes of milk from 98,685 milk heads in Serbia. Statistical yearbook of the Republic of Serbia’s officially published data for year 2019 was 31,000 tonnes of goat milk. Territorially, most of the sheep’s and goat’s milk is produced in the Region of Eastern and Southern Serbia, followed by the Region of Šumadija and Western Serbia *(Sredojević et al., 2020)*. Goat meat production in Serbia was 3,192 tonnes from 238,591 producing/slaughtered animals *(FAOSTAT, 2019)*.

The value of goat’s milk and head for slaughter is only 0.7% of the total value of livestock production. In the total milk production in Serbia, cow’s milk makes 96.84%, then goat’s milk 2.20, and in a smaller amount sheep’s milk with 0.96% *(Sredojević et al., 2020)*. However, since official statistics do not include home consumption or informal market sales where records are not kept, it is complicated to measure the real value of goats. In majority of countries, goat milk is more likely to be consumed locally, whereas cow milk is more likely to enter formal markets for processing *(Miller and Lu, 2019),* and that is no exception in Serbia.

Goat population and production in Europe

European countries leading in regard to their goat populations are Greece (3,580,000 heads), Spain (2,659,110), Russian Federation (1,992,896), Romania (1,598,800), France (1,242,000) and Italy (1,058,720). As for Eastern Europe, the highest-ranking countries in regard to goat populations are Russian Federation and Romania, followed by Ukraine *(FAOSTAT, 2019).*

When looking into Serbia's neighbouring countries, Romania and Bulgaria are ahead of Serbia, while other countries have fewer goats (Table 4). The same accounts for milk production.

Table 4. Number of goats in Serbia's neighbouring countries in 2019

Country	**Heads of goats**	**Milk production (in tonnes)**
Hungary	63,000	3,010
Bulgaria	228,490	37,000
Romania	1,598,800	236,400
Croatia	82,000	9,000
Montenegro	28,700	No data
Bosnia and Herzegovina	72,362	No data
North Macedonia	87,581	16,865

Source: *FAOSTAT, 2019*

Europe is characterized by the widest caprine biodiversity, compared to other continents, with 187 goat breeds making up 33% of the goat breeds recognised worldwide *(Gahal, 2005).* There are breeds which are large in population sizes, characterised by high milk production leading to exports to other countries, as is the case with Saanen and French Alpine, or the Murciano-Granadina from Spain. On the other hand, there are breeds in the opposite position, with small population sizes, often in a critical situation and on the brink of extinction *(Dubeuf and Boyazoglu, 2009; Ruiz Morales et al., 2019)*. In this context of genotype diversity, traditional systems of meat–milk production co-exist with intensive milk production systems.

Europe accounts for only 1.9% of world goat population, but produces 15.1% of the world's goat milk and 35.1% of the world's goat cheese *(Ruiz Morales et al., 2019* referring to FAOSTAT data*)*. So, milk is dominant product of goat farming in Europe and most of goat milk is transformed into cheese. According to *FAOSTAT (2019)* data, Europe has 11,100,356 heads of dairy goats producing 3,075,264 tonnes of milk.

Table 5. Dairy goat population and milk production in leading European countries

Country	**No of heads (milk animals)**	**Milk production (in tonnes)**
France	1,058,000	656,740
Spain	1,896,890	535,790
Netherlands	509,000	386,000
Greece	2,588,000	355,760
Romania	1,297,600	236,400
Russian federation	775,204	247,728
Ukraine	422,600	200,500

Source: *FAOSTAT, 2019*

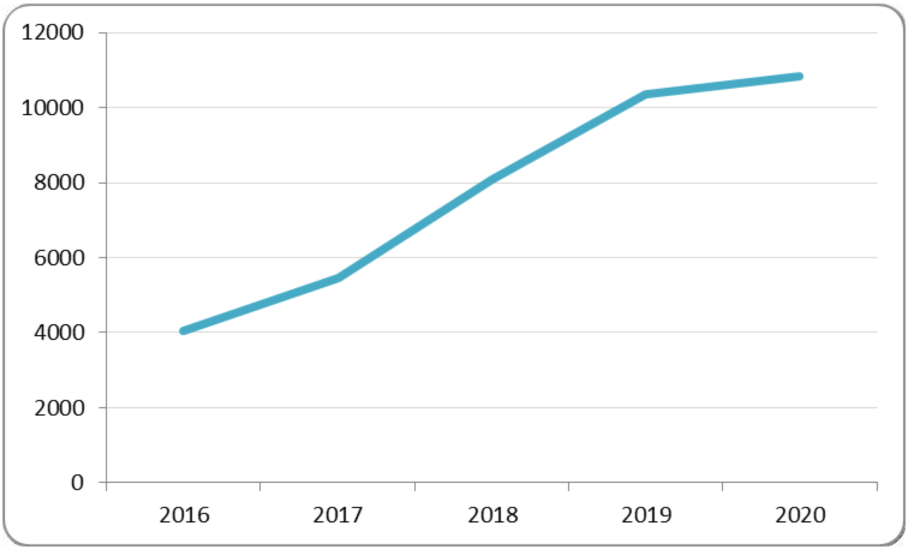
As can be seen from Table 5, France is an absolute leader in Europe's goat milk production with 656,740 tonnes of milk produced in 2019 from 1,058,000 dairy goats. Spain comes second when it comes down to overall quantity of goat milk production in Europe, but Netherlands, which comes third on this list with overall quantity of milk yield, produces more milk from fewer dairy animals. Production systems in France and Netherlands are more based on the use of high-yielding breeds kept in confined systems which provide more intensified production and therefore more milk from fewer animals. The dairy goat sector in the Netherlands has been expanding since 1984 when many farmers transitioned from cows to goats due to quotas for cow milk *(Van Dijk, 1996)*, and so the Dutch became important producers of goat milk in Europe. Because of their experience with intensive dairy cow management, Dutch dairy goat breeders use more technology, such as artificial insemination, confinement rearing, and computerized record-keeping, compared to other European farmers *(Miller and Lu, 2019)*.

Spain and Netherlands are exporting great amount of their goat milk to France for processing, as France is the leader in goat milk processing and cheese production. In Spain, goat milk is used for making mixed cheese (with cow or sheep milk) and in Greece traditional "feta" cheese is made of goat milk.

As for goat meat production, in 2019, Europe produced 96,310 tonnes of goat meat from 8,460,324 heads of goats, with Greece being the leader in this sector with 26,480 tonnes of meat from 2,367,770 heads, followed by the Russian federation and Spain with 18,676 and 10,420 tonnes of goat meat produced in 2019, respectively *(FAOSTAT, 2019).*

Herd book goat population in Central Serbia

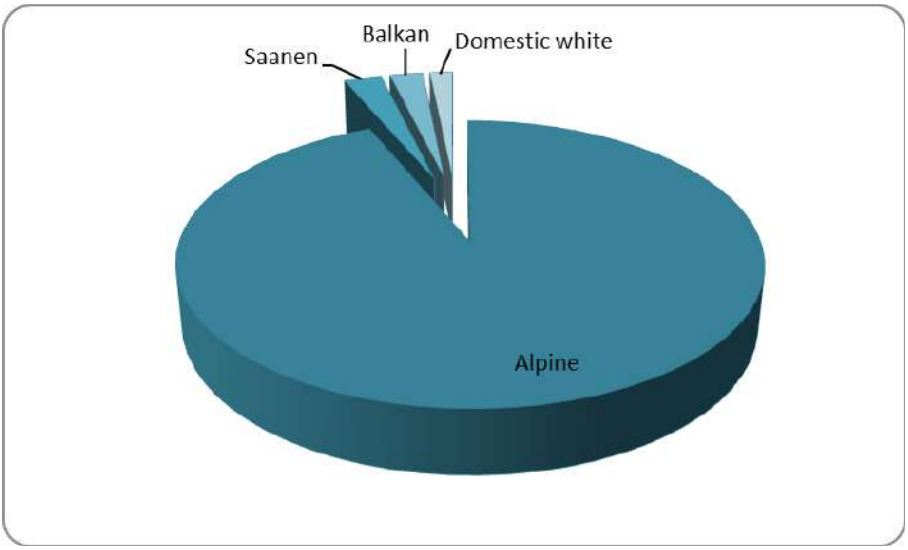
In Central Serbia, herd books are kept for goats raised on this territory. Quality breeding animals are registered in the Central herd book and are under productivity control. In 2020 there were 10,851 heads of goats and 368 bucks under productivity control *(IAH, 2020)*. This accounts for 7.73% of total number of goats raised on this territory. Contrary to decreasing goat population in total, in the country, number of herd book animals has been increasing constantly.



Graph1. Number of goats (♀) under selection control in the period 2016 to 2020; Source: IAH, 2020

As seen from Graph 1, number of animals under selection control has increased significantly during the past 5 years. This period brought increase of roughly 169% in the number of controlled animals. In 2016, number of registered goats accounted for 2.35% of total goat population in Central Serbia *(Maksimović* *et al., 2017)*, while in 2020, a significant increase to roughly 7.7 is recorded. The main reason behind this increase is government founding of goat breeders, which is currently almost the only thing keeping goat sector alive. Farmers receive roughly 59 Euros a year per head for animals registered in the Central heard book plus additional 38 Euros for goats of endangered breeds (genetic resources) that are under selection control.

Within breed structure, the most dominant breed of registered goats is Alpine which accounts for 93.65%, while the rest is divided among three other breeds, with Saanen breed participating with 2.54%, Balkan with 2.26% and Domestic white goat with 1.55% *(IAH, 2020)*.



Graph 2. Breed structure of registered goats in Central Serbia

All animals registered in the Central herd book are under productivity control, including milk production, body development and reproductive traits. Controls are conducted annually and are done following ICAR Recording Guidelines. Tables 6 and 7 include the production data for heard book animals under productivity control.

Table 6. Average values for body weight and fertility of goats of different breeds in 2020

Breed	Body weight of goats (kg)	Fertility	Body weight of kids (kg)		
			at birth	30 days of age	at weaning
Alpine goat	50.97	1.5	2.63	8.23	18.06
Saanen	53.46	1.33	3.49	8.96	18.60
Domestic (Serbian) white goat	50.15	1.63	2.56	7.07	14.43
Balkan goat	41.55	1.22	2.48	6.72	15.30

Source: *IAH, 2020*

Table 7. Average milk production and composition of different goat breeds in 2020

Breed	Lactation duration (days)	Total milk yield (kg)	Milk fat (%)	Protein (%)	Daily milk yield (kg)
Alpine goat	219	459.52	3.66	3.18	2.10
Saanen	231	651.37	3.59	2.99	2.82
Domestic (Serbian) white goat	230	484.04	3.64	3.31	2.11
Balkan goat	245	255.19	3.98	3.15	1.04

Source: *IAH, 2020*

The presented data shows that the productivity of high yielding breeds, such as Alpine and Saanen goat, is not high, including all recorded traits, i.e. their body weight, fertility and milk production. The reason behind this is that these animals are mostly reared under traditional systems of milk–meat production in more extensive or semi-intensive manner and therefore their genetic potential is not completely utilized. Also, milk production is not consistent because of the inconsistent market which imposes its rules on primary production through prices and demand for goat products. When the raw milk price is low, producers don’t milk goats and let kids suck for long period of time. This can cause some udder problems and leads to lower subsequent production. As for local breeds, their productivity is within standard for each breed and is considered good due to the fact that both breeds are reared almost exclusively extensive.

Prospects for goat breeding

There is a rich diversity in goat breeds in European countries. Due to the very different environments and cultural peculiarities many different breeds of goats were formed across the Europe. In fact, alongside sheep, goats are probably the most diverse livestock species in Europe. And therefore they represent particularly important biodiversity factor, not just within world of fauna (i.e. animal sector), but also for ecosystem in general. According to *Gahal (2005)*, Europe is a continent with the widest caprine biodiversity, with 187 breeds making up to 33% of the goat breeds recognized worldwide. Majority of these European goat breeds are of local character and importance, low in their productivity, but very resilient and well adapted to harsh conditions of environment and nutrition. Unfortunately, they are considered endangered and/or at the brink of extinction. And yet, they are of most importance as genetic resources. On the other hand, there are breeds large in population sizes, characterised by a high milk production exported to other countries, as is the case with Saanen and French Alpine, or the Murciano-Granadina from Spain *(Ruiz Morales et al., 2019)*.
As pointed out previously, in Serbia there are both local and imported breeds, with local being very small in population size and imported ones (French Alpine in particular) dominating the breed structure. While high yielding breeds are important for closed intensive production systems, it is necessary to preserve these local genotypes and to utilise them in organic and ecology friendly production systems. Goats in general are important socio-economic factor in marginal and remote rural areas, as they provide entire families' incomes, but also have socio-cultural impact for the rural community. In these less favoured and remote areas, raising small ruminants can often be the last possible economic activity. In Serbia, as in most other European countries, goat milk could never compete with cow milk in terms of both consumption and the price, as bovine dairy industry is far more advanced. However, goats are perfect resource for cleaner organic production. When reared extensively in natural grazing systems, goats help manage the land, shape the landscape and reduce biomass fuel, reduce non-renewable energy used as well as net greenhouse gas emissions *(Mancilla-Leyton et al., 2013; Perez-Neira et al., 2018; Gutiérrez-Peña et al., 2019)*. In terms of cleaner ecological production, indigenous breeds are of most importance as they are well adapted to a range of pasture-based ecosystems. They also help maintain cultural and ethnological traditions and typical products. In order to contribute to the economic sustainability of goat farming, it is important for the environmental and social role to be recognized, but it should also be paid for, thus diversifying and increasing the income received by farmers.

One of the most important and applicable prospects for goat breeding in Central Serbia should be conservation of goat genetic resources and their utilisation in clean ecological farming systems. Production of so called typical products with geographical origin and creation and reinforcement of markets for such products should be priority in goat sector in Central Serbia. Goats and sheep are the only domestic species left in Serbia that are still so called grass fed, which makes their products, i.e. meat and milk, valuable for healthy human nutrition. Government founding should be directed toward environmental and social role of goat production, rather than just giving the money for herd book animals in equal amounts. Preserving of animal genetic resources is investment in future.

Conclusion

Number of goats in the country has been decreasing for years. Goats are kept in small herds, with very small number of specialized goat farms. Small portion of goat population is involved in nucleus breeding, while the productivity of majority of goats is unknown. Contrary to decrease of goat population in total, in the country, number of herd book animals has been increasing constantly. But, even though there is a positive trend, this number needs to be even higher and more animals need to be included in record-keeping. The market for goat products is poorly organized, with meat market being particularly weak. Milk is the dominant income source for most goat farms, but meat becomes the main product in some situations, such as is the case in those years when the purchase price of milk is low for example. Producers are vulnerable to market fluctuations, which makes goat production unpredictable and insecure. High yielding breeds are not fully exploited and their productivity is far below their genetic limits. Local indigenous goat breeds are considered endangered, although the real number of these animals is not known. Their breeding was abandoned mostly because of their lower productivity and also because of the changes in demographic dynamics and abandonment of mountainous rural areas.
The only sustainable prospects for preservation of the goat sector in Central Serbia for the future are clean organic production, reinforcement of markets for goat products and smart government founding. Scientific, as well as commercial promotion of healthy and safe goat products for human nutrition should be a priority.

Trenutno stanje kozarske proizvodnje u Centralnoj Srbiji

Nevena Maksimović, Dragana Ružić-Muslić, Violeta Caro Petrović, Bogdan Cekić, Ivan Ćosić, Nikola Delić, Marina Lazarević

Rezime

U radu je prikazano trenutno stanje kozarske proizvodnje u Centralnoj Srbiji uz osvrt na izazove koji su prisutni i prospekte za budućnost ove grane stočarske proizvodnje. U Srbiji je prisutan trend smanjenja ukupnog broja grla iz godine u godinu. Proizvodni sistemi u kozarskom sektoru su predominantno ekstenzivni ili poluintenzivni, a koze se gaje u malim stadima, naročito u brdsko-planinskim regijama. U Centralnoj Srbiji najzastupljenija su grla alpske rase, a u znatno manjem broju gaje se još i sanska, balkanska i srpska (domaća) bela koza. Kozarska proizvodnja je usmerena u pravcu mleko-meso, ali je mleko dominantno. U Centralnoj Srbiji nema tipičnih tovnih rasa koza, a meso se proizvodi uglavnom samo kao alternativa u formi jaradi za klanje. Matična evidencija vodi se za koze koje se gaje na teritoriji Srbije. Kvalitetna priplodna grla koza upisuju se u Glavnu matiču evidenciju i nalaze se pod kontrolom proizvodnih svojstava. Ipak, tu spada samo jedan mali deo koza dok je većina koza van sistema kontrole i nepoznate produktivnosti. Proizvodnost visokoproduktivnih genotipova poput alpske i sanske koze u Centralnoj Srbiji je niska, posebno proizvodnja mleka. Autohtone rase koza se smatraju visoko ugroženim u svom opstanku i njihovo gajenje je gotovo potpuno napušteno. Čitav sektor kozarstva je gotovo potpuno zavistan od subvencionsanja od strane države. Jedan od najrealnijih prospekata za održivu kozarsku proizvodnju u budućnosti na teritoriji Centralne Srbije je čista organska proizvodnja uz jačanje tržišta za kozarske proizvode i pametno državno finansiranje upravo ovakvog vida proivodnje.

Ključne reči: mlečne koze, Centralna Srbija, autohtone rase, produktivnost, matična evidencija

Acknowledgements

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

References

DUBEUF J.P. (2010): Characteristics and diversity of the dairy goat production systems and industry around the world. Structural, market and organisational conditions for their development. Tecnologia & Ciência Agropecuária 4, 25-31.
DUBEUF J.P., BOYAZOGLU J. (2009): An international panorama of goat selection and breeds. Livestock Science 120, 225-31.
EUROSTAT: Organic production in the European goat sector. Statistical Office of the European Union. https://ec.europa.eu/eurostat
FAOSTAT (2019): Live animals and livestock processed stats. Rome, Italy: Food and Agriculture Organization (FAO). http://www.fao.org/faostat/en/
GAHAL S. (2005): Biodiversity in goats. Small Ruminant Research 60, 75-81.
GUTIÉRREZ-PEÑA R., MENA Y., BATALLA I., MANCILLA-LEYTON J.M. (2019): Carbon footprint of dairy goat production systems: A comparison of three contrasting grazing levels in the Sierra de Grazalema Natural Park (Southern Spain). Journal of Environmental Management 232, 993-8.
IAH (2020): ACTIVITIES REPORT AND RESULTS OF THE CONTROL OF REALIZATION OF BREEDING PROGRAMS IN 2020. Institute for animal husbandry.
IVANOVIC S., POPOV-RALJIC J., BALTIC M.Z., ZUJOVIC M., TOMIC Z., LILIC S., PAVLOVIĆ I. (2011): Chemical and sensory charac-teristics of Bunte deutsche edelziege and Balkan goat meat. African Journal of Biotechnology 10, 18433–18439.
MAKSIMOVIĆ N., BAUMAN F., PETROVIĆ P.M., CARO PETROVIĆ V., RUŽIĆ-MUSLIĆ D., MIĆIĆ N., MILOŠEVIĆ-STANKOVIĆ I. (2015): Productive characteristics and body measurements of Alpine goats raised under smallholder production systems in central Serbia. Biotechnology in animal husbandry 31, 2, 245-253.
MAKSIMOVIĆ N., PETROVIĆ P.M., RUŽIĆ-MUSLIĆ D., CARO PETROVIĆ V., CEKIĆ B., LAZAREVIĆ M., STEFANOV R. (2017): Sheep and goat flock book and herd book record keeping in Central Serbia. Invited Paper. 11th International symposium ’’Modern trends in livestock production’’, October 11-13, Belgrade, Serbia. Proceedings, 90-98.
MAKSIMOVIĆ N., RUŽIĆ-MUSLIĆ D., CARO PETROVIĆ V., PANTELIĆ V., CEKIĆ B., ĆOSIĆ I., LAZAREVIĆ M. (2019): Performance records of autochthonous goats in Central Serbia. Biotechnology in Animal Husbandry 35, 4, 357-365.

MANCILLA-LEYTÓN J.M., PINO MEJÍAS R., MARTÍN VICENTE A. (2013): Do goats preserve the forest? Evaluating the effects of grazing goats on combustible Mediterranean scrub. Applied Vegetation Science 16, 63-73.

MILLER B., LU D.C. (2019): Current status of global dairy goat production: an overview. Asian-Australasian Journal of Animal Sciences 32, 8, 1219-1232.

NASTIĆ L., POTREBIĆ V. (2015): Sustainable Use of Resources in Sheep and Goat Production in Serbia and EU Countries. International Scientific Conference: "Sustainable Agriculture and Rural Development in Terms of the Republic of Serbia Strategic Goals Realization within the Danube Region: Regional specificities. December 10-11th, Belgrade, Serbia. Proceedings, 104-120.

PEREZ-NEIRA D., SOLER-MONTIEL M., GUTIÉRREZ-PEÑA R., MENA-GUERRERO Y. (2018): Energy Assesment of Pastoral Dairy Goat Husbandry from an Agroecologycal Economics Perspective. A Case Study in Andalusia (Spain). Sustainability 10, 2838.

RUIZ MORALES A.F., CASTEL GENÍS M.J., MENA GUERRERO Y. (2019): Current status, challenges and the way forward for dairy goat production in Europe. Asian-Australasian Journal of Animal Sciences 32, 8, 1256-1265.

RZS: Statistical yearbook of the Republic of Serbia, 2020. https://www.data.stat.gov.rs

RZS: Census on Agriculture 2012. https://www.data.stat.gov.rs

SREDOJEVIĆ Z., VUJIĆ T., JEVREMOVIĆ M. (2020): Economic indicators of goat breeding on family holdings in the Republic of Serbia. Economics of Agriculture 67, 4, 1297-1308.

VAN DIJK D. (1996): The production of artisanal and on-farm cheeses in Holland (La production des fromages fermiers et artisanaux en Hollande). In: FNEC (Ed). Proceedings of Fromagora, the national competition of goat cheese in France; Paris, France, 171-4.

ŽUJOVIĆ M., MEMIŠI N., IVANOVIĆ S. (2011): Present status, possibilities and perspective of development of goat production in republic of Serbia. Biotechnology in Animal Husbandry 27, 3, 431-443.

QUALITY OF ABORIGENOUS KARACHAY GOAT MEAT UNDER DIFFERENT CONDITIONS

Marina I. Selionova[1], Tatyana V. Mamontova[2], Ali-Magomet M. Aybazov[2], Violeta Caro Petrovic[3], Milan P. Petrovic[3]

[1]Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, 49, Timiryazevskaya st., Moscow, 127550 Russian Federation
[2]North Caucasus Federal Agrarian Research Centre,12, Zootechnical lane, Stavropol, 355002 Russian Federation
[3]Institute for Animal Husbandry, Autoput 16, Belgrade. 11080 Zemun, Serbia
Corresponding author: Marina Selionova, e-mail: m_selin@mail.ru
Invited paper

Abstract: In this study, the influence of different conditions of keeping and feeding on meat productivity and meat quality of indigenous Karachai goats kept in different geographical regions of the Karachay-Cherkess Republic was assessed. The experiment involved three populations of Karachai goats: Group I was kept at an altitude of 500-600 meters above sea level, II - 900-1000, III - 1600-2000 meters above sea level. There were no significant differences in slaughter indicators (P≥0.05). However, there was a tendency to a decrease in the pre-slaughter weight, the area of the muscle eye in carcasses obtained from goats of the foothill and mountain zones (on average by 5.15% and 5.55%), the meat content coefficient from the foothill zone (7.3%) and several a greater proportion of bones in the carcasses of goats in the foothill and mountain zones (on average by 3.9%). Morphological cutting of carcasses showed the predominance of muscle tissue over bone - 76.8% versus 23.2%. The data of microstructural analysis of the longest muscle of the back of Karachai goats showed a large number of muscle fibers (on average 404.5 pieces per 1 mm2 with their small diameter (on average 26.3 microns). Based on the assessment, it is concluded that the higher the height above the level the sea of the animal habitat, the lower the meat productivity and the lower the fat content in the meat.

Key words: Goat, Goat meat, Meat quality, Chemical composition

Introduction

Goats are one of the earliest domesticated ruminants. They are traditional sources of meat, milk, wool, fluff, sheepskin and other animal by-products. The unique abilities of goats, such as unpretentiousness in keeping and feeding, the ability to adapt to almost any natural and climatic conditions of the environment have made them widespread. Due to the variety of products obtained from them, goats have become the main source of resources and an integral part of the culture of many peoples of the world.

Also, goats are the most diverse species of livestock with pronounced morphological and physiological characteristics. To date, there are over 576 goat breeds registered in the world *(FAO, 2019)*. Most of the goats are native and perfectly adapted to breeding conditions. The exceptional ability of native goats to develop territories unsuitable for breeding other types of productive animals made them indispensable sources of milk and meat in mountainous, arid and inaccessible places with scarce vegetation.

In many developing countries, goat meat is an important food source. Despite the numerical importance of the livestock (807.6 million head worldwide), the consumption of goat meat (4.5 million tons) is low compared to the consumption of beef or poultry *(FAO, 2019)*. Countries with developed goat breeding have developed their own culture of goat meat consumption. For example, in North America and in western India, the so-called "Sabrito meat" is used. As the locals themselves say: "All cabrito is a goat, but not all goat is cabrito." This is a kind of veal in the goat world. Animals are fed only milk until 4-6 weeks of age and then slaughtered *(Exotic Meat Market, 2021)*. In Africa, the Middle East and the countries of South-West Asia, animals are killed for consumption at the age of 12-24 months with a carcass weight of 13-25 kg males, 11-20 kg females. Adult goat meat is prized in Africa and India (animals age 2-6 years, carcass weight 20-30 kg) *(Devendra and Owen, 1983)*.

As reported by *Stanisz et al. (2009)* the main lovers of young goat meat, with an increased demand during the Christmas and Easter periods, are concentrated in Greece, Italy, France, Spain and Portugal.

In Russia, goat breeding is not of great interest, especially meat. However, in several regions of the North Caucasian and Southern Federal Districts, the population of local Karachai goats is widespread. These animals perfectly combine all the valuable qualities of goats, plus they have good immunity and resistance to some diseases. At the same time, despite the fact that in some regions in some seasons of the year rather extreme weather conditions occur, Karachai goats, thanks to their unique characteristics, help to maintain production and are the main source of animal protein in the diet of local residents.

The aim of the study was to study the effect of different conditions of keeping and feeding on meat productivity and meat quality of indigenous Karachai goats kept in different geographic regions of the Karachay-Cherkess Republic.

Materials and Methods

The Animal Ethics Committee of the North Caucasus Federal Agrarian Center has approved the procedures outlined in this document. The studies were carried out in 2019-2020 on three groups of typical Karachai goats bred in different regions of the KChR. The research scheme is presented in Table 1. The conditions of keeping and feeding the animals were different depending on the group and the norms adopted in the farms.

Table 1. Experimental Scheme

Containment area	Height above sea level	Groups of animals		
		group number	quantity	quantity for slaughter
Prikubansky district	500-600 m	I	53	3
Zelenchuksky district	900-1000 m	II	33	3
Karachaevsky district	1600-2000 m	III	73	3

In November 2020, at the age of 8 months, 3 goats from each group were slaughtered.

The pre-slaughter live weight was determined by weighing the animals after 24-hour fasting with an accuracy of 0.1 kg. At the same time, access to water was limited 2-3 hours before slaughter. The slaughter was carried out through a transverse incision of the skin of the neck and by cutting the carotid artery and jugular vein. Immediately after gutting, the weight of the paired carcass was determined, after cooling at a temperature of 4 ° C for 24 hours, the weight of the cooled carcass was measured with an accuracy of 0.1 kg. Each carcass was divided into 2 longitudinal halves. Sampling of meat for research was carried out in pieces weighing at least 200 g from the incision, the area opposite the 4-5th cervical vertebrae, in the area of the shoulder blade, in the area of the thigh.

The varietal composition of the carcass was established on the basis of the cut of the carcass in accordance with GOST 7596-81. The morphological composition of the carcass was determined during deboning. The chemical composition of muscle tissue was determined by the average sample of the pulp of the carcass according to generally accepted methods, the mass fraction of moisture - according to GOST 9793-74, fat - according to GOST 23042-86, protein - according to the Kjeldahl method, ash - by burning a sample, meat pH - according to GOST R 51478-99, caloric content by calculation according to the formula of V.A. (1951). Histological assessment of meat was carried out in accordance with GOST 19496-93.

Statistical processing was carried out using Microsoft Excel 2016 (Microsoft, USA). Results were expressed as arithmetic mean ± standard deviation (Mean ± SD). To determine the statistical significance of the differences in the mean values, the Student's t-test was used under three conditions of probability "P" and different numbers of degrees of freedom.

Results

It was found that, on average, for three populations of Karachai goats, carcasses obtained from young animals had a mass of 13.4 kg, after cooling - 13.1 kg; the yield of cuts of grades 1 and 2, pulp and bones, respectively, amounted to 81.1 and 18.9%; 76.8 and 23.2%. The most significant indicators of meat productivity are the slaughter yield, the coefficient of meat content and the area of the muscle eye, which were 44.76%, 3.31 and 12.24 cm2, respectively. These indicators characterize carcasses as typical carcasses obtained from small ruminants (Table 2).

Table 2. Indicators of meat productivity of young goats in different breeding zones (8 months)

Indicator	Breeding zone			Average
	flat-hilly	foothill	mountain	
Pre-slaughter weight, kg	33.5 ±0.41	32.0±0.34	31.5±0.52	32.3±0.30
Carcass weight, kg	13.8 ±0.38	13.5±0.44	13.0±0.31	13.4±0.29
Internal fat mass, kg	1.05 ±0.09	1.10±0.12	1.0±0.11	1.05±0.07
Slaughter weight, kg	14.85 ±0.32	14.60±0.24	14.00±0.38	14.48±0.22
Lethal output,%	44.3	45.6	44.4	44.76
Chilled carcass weight, kg	13.5 ±0.31	13.2±0.29	12.8±0.39	13.16±0.21
Muscular eye area, cm2	12.72 ±0.21	12.11±0.11	11.90±0.26	12.24±0.18
Output, %				
cuts of 1 grade	80.6	81.0	81.7	81.1
2 grade cuts	19.4	19.0	18.3	18.9
pulp	77.4	76.0	77.0	76.8
bones	22.6	24.0	23.0	23.2
Meat factor	3.42	3.17	3.34	3.31

Results are presented as mean ± SD

Chemical analysis of the average sample of muscle tissue showed that the protein content was 19.6, fat - 9.7, dry matter - 30.3, ash - 1%, which characterizes meat as a product with a low fat content and allows it to be classified as a dietary category.

Comparison of the parameters of goat carcasses from different breeding zones did not reveal significant differences. It should be noted a tendency for a decrease in the pre-slaughter weight, the area of the muscle eye in carcasses obtained from goats of the foothill and mountain zones (on average, by 5.15% and 5.55%), the meat content coefficient from the foothill zone (7.3%) and a slightly higher specific the weight of bones in the carcasses of goats in the foothill and mountain zones (on average by 3.9%). However, in general, this did not affect the slaughter yield, which was at the level of carcasses obtained from goats from the flat-hilly area (45.6 and 44.4 versus 44.3%, respectively).

No significant differences were found in the indicators of the chemical composition of meat. There was a slight decrease in the amount of fat and calorie content in carcasses obtained in the foothill and mountain zones, respectively by 0.7 and 1.5 abs. percent, 2.3 and 6.7%. The established low calorie content for the meat of experimental animals - 1703.9 kcal, testifies to the high modern consumer (dietary) properties of goat meat (for reference: the calorie content of 1 kg of broiler meat is 1800 kcal, turkeys - 1940 kcal, veal - 970 kcal) (Table 3).

Table 3. Chemical composition of young goat meat in different breeding zones (8 months)

Indicators	Breeding zone			Average
Content,%	flat-hilly	foothill	mountain	
Moisture	69.3±0.67	69.5±0.72	70.2±0.84	69.7±0.47
Dry matter	30.7±0.34	30.5±0.35	29.8±0.43	30.3±0.25
Fat	10.4±0.18	9.7±0.26	8.9±0.21	9.7±0.16
Ash	1.1±0.11	1.0±0.09	1.0±0.12	1.0±0.08
Squirrel	19.2±0.34	19.8±0.43	19.9±0.62	19.6±0.28
Caloric content of 1 kg of pulp, kcal	1754.2±2.55	1713.9±4.77	1643.6±3.63	1703.9±2.52

Results are presented as mean ± SD

Morphometric analysis at the histological level of the longest back muscle made it possible for the first time to determine in Karachai goats the number of muscle fibers per square millimeter, their diameter, the content of connective tissue, which, respectively, amounted to 404.5 pcs., 26.3 μm and 8.47% (Table 4).

Table 4. Microstructural analysis of the longissimus dorsi muscle (m. Longissimus dorsi) of young goats in different breeding zones (8 months)

Indicators	Breeding zone			Average
	flat-hilly	foothill	mountain	
Number of muscle fibers, pcs. per mm2	398.7	402.4	412.5	404.5
Muscle fiber diameter, μm	25.2	26.1	27.8	26.3
Marbling score, point	24.4	22.6	20.8	22.6
Connective tissue content,%	7.8	8.7	8.9	8.47

The assessment of "marbling", determined according to the original method developed by the staff of the All-Russian Research Institute of Sheep and Goat Breeding for Sheep and which is based on the registration and branching of fatty interfiber and interbeam inclusions made it possible to establish an assessment of 22.6 points out of 40.0 possible. Comparison of the obtained results with similar ones for sheep showed that goats per square millimeter have 5.39% more muscle fibers with their diameter smaller by 12.0%, which can be interpreted as a result indicating that goat meat is more tender. At the same time, due to the lower content of fat inclusions, goat meat received 8.1 points less marbling than sheep meat. However, it is possible for goats to develop their own scale for assessing "marbling" and this requires a much larger amount of experimental data and their correlation with the tasting assessment of goat and sheep meat.

Discussion

In our research, we state that carcasses obtained from 8-month-old Karachai goats can be characterized as typical for small ruminants. However, *Sheridan et al. (2003a)* argues that, compared to sheep of the same age and sex, goat carcasses are smaller and have less covering fat. Differences in the quality of goat meat depend on its physical and chemical properties *(Webb et al., 2005)*, biological factors such as age, sex and breed of the animal, and non-biological factors such as pre-slaughter stress, slaughter techniques, chilling and freezing of carcasses *(Leo et al., 2020)*. The physiological state of a living animal and the biochemistry of postmortem processes in muscles, fat, and connective tissue also directly affect the taste of meat *(Mamontova, 2012)*. Nutrition also influences the quality of meat through species-specific flavonoids from feed sources, because the taste of meat is due to the ratio of fatty acids and the ratio of muscle to fat. Thus, most researchers agree that determining the quality of meat is multifaceted and difficult.

The percentage ratio of pulp to bones obtained by us (76.8% versus 23.2%) with morphological cutting of the carcass with a large predominance of muscle tissue can be explained by the rather young age of the experimental animals. The same conclusion was reached by *Santos et al. (2008)* and *Stanisz et al. (2009)*. They state that the carcasses of young goats contain a high percentage of muscle mass and a low percentage of bone tissue, intramuscular and subcutaneous fat.

The data of microstructural analysis of the longest muscle of the back of Karachai goats indicate a large number of muscle fibers and their small diameter in comparison with sheep, which allows us to conclude that the meat of goats is more tender. However, *Webb et al. (2005)* believe that goat meat is less tender than sheep meat. At the same time, tenderness decreases with increasing age of slaughter. The nature of the decrease in the tenderness of meat with age can be explained by the function of collagen in the body of the animal - the collagen molecule becomes less soluble due to covalent cross-links between collagen fibers. *Yarmand and Homayouni (2010)* in studies of microstructural analysis of muscle fibers of goats found that the fibers run parallel to each other, the collagen fibers surrounding the muscle fiber are fuzzy, the surfaces of myofibrils seem to be normal. At the same time, the authors do not give a conclusion about the relationship between the microstructural structure and the tenderness of meat and do not indicate the breed used.

A lower fat content as a result of chemical analysis of the meat of Karachai goats was found in animals from group III (highlands) compared to the rest. This can be explained by a more active motor ability, in connection with the conditions of detention and more scarce forage resources. Our hypothesis is confirmed by colleagues from Brazil who have carried out work on Brazilian native goats. They explain the changes in fat content in animals with an association with the balance between dietary energy and nutritional requirements of goats *(Lopes et al., 2014).*

Conclusions

Different conditions of detention did not reveal large differences in the quality of meat and meat productivity of Karachai goats. Comparison of morphometric parameters of muscle tissue in animals from different breeding zones showed that the higher the height above sea level of the area of their content, the greater the number of muscle fibers per unit area, respectively, with a smaller diameter and less fat inclusions. It is possible to further study the quality of the meat of Karachai goats with a detailed analysis of the profile of fatty acids, which will make it possible to give a clearer characterization of the qualitative analysis of meat.

Kvalitet mesa autohtone rase koza karačaj pod različitim uslovima

Marina I. Selionova, Tatyana V. Mamontova, Ali-Magomet M. Aybazov, Violeta Caro Petrovic, Milan P. Petrovic

Rezime

U ovom istraživanju rađena je procena uticaj različitih uslova držanja i ishrane na mesnatost i kvalitet mesa autohtonih karačaj koza koje se drže u različitim geografskim regionima Karačajsko-čerkeške republike. U eksperiment su bile uključene tri populacije karačaj koza: I grupa je držana na 500-600 metara nadmorske visine, II grupa na 900-1000, III grupa na1600-2000 metara nadmorske visine. Nije bilo značajnih razlika u klaničnim pokazateljima (P≥0,05). Međutim, postojala je tendencija smanjenja težine pred klanje, površine mišića u trupovima dobijenih od koza iz zona podnožja planina i planinskih zona (u proseku za 5,15% i 5,55%), koeficijenta sadržaja mesa iz zona podnožja planina (7,3%) i nekoliko puta veći udeo kostiju u trupovima koza u zonama podnožja planina i planinskim zonama (u proseku za 3,9%). Morfološko rasecanje trupova pokazalo je prevagu mišićnog tkiva nad kostima - 76,8% naspram 23,2%. Podaci mikrostrukturne analize najdužeg mišića leđa karačaj koza pokazali su veliki broj mišićnih vlakana (u proseku 404,5 po 1 mm^2 sa njihovim malim prečnikom (u proseku 26,3 mikrona). Na osnovu procene zaključuje se da što je veća nadmorska visina životinjskog staništa, manja je produktivnost mesa i niži sadržaj masti u mesu.

Ključne reči: koza, kozje meso, kvalitet mesa, hemijski sastav

Acknowledgments

The research was supported by a grant from the Russian Science Foundation (project No. 19-76-20006).

References

DEVENDRA C., OWEN J.E. (1983): Quantitative and qualitative aspects of meat production from goats. World Anim. Rev., 47, 19-25.

Exotic Meat Market (2021): // URL: https//www.exoticmeatmarkets.com (retrieved on 15.04.2021).

FAO. (2019): The State of the World's Biodiversity for Food and Agriculture, J. Bélanger & D. Pilling (eds.). FAO Commission on Genetic Resources for Food and Agriculture Assessments. Rome. 572 pp. // URL: http://www.fao.org/3/CA3129EN/CA3129EN.pdf.

GIGLI S., SALTALAMACCHIA F. (2007): Goat meat quality characteristics and affecting factors. Proceedings of the International Symposium, The Quality of Goat Products: Models and Tools for Evaluation and Promotion, CRA-ZOE, Bella, Italy, 97-104.

LEO N., OBERT C., CHENAIMOYO L.F., MUNYARADZI C., ADEYEMI O., CLETOS M. (2020): Sericea lespedeza (Lespedeza juncea var. sericea) for sustainable small ruminant production: Feed, helminth suppressant and meat preservation capabilities. Animal Feed Science and Technology, 270, 114688, doi: 10.1016/j.anifeedsci.2020.114688.

LOPES L.S., MARTINS S.R., CHIZZOTTI M.L., BUSATO K.C., OLIVEIRA I.M., MACHADO NETO O.R., PAULINO P.V.R., LANNA D.P.D., LADEIRA M.M. (2014): Meat quality and fatty acid profile of Brazilian goats subjected to different nutritional treatments. Meat Science, 97, 4, 602-608, doi: 10.1016/j.meatsci.2014.03.005.

MAMONTOVA T.V. (2012): Продуктивные, конституциональные и биологические особенности карачаевских коз в разных условиях содержания. Кандидатская диссертация. Ставропольский научно-исследовательский институт животноводства и кормопроизводства, Ставрополь.

SANTOS V.A.C., SILVA S.R., AZEVEDO J.M.T. (2008): Carcass composition and meat quality of equally mature kids and lambs. Journal of Animal Science, 86, 1943-1950.

STANISZ M., ŚLÓSARZ P., ADAM G. (2009): Slaughter value and meat quality of goat kids with various share of Boer blood. Animal Science Papers and Reports, 27, 189-197.

WEBB E.C., CASEY N.H., SIMELA L. (2005): Goat meat quality. Small Ruminant Research, 60, 153-166.

YARMAND M.S., HOMAYOUNI A. (2010): Quality and microstructural changes in goat meat during heat treatment. Meat Science, 86, 2, 451-455, doi: 10.1016/j.meatsci.2010.05.033.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

THE IMPORTANCE OF EGGS IN THE DIET, CONSUMER PREFERENCES, THE PRODUCTION AND MARKET OF TABLE EGGS IN SERBIA

Nataša Tolimir[1], Zdenka Škrbić[2], Marijana Maslovarić[1], Miloš Lukić[2] , Dragan Milić[3]

[1]Institute for Science Application in Agriculture, Boulevard of Despot Stefan 68b, 11000, Belgrade, Republic of Serbia
[2]Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Republic of Serbia
[3]Erber Biotech doo, Neimarova 28, 21000 Novi Sad, Republic of Serbia
Corresponding author: Nataša Tolimir, ntolimir@ipn.bg.ac.rs
Invited paper

Abstract: Nutrition is very important for maintaining the good health of people at all stages of life and as such should be the subject of interest not only for nutritionists, but also for each individual. The choice of food stuffs in the diet is significantly influenced by habits, and in recent decades it has been noticed that consumers are increasingly concerned about the way food is produced, its safety and harmlessness. Although eggs, as a food, have an excellent nutritional composition, they have had a bad reputation for decades due to the high content of cholesterol, which was considered bad, which is why many consumers avoided them or ate only egg whites. The results of research indicating that cholesterol in food such as eggs has an insignificant effect on blood cholesterol have contributed to solving the problem of cholestephobia, so that official nutrition organizations, around year 2000, recommended the abolition of restrictions on egg consumption. Egg consumption and method of preparation vary significantly among different countries, which may be related to dietary traditions and consumer preferences. During the last decades, the attitudes of consumers are gaining in importance and are being studied in relation to the characteristics of egg quality, according to production systems, the welfare of chickens, etc. A comprehensive view of consumers' perceptions, their habits and understanding of the role of eggs in the diet requires a systematic approach, at the level of the entire social community, including education, which is especially important for younger populations. The aim of this paper is to point out the importance of eggs in the diet, to show the situation in the sector of table egg production in Serbia, with reference to EU countries, including expected changes caused by changes in the legislation, which may have far-reaching consequences for this sector, consumers and the market situation.

Key words: nutrition, eggs, welfare, rearing systems, consumer preferences

Introduction

Eggs as a food stuff play a significant role in the diet from early childhood to old age. Although they are a high-value biological food, attitudes about the role of eggs in human nutrition have changed several times over the last decades. Egg confusion results from a cholesterol content of 200 to 300 mg per 100g (*Miranda et al., 2015*), or an average of 141 to 234 mg per egg, which is about two-thirds of the recommended daily limit of cholesterol intake, which several a decade ago was 300 mg/day (*Clayton et al., 2017*). Cholesterophobia, at one time, was primarily related to consumer fear that consuming eggs could lead to coronary heart disease, which could be linked to a decline in egg consumption (*Ruxton et al., 2010; Kuang et al., 2018*). Only after scientific studies established that cholesterol in foods such as eggs had only a small and clinically insignificant effect on blood cholesterol, and that egg consumption was not associated with an increased risk of cardiovascular diseases (*Qureshi et al., 2007; Gray and Griffin, 2009*), official nutrition organizations have recommended lifting restrictions on egg consumption. In 2002, the American Heart Association (AHA) withdrew a recommendation to limit egg consumption to 3 to 4 per week. In 2015, the Dietary Guidelines Advisory Committee (DGAC) officially withdrew its recommendation to limit cholesterol intake to 300 mg per day, as there was no evidence of a link between dietary cholesterol and blood serum cholesterol (*Carson et al., 2020*). However, as the dietary recommendations for Americans for the period 2015-2020 are such that it is recommended to consume as little cholesterol as possible, while respecting healthy eating habits, but that cholesterol is not an ingredient that, if consumed excessively, does represent a concern, one gets the impression that confusion related to cholesterol intake to some extent still remains (*Zhuang et al., 2021*).

Egg production and consumption vary significantly and from country to country. Production in the last few years, at the world level, according to FAO data, has recorded a steady increase (*FAOSTAT, 2015, 2016, 2017, 2018, 2019*). After China, the European Union is the world's largest producer of table eggs (*Augére Granier, 2019*). Among the largest producers in Europe are the Russian Federation, Ukraine, France, Spain, Germany, the United Kingdom, Italy, the Netherlands and Poland, and of the surrounding countries Romania, Hungary and Bulgaria. Serbia is on the first place among the countries of the Western Balkans in terms of egg production, and in 2019 it was 17th in Europe (*FAOSTAT, 2017, 2018, 2019*). In Serbia, egg production in 2019 amounted to 7,600 tons, or 1,775,000 pieces, according to the Statistical Yearbook (*Statistical Office of the Republic of Serbia, 2020*). Egg consumption per capita on an annual basis, according to IEC (*2018*) data, is the highest in Mexico (368) and Japan (337), followed by China (255), Spain (273) and Denmark (248). The average consumption of eggs in EU countries in 2018 was 210, and in the USA 287.5 eggs per capita (*IEC, 2018; Shahbandeh, 2021*).

Egg consumption in Serbia in 2007 was about 8 kg, or 135 eggs, while in the same year in Europe it was 12 kg or 200 eggs per capita, and in the United States 14 kg or 240 eggs (*Milošević and Perić, 2011*). In Serbia, egg consumption recorded a slight increase in 2013 (*Zlatanović, 2015*) and amounted to 222 eggs per household member, which is more compared to countries in the region, in Macedonia 168, in Croatia 153 and in Slovenia 76 eggs. According to the Household Budget Survey for the period from 2015 to 2019, the average consumption of eggs per capita in Serbia was 219.4 (*Statistical Office of the Republic of Serbia, 2015, 2016, 2017, 2018, 2019*). The reason for the still lower consumption of eggs in Serbia compared to some developed countries may be the continued presence of cholesterol phobia, given that according to a survey by *Tolimir et al. (2016)*, 18.66 % of consumers cite fear of high cholesterol as an egg deficiency, which opened the question, i.e. the need for better information of consumers. In the coming period, poultry meat and eggs are recognized as the leading foods in the diet, since their production is up to 50% cheaper compared to the same amount of protein from the meat of other domestic animals, which makes them available to the population, regardless of material status (*Milošević and Perić, 2011*).

The table egg production sector, in the last two decades, is facing various challenges due to changes in legislation and numerous regulations in the field of food safety, ecology, poultry welfare, sustainability of production, as well as socio - economic changes. Major changes in this sector have been brought about by the EU Directive 1999/74/EC, which has banned, starting from 2012, the rearing of laying hens in classic conventional cages. This complex process is influenced by a large number of factors and takes place differently in individual EU countries. Serbia has a legal framework harmonized with EU regulations, and the process of transition from conventional cage systems for raising chickens to permitted systems is slow. According to the research of *Tolimir et al. (2020)*, in the transition period (2014-2020), about 85% of producers failed to harmonize their production with regulations, which led to the extension of the deadline.

Increased consideration of consumer attitudes has led to an increase in research, mainly surveys and studies focused on consumer preferences of table eggs (*Guyonnet, 2012; Mizrak et al., 2012*, *Kralik et al., 2014*, *Tolimir et al., 2016*, *Zelić et al., 2016*). Also, changes in the egg production sector, conditioned by legislation, have opened up a number of questions regarding consumer attitudes about animal welfare (*Verbeke, 2009; European Commission, 2007; de Roest et* *al., 2010; Hansstein, 2011*). Research indicates that European consumers generally have similar attitudes towards domestic animal welfare and agree that welfare needs to be raised to a higher level (*Blokhuis et al., 2008; Alonso et al., 2020*). However, there are differences between individual countries (*European Commission, 2005; Martelli, 2009*) which are a consequence of different levels of knowledge and awareness of consumers about animal welfare, i.e. about farming systems and different willingness to allocate more funds for purchasing products obtained by welfare principles. The contribution to raising awareness of the importance of food production according to the welfare principles and the impact of animal welfare on the quality of animal products could be achieved through better information and education of consumers (*Autio et al., 2017*).

The aim of this paper is to point out the very important role of eggs in the diet and to contribute to overcoming the problem of cholestephobia, to show the situation in the sector of egg production in Serbia and EU countries, cuased by changes in legislation related to poultry welfare, including consumer preferences and the situation on the market in Serbia.

The importance of eggs as food stuff

The human nutrition has always been important - Hippocrates, a Greek philosopher and physician, who is considered the founder of medicine as a science, said: "Let food be your medicine, and medicine be your food". Eggs, as a food stuff, have a wide application, due to their great nutritional value and numerous possibilities of use in the preparation of various salty and sweet dishes. In addition to differences among countries in the consumption of eggs as food stuff (*Magdelaine, 2011*), there are also differences in the way of preparation, i.e. consumption of eggs, which are most often conditioned by the tradition of diet and habits in preparing eggs. *Tolimir et al. (2016)* indicate a certain similarity between Serbia and the surrounding countries, i.e. Croatia, and differences in relation to Turkey. In order to fully understand the importance of eggs as a food stuff in the diet, which could affect the increase in egg consumption in Serbia, which is lower compared to some developed countries, it is necessary to educate consumers in several different areas, i.e. the nutritional value of eggs, the role eggs within a balanced, healthy diet, the importance of properly combining eggs with other foods, as well as the impact of heat treatment on nutritive value (*Farjami et al., 2021*).

The egg contains almost all the nutrients needed by man and as such is considered important in the diet, during all life stages. It has the same ratio of egg yolks, egg whites and shells, as the basic parts, regardless of the breed, age of the hens and nutrition (*Milošević and Perić, 2011*). The egg has a high protein content, as the basic building blocks necessary for the growth and regeneration of all living cells in the body (*Pal and Molnar, 2021*). The egg proteins are among the most biologically valuable because they are easily digestible and therefore serve as a standard for measuring the quality of protein in food (*Sakanaka et al., 2000*). The amino acid composition of egg proteins is favourable, i.e. they contain all the essential amino acids necessary for the organism (*Lesnierowski and Stangierski, 2018; Wang et al., 2018*). In addition to being an excellent source of first-class proteins, eggs contain significant amounts of vitamin A, D, E, K and B complexes, especially riboflavin, biotin, vitamin B12, folic and pantothenic acid, minerals, especially iron, zinc, calcium, phosphorus and sodium (*Anton et al., 2005; Lopéz-Fandino, 2007),* also bioactive and antimicrobial compounds, such as phospholipids, immunoglobulin, fosvitin, lysozyme and cystatin (*Lesnierowski and Stangierski, 2018*). Also, eggs are foods of moderate caloric value (about 150 kcal/100 g) and their regular use is a healthy eating habit, because they provide satiety and can contribute to weight loss (*Bertechini and Mazzuco, 2013; Miranda et al., 2015*).

The role of eggs, as high-value food stuff, in maintaining good health is indicated by a large number of researches. Eggs are one of the best sources of lutein, a pigment that enables better vision, and is especially important for slowing down some degenerative processes that can affect the eyes, i.e. reduce the risk of cataracts and macular degeneration (*Goodrow et al., 2006*). In terms of vision, eggs are also rich in vitamin A, which, according to the World Health Organization (WHO), is a public health problem, especially in developing countries, where it is the most common cause of blindness in children (*Mendonça Jr et al., 2002; WHO, 2009)*. Also, egg is an excellent source of choline, one egg contains more than 100 mg of this very important nutrient used to build cell membranes and plays a role in the production of signalling molecules in the brain (*Penry and Manore, 2008; Caudill, 2010*). Eggs contain LDL or "bad" cholesterol, but also HDL or "good" cholesterol, which provides strong protection against LDL cholesterol in the arteries, i.e. from oxidative damage induced by free radicals (*Kosmas et al., 2018*). According to one study, consuming 2 eggs a day for 6 weeks resulted in a 10% increase in HDL levels (*Scänohr et al., 1994*). Although recent research indicates the unfounded fear of cholesterol, and opinion that eggs can be a key element of a healthy diet, a number of consumers still view eggs with suspicion (*Tolimir et al. 2016; Ahnen and Slavin, 2019*). It is crucial to inform consumers that according to research, dietary cholesterol and blood cholesterol are only weakly related (*McNamara, 2000*) and that based on these studies, recommendations are given by official health organizations to remove restrictions on egg consumption in healthy people, except in people with diabetes (*Carson et al., 2020; Qureshi et al., 2007;*

Guo et al., 2018). According to *Lopez-Sobaler and Gonzalez-Rodriguez (2015)*, omitting eggs in the diet would be unnecessary and undesirable.

So called „enriched eggs“, which belong to functional foods, can have particularly positive impact on human health, and even in the treatment of some diseases, (*Perić et al., 2011*), and which, in addition to their basic nutritional function, have some additional substance or some substances that are normally contained in the egg are increased to levels that can have a positive effect on health. The goal of increasing the levels of omega 3 fatty acids in eggs is to meet the daily human requirements for this ingredient by eating one egg (*Grashorn, 2005*). Studies indicate that the consumption of omega - 3 eggs can lead to improved lipid status, i.e. lowering levels of triglycerides, total and LDL cholesterol, and increasing HDL cholesterol levels in the blood, and thus to protection against atherosclerosis and lower blood pressure (*Yannakopoulos et al., 2005; Bovet et al., 2007; Shakoor et al., 2020)*. Selenium-enriched eggs, which also belong to functional foods, are also important in the diet (*Perić et al., 2009, Tolimir et al., 2012*), especially bearing in mind that there is a selenium deficiency in the soil. The addition of selenium must be carefully dosed, because this element in higher concentrations is harmful to human health (*Surai, 2002*), and when enriching eggs, it is recommended to use organic forms of selenium, which are better absorbed (*Tolimir et al., 2012*). It is recommended that eggs enriched with selenium contain no more than half of the recommended human daily needs, i.e. 35 μg Se (*Yaroshenko et al., 2004*), which according to *Fisinin et al. (2008)* can be achieved by introducing 0.3 to 0.5 ppm of organically bound selenium into feed for layer hens.

Situation in the production of table eggs and expected changes

According to FAO data, egg production, worldwide, is growing steadily and in 2019 amounted to over 83 million tons of eggs (*FAOSTAT, 2019*). In recent decades, the egg production sector has faced a number of challenges, among which changes in legislation related to poultry welfare, food safety and the environment protection have a major impact, to which the growing demands of consumers can be added. In the European Union, the implementation of legislation, i.e. EU Directive 1999/74/EC which has banned the rearing of laying hens in classic conventional cages in all EU countries since 2012, has varied in individual countries, under the direct influence or consequence of interaction between producers, retailers, consumers, lawmakers, the media, and public pressure in general (*Appleby, 2003; Frewer et al., 2005*). By looking into the structure of production, and depending on the rearing system, it can be stated that out of the total 365 million laying hens in the European Union in 2019, the largest share of poultry is still in the cage system, i.e. in "enriched" cages about 49.50%, approx. 32.5% in the floor system, 11.8% in the so-called "Free range" or free keeping system and the least is in the organic production system, about 6.20% (*European Commission, 2020*), with a tendency to increase the percentage of individuals from "non-caged" systems (*Committee for the Common Organization of the Agricultural Markets, 2017*). Enriched cages are still dominant in EU Member States from Eastern, Central and Southern Europe, while they are very underrepresented in Northern and Western European countries (*Kollenda et al., 2020*).

The abolition of cage systems proceeded with different dynamics in the EU countries. Some countries, such as Belgium, which had a deadline of 2012, have been slow to adopt alternative chicken farming systems (*Tuyttens et al., 2011*), while some members have decided to go beyond EU standards by introducing stricter national or regional laws (*Van Horne and Bondt, 2017*). Strategies for waiting and producer requests for extensions, in some countries, have proven to be a poorer choice in relation to acceptance and quicker adaptation to new conditions, and *Rodić et al. (2014)* indicate that it is not realistic that the rules for Serbia could be different, if we take into account how the process took place in EU countries and considering the persistence of decision makers.

In Serbia, adaptation to European standards (*Directive 1999/74/EC*) is related to the Law on Animal Welfare *(Official Gazette of the Republic of Serbia No. 41/2009*) and the Rulebook on rearing conditions (*Official Gazette of the Republic of Serbia No. 6/10; Official Gazette of the Republic Of Serbia No. 57/2014-27*). The process of abandoning the conventional battery system and switching to permitted systems is slow, with extensions of deadlines on several occasions, and given that the changes are major, with high costs, they can be considered a danger to the egg production sector in Serbia. In the last two decades, it can be stated that the number of laying hens has decreased, which according to the data from 2019 is lower by 11.11% compared to the 2008-2017 average. The negative trend can also be related to the inability of producers to meet the new high requirements of regulations in Serbia, harmonized with EU regulations (*Krnjaić, 2019*). According to the results of *Tolimir et al. (2020),* until the fall of 2020, when the previous deadline for abandoning of production in conventional cages was, only 16.3% of producers switched to permitted systems, of which 2% in full and 14.3% in part. Similar results are part of a study conducted in 2019, according to which it is estimated that only about 15% of conventional cage equipment has been replaced in Serbia (*Krnjaić, 2019*). This situation resulted in a new extension of the deadline for abandoning of the conventional cage system, until the end of 2023, at the request of the producer, i.e. the Group for the production of poultry meat and eggs.

In the coming years, major changes are expected in the egg production sector in Serbia, with a far-reaching impact on the primary production of table eggs, which will be reflected in the market. The threat to the self-sufficiency of production, i.e. the danger of a deficit of table eggs in Serbia may be, on the one hand due to the reduction in the number of hens, caused by the transition to systems complying with poultry welfare, and on the other hand due to producers forced to leave production, mainly due to high initial cost for the procurement of equipment, which according to the research of *Tolimir et al. (2020)* accounts for about 20%. Knowledge of the attitudes of manufacturers is important for monitoring and directing the entire process of implementing regulations. According to the research in 2020 (*Tolimir et al., 2020*), all surveyed producers in Serbia (100%) have enriched cages as a choice when switching from conventional cages, with 65.11% of them believing that switching to enriched cages will not affect the welfare of layer hens. The same authors, during the survey, got the impression that producers would not switch from the classic cage system, if they were not forced due to legal regulations, which was also established by *Stadig et al. (2016),* who state that only 8.2% of the surveyed egg producers would switch from a battery system to one of the alternative systems of laying laying hens if it was not required by law. *Tuyttens (2011)* indicates that the problem of non-acceptance was more pronounced in older farmers. In Serbia, the age structure of surveyed producers, based on surveys in 2020, is as follows: 50% are aged 56 to 65, 26.09% are 46 to 55, 15.22% are 36 to 45 and 8.7% of the youngest category, with about 50% of producers stating that they have a successor in their business (*Tolimir et al., 2020*).

One of the key issues in the implementation of regulations, i.e. for further production of table eggs in Serbia, is decision of the producer on the rearing system, from the group of permitted systems - enriched cages, alternative systems, free range and organic production. The choice of producers in Serbia may also be influenced by the outcome of a civic initiative launched in Europe, called "End the Cage Age", which calls for the abolition of cage systems for keeping laying hens, for which over a million signatures have been collected, presented to the European Parliament in 2019 and discussed in the European Parliament in 2021 (*European Parliament, 2021)*. According to the research *of Tolimir et al. (2020*), 61.7% of producers in Serbia were aware of this initiative, while the awareness of producers with smaller farm capacities was lower. Also, for the choice of rearing system, the experience of producers who have already implemented legislation is important, based on which it can be determined whether legally imposed changes in the rearing environment really result in improving the welfare of laying hens in practice (*Tyttens et al., 2011*).

It is important to consider the research results to make a decision on the choice of rearing system, and according to the available literature, some scientists are not convinced that the welfare of laying hens is better in alternative systems compared to battery/tier system (*Duncan, 2001*), but the fact is that both systems have advantages and disadvantages. The results of the research indicate that laying hens in non-cage systems have a greater possibility to show their natural behavior, but also increased risk of injuries, parasites, diseases, predators in relation to cage systems (*Laing, 1988*). Since the adoption of regulations in Serbia, scientists have repeatedly (*Pavlovski et al., 2011; Rakonjac, 2016*) pointed out the importance and need, for more efficient implementation of expected changes in the egg production sector, to focus attention to scientific research and application of new, alternative poultry farming systems, for laying hens and poultry welfare. *Đokovic et al. (2018)* indicate that in Serbia, for the process of implementing legislation related to the transition from conventional cages to permitted systems of hen rearing, a synergistic action of the state through financial support programs would be necessary, on the one hand and scientific institutions on the other hand, through scientific -professional teams in several fields, including investment design, development of solutions for the construction of new and reconstruction of existing farms and monitoring of production performance indicators from several aspects. In Serbia, the government, as one of the measures to support producers, for the transition of production to permitted systems, has enabled the use of IPARD funds, since 2020 (*IPARD program, Serbia, 2021*).

Consumer preferences and market conditions

Studies with consumers of table eggs are mainly surveys/questionnaires and refer to consumer preferences regarding certain characteristics of egg quality, as well as factors that influence consumer choice when buying (*Kralik et al., 2014; Mizrak et al., 2012; Tolimir et al., 2016; Zelić et al., 2016*). The research results indicate that consumers are more and more demanding, with specific requirements in terms of production of eggs with special properties (functional food), welfare of domestic animals, food safety, etc. Research on consumer preferences indicates that in relation to the characteristics of egg quality, attention is primarily on egg weight, shell colour and yolk colour, egg white quality and the absence of meat and blood stains (*Tolimir et al., 2016*), contrary to producers who primarily focus on egg mass and shell quality, as prerequisites for good prices and placement. According to the study by *Tolimir et al. (2017*), freshness of eggs is very important to consumers in Serbia (73.28%), they prefer to buy larger eggs (classes SS, S and A are the choice for 69.82%) and prefer eggs of extremely yellow colour (62.76%). In the previous period, consumers in Serbia preferred eggs of a more intense yellow colour, i.e. (56.5%) prefer the yellow colour of the yolk (up to 9 Roche units), and 27% prefer a dark yellow color (more than 9 Roche units) (*Pavlovski* *and Mašić, 1994*). Also, in most EU countries, a more intense (darker) yellow colour is valued (*Parrott et al., 2013; Hernandez et al., 2005*). Consumer interest in egg safety and quality is on the rise and the literature indicates that in a number of European countries (France, Germany, Italy, UK, Spain, Poland and Greece), consumer safety and egg freshness are the most important factors, and from the point of sensory traits of egg quality, strong shell, egg white consistency and yolk colour are characteristics that are especially appreciated by consumers *(Hernandez et al., 2005; Hernandez, 2006)*.

Examining consumer attitudes about the production system in obtaining products of animal origin is of great importance because together with sensory characteristics, impact on human health and ease of preparation, they are among the four most relevant criteria in consumer choice for products (*Grunert et al., 2000). Stadig et al. (2016)* points out the significant influence of consumers attitudes when producers are deciding on rearing systems. Analysing the results of consumer surveys on the welfare of laying hens (*Bejaei, 2009; Vecchio and Annunziata, 2011; Kehlbacher et al., 2012; Heng et al., 2013; Mulder and Zomer, 2017)*, differences between respondents can be found depending on gender, education, regions in which they live, i.e. whether it is an urban and rural environment, and other socio-demographic differences. According to the *European Commission (2005)*, there are differences between countries and in the north of Europe greater importance is attached to welfare compared to the countries of the south and the newly acceded EU member states, which may be due to differences in levels of knowledge and willingness to allocate more resources to products obtained according to welfare principles. In Serbia, there are, but few studies on the attitudes of consumers of table eggs on the welfare of poultry/laying hens (*Rodić et al. 2010; Stojanović et al., 2014, Tolimir et al., 2019a*). Comparing data with research in earlier periods in Serbia (*Pavlovski et al., 2011*), there is a decrease in the share of consumers for whom the cage system is acceptable in egg production, from 70.6% to 35.6% in the period from 1981 to 2001, which is in favour of changing consumer attitudes. The attitude of consumers of table eggs in Serbia differed depending on the influence of gender, education, age and number of children in the family (*Stojanović et al., 2014; Tolimir et al., 2019a*).

Welfare and rearing systems are closely related, and according to *Tactacan et al. (2009)* one of the factors that led to the development of improved production systems is the concern for welfare and the desire for laying hens to show their natural behaviour. According to the research of *Tolimir et al. (2019a)*, the same group of respondents attach greater importance to the welfare of domestic animals (50.77%) compared to the rearing system (39.49%), which raises the question of whether consumers associate the rearing system with the welfare of laying hens and understand their connection. Also, some authors who have examined consumer attitudes about welfare point to a lack of consumer knowledge about welfare standards and its relationship to product quality (*Nocella et al., 2010*). *Autio et al. (2017)* also point to the need for consumers to be better informed and educated about the importance of welfare of domestic animals, which would also include rearing systems. Also, *Binnekamp and Ingenbleek (2006)* point to the lack of consumers' full understanding of the concept of animal welfare and the lack of information on the quality of these products, and recognize this as market barriers for products produced according to welfare standards.

One of the issues related to the production of food of animal origin in accordance with welfare standards is the willingness of consumers to allocate more funds for products obtained with respect to welfare. According to a large number of studies, consumers have expressed this readiness (*Nocella et al., 2010; Heng et al., 2013; Stojanović et al. 2014*), which is of great importance given that the application of welfare standards results in increased production costs, also, as stated by *Rodić et al. (2010)*, eggs from non-caged systems can be competitive only if there is a willingness of consumers to pay a higher price for eggs produced in this way. The publication *European Commission (2005)* it is stated that 81% of consumers in the EU express their readiness to allocate more funds for eggs produced according to the principles of welfare, for a price higher than 5 to 10%. According to the results of research in Serbia, *Pavlovski et al. (2011)* indicate that the share of consumers who are willing to pay a higher price for eggs from non-battery systems increased from 46 to 71.5%, and *Rodić et al. (2010)* state that the willingness of consumers to pay more for eggs from non-cage systems exists, but it is up to 20% more money. According to the research by *Tolimir et al. (2019a)*, the share of consumers in Serbia who expressed readiness to allocate more funds for eggs produced with respect to welfare compared to eggs from conventional production was 78.53%, and differed between regions, from 69.9% in Southern and Eastern Serbia up to 86.76% in the region of Western Serbia and Šumadija. It is also important to what extent the consumers are willing to pay more and according to the research of *Tolimir et al. (2019b)* in Serbia, 71.2% expressed readiness to allocate more funds for eggs from organic production, however 58.8% consumers would pay up to 30% higher price, and only 12.4% of them price higher by more than 30%. *The European Commission (2009)* states that although there is a willingness of consumers to pay a higher price, they do not always implement it in practice. In the research of *Tolimir et al. (2019a)*, producers were surveyed about the readiness of consumers to allocate more money for eggs from permitted systems, who, when asked: "How much are consumers willing to pay a higher price for eggs from non-battery systems“, expressed skepticism and stated that for consumers in Serbia, the most important thing is the lowest possible price of eggs, except for a very small number of consumers, mainly in the city of Belgrade. The authors of this study noted the differences between producers and consumers in terms of consumer willingness to pay more for eggs from alternative systems and concluded that in the coming period, attention should be paid to their better communication and consumer education.

For the table egg production sector, it is important to know the consumer's commitment to purchase, i.e. their choice of place of purchase, as well as the choice of egg type depending on the producer and production system - conventional production, alternative systems in accordance with animal welfare or functional food programs. The supply of table eggs in Serbia, based on market insights by the authors, is in line with current production, in terms of representation of individual systems, i.e. mostly present are eggs from conventional cage systems, and eggs from free systems, from free range systems, as well as organic, and eggs from the functional food program, these are eggs enriched with omega acids and selenium. Also, it can be noticed that the supply of the market is different, with the supply from one to five producers, within one market, with eggs being sold in special sections on markets and specialized shops with organic products. In European countries, supermarket supply is given a lot of attention, and market chains are considered factors that drive the product market, which results in the effort provide for consumers a larger range of specific, targeted products, such as organic products (*Rader, 2018*). The market for table eggs in Serbia is very important, as indicated by the research of *Tolimir et al. (2017)*, according to which in the Belgrade market, the majority of respondents (39.62%) stated that they buy eggs in supermarkets/hypermarkets, similar to neighbouring countries, where supermarkets are locations of purchase of eggs for 38.78% of respondents (*Kralik et al., 2014*). Markets should be observed through the impact on egg quality, through the aspect of conditions in the facility and the time from supply to sale of eggs, given that the initial quality of eggs, at the time of laying is the highest and subsequently the internal quality of eggs begins to decline depending on further manipulation (*Jin et al., 2011*). Examination of egg quality in retail establishments is research subject of a number of authors, mainly with the aim of determining egg quality characteristics, i.e. egg age, weight, egg white height, Haugh units, yolk colour, number of cracked eggs (*Bell et al., 2001; Burley and Johnson, 2013*). Studies also indicate the dependence of egg quality on producers *(Škrbić et al., 2006*), and according to the results of market research in the City of Belgrade, between supermarkets/hypermarkets there is a difference in supply and quality of eggs (*Tolimir et al., 2017*), number brands/producers within the market varied from 1 to 5, and the analysis of eggs of all producers, aged up to 1 to 10 days, according to the values of egg quality parameters, a satisfactory quality of eggs was recorded.

Conclusion

A balanced diet is the key to good health, within which eggs as a high-value biological food are of great importance for all age groups. If the eggs are enriched, by adding certain nutrients, vitamins, antioxidants and omega 3 acids to food for laying hens, which are converted into eggs, they are considered to be functional foods, of special importance for maintaining health or treating certain diseases.

The increase in egg consumption in Serbia should be paid attention to, given that it is significantly lower compared to some developed countries, and in that regard, educational and better information measures should be taken at the level of the entire community, through a systematic approach. Special emphasis should be placed on eliminating the unfounded fear of an increase in blood cholesterol due to egg consumption, given that some consumers in Serbia still have misconceptions on this issue.

The egg production sector in Serbia is under strong pressure aimed at harmonizing production with international regulations, i.e. the implementation of welfare laws, which will cause major changes in the coming years, often considered as a potential threat to this sector. At the same time, consumers are increasingly demanding in terms of welfare, rearing systems, egg quality, food safety, but there is a need to better inform them, as well as increase their awareness of the importance of welfare and the relationship between welfare and product quality.

The willingness of more than three quarters of surveyed consumers in Serbia to pay more money for eggs produced in systems that are based on the principle of welfare of poultry, can be a guideline for producers who are obliged to establish a system of rearing standards according to welfare standards, as well as other actors in market chains in Serbia. However, the conflicting position of producers in Serbia regarding the expressed readiness of consumers to actually do so imposes the need to establish better communication between them.

Značaj jaja u ishrani, preference potrošača, stanje u proizvodnji konzumnih jaja i na tržištu u Srbiji

Nataša Tolimir, Zdenka Škrbić, Marijana Maslovarić, Miloš Lukić, Dragan Milić

Rezime

Ishrana je veoma važna za održavanje dobrog zdravlja ljudi u svim životnim fazama i kao takva ne bi trebala da bude predmet interesovanja samo nutricionista, već i svakog pojedinca. Na odabir namirnica u ishrani značajno utiču navike, a u poslednjim decenijama zapaža se da potrošači sve više brinu i o načinu na koji je hrana proizvedena, o njenoj bezbednosti i neškodljivosti. Iako se jaja, kao namirnica, odlikuju odličnim nutritivnim sastavom decenijama su imala lošu reputaciju zbog visokog sadržaja holesterola koji se smatrao lošim, zbog čega su ih mnogi potrošači izbegavali ili su jeli samo belance. Rešavanju problema holesterofobije doprineli su rezultati istraživanja koja ukazuju da holesterol u hrani kao što su jaja ima beznačajan uticaj na holesterol u krvi, tako da su i zvanične organizacije za ishranu, oko 2000. godine, dale preporuku za ukidanje ograničnja u konzumiranju jaja. Potrošnja jaja i način pripreme u velikoj meri variraju među različitim zemljama, što se može povezati sa tradicijom ishrane i preferencama potrošača. Tokom poslednjih decenija, stavovi potrošača dobijaju na značaju i proučavaju se u odnosu na osobine kvaliteta jaja, prema sistemima proizvodnje, dobrobiti kokoši i dr. Celovito sagledavanje percepcije potrošača, njihovih navika i shvatanja o ulozi jaja u ishrani zahteva sistemski pristup, na nivu celokupne društvene zajednice, uključujući i edukaciju, posebno važnu za mlađe populacije. Cilj ovog rada je da ukaže na značaj jaja u ishrani, da prikaže stanje u sektoru proizvodnje konzumnih jaja u Srbiji, sa osvrtom na zemlje EU, uključujući i očekivane promene uslovljene zakonskom regulativom, koje mogu imati dalekosežne posledice po ovaj sektor, sa osvrtom na preference potrošača i situaciji na tržištu.

Key words: ishrana, jaja, dobrobit, sistemi gajenja, preference potrošača

Acknowledgment

This work was created as a result of research within the contract of the realisation and financing of scientific research work in 2021 between the Ministry of Education, Science and Technological Development of the Republic of Serbia and the Institute for Science Application in Agriculture (contract number: 451-03-9/2021-14/ 200045), as well as the Institute for Animal Husbandry (contract number: 451-03-9/2021-14/200022)

References

AHNEN R. T., SLAVIN J. L. (2019): Eggs as part of a healthy eating pattern. In: Eggs as Functional Foods and Nutraceuticals for Human Health. Ed Ju W. The Royal Society of Chemistry, Croydon, UK, 1-21.

ALONSO M. E., GONZÁLEZ-MONTAÑA J. R., LOMILLOS J. M. (2020): Consumers' Concerns and Perceptions of Farm Animal Welfare. Animals 10, 3, 385.

ANTON M., NAU F., NYS Y. (2005): Bioactive egg components and their potential uses. Proceedings of the XIth European Symposium on the Quality of Eggs and Egg Products, May 23-26, Doorwerth, Netherlands, 237-244.

APPLEBY M. C. (2003): The European Union ban on conventional cages for laying hens: History and prospects. Journal of Applied Animal Welfare Science 6, 2, 103-121.

AUGÈRE-GRANIER M.L. (2019): The EU poultry meat and egg sector - Main features, challenges and prospects (In depth analysis), PE 644.195, EPRS - European Parliamentary Research Service, European Parliament, retrieved from https://www.europarl.europa.eu/RegData/etudes/IDAN/2019/644195/EPRS_IDA(2019)644195_EN.pdf, on 20.06.2021

AUTIO M., AUTIO A., KUISMIN A., RAMSINGH B., KYLKILAHTI E., VALROS A. (2017): Bringing Farm Animal Welfare to the Consumer's Plate – The Quest for Food Business to Enhance Transparency, Labelling and Consumer Education. In: The Business of Farm Animal Welfare. Eds Amos N. and Sullivan R. Greenleaf Publishing, Taylor & Francis / Routledge, 120-136.

BEJAEI (2009): Attitudes and preferences of consumers/purchasers toward different types of table eggs. Master of Science Thesis, University of British Columbia.

BELL D. D., PATTERSON P. H., KOELKEBECK K. W., ANDERSON K. E., DARRE M. J., CAREY J. B., KUNEY D. R., ZEIDLER G. (2001): Egg marketing in national supermarkets: Egg quality—Part 1. Poultry Science 80, 383-389.

BERTECHINI A. G., MAZZUCO H. (2013): The table egg: a review. Ciência e Agrotecnologia, 37, 2, 115-122.

BINNEKAMP M. H. A., INGENBLEEK P. T. M. (2006): Market barriers for welfare product innovations. NJAS - Wageningen Journal of Life Sciences 54, 2, 169-178.

BLOKHUIS H. J., KEELING L. J., GAVINELLI A., SERRATOSA J. (2008): Animal welfare's impact on the food chain. Trends in Food Science & Technology, 19, 1, S79-S87.

BOVET P., FAEH D., MADELEINE G., VISWANATHAN B., PACCAUD F. (2007): Decrease in blood triglycerides associated with the consumption of eggs of

hens fed with food supplemented with fish oil. Nutrition, Metabolism and Cardiovascular Diseases 17, 4, 280-287.
BURLEY H. K., JOHNSON C. L. (2013): Market survey of quality and freshness of eggs produced under an enhanced hen nutrition and egg production program. The Journal of Applied Poultry Research 22, 929-933.
CARSON J. A. S., LICHTENSTEIN A. H., ANDERSON C. A. M., LAWRENCE J. A., PENNY M. K. E., MEYER K. A., PETERSEN K., POLONSKY T., VAN HORN L. (2020): Dietary cholesterol and cardiovascular risk; A science advisory from the American Heart Association. Circulation, 141, e39-e53.
CAUDILL M. A. (2010): Pre- and postnatal health: Evidence of increased choline needs. Journal of the American Dietetic Association 110, 1198-1206.
CLAYTON Z.S., FUSCO E., KERN M. (2017): Egg Consumption and Heart Health: A Review. Nutrition, 37, 79-85.
COMMITTEE FOR THE COMMON ORGANISATION OF THE AGRICULTURAL MARKETS 2017. (Ed.): EU Market Situation for Eggs. Brussels, August 27th, retrieved from: https://circabc.europa.eu/sd/a/18f7766e-e9a9-46a4-bbec-94d4c181183f/23.03.2017_eggs_Europa.pdf, on 25.05.2021.
DE ROEST K., FERRARI P., MONTANARI C., BOKMA M., HEUTINCK L., VAN REENEN K., et al. (2010): Report on consumers' attitudes towards animal welfare standards based on the main findings of EU and national research projects. Resource document, retrieved from https://www.researchgate.net/publication/314153758_Report_on_consumers'_attitudes_towards_animal_welfare_standards_based_on_the_main_findings_of_EU_and_national_research_projects/citations, on 16.07.2021.
DIRECTIVE 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. European Union, retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31999L0074&from=EN, on 05.07.2021.
ĐOKOVIĆ J., MUNĆAN M., PAUNOVIĆ T. (2018): Proizvodnja konzumnih jaja u Srbiji – sadašnje stanje, aktuelni problemi i mogućnosti unapređenja. Agroekonomika, 47, 81, 49-57.
DUNCAN I. J. H. (2001): The pros and cons of cages. World's Poultry Science Journal 57, 4, 381-390.
EUROPEAN COMMISSION (2005): Attitudes of Consumers towards the Welfare of Farmed Animals. Special Eurobarometer, 229, 1-138.
EUROPEAN COMMISSION (2007): Attitudes of EU citizens towards animal welfare. Special Eurobarometer 270, Wave 66.1.TNS Opinion and Social.
EUROPEAN COMMISSION (2009): Feasibility study on animal welfare labeling and establishing a Community Reference Centre for Animal Protection and Welfare, DG SANCO, Brussels.

EUROPEAN COMMISSION (2020): DG Agriculture and Rural Development, EU Market Situation for Eggs, retrieved from: https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/eggs_en, on 17.07.2021

EUROPEAN PARLIAMENT (2021): European Citizens' Initiative "End the cage age", retrieved from https://www.europarl.europa.eu/doceo/document/TA-9-2021-0295_EN.html, on 10.07.2021.

FAOSTAT (2015, 2016, 2017, 2018, 2019): Crops and livestock products, retrieved from http://www.fao.org/faostat/en/#data/QCL, on 08.05.2021.

FAOSTAT (2017, 2018, 2019): Crops and livestock products, retrieved from http://www.fao.org/faostat/en/#data/QCL, on 03.06.2021.

FARJAMI T., BABAEI J., NAU F., DUPONT D., MADADLOU A. (2021): Effects of thermal, non-thermal and emulsification processes on the gastrointestinal digestibility of egg white proteins, Trends in Food Science & Technology 107, 45-56.

FISININ V. I., PAPAZYAN T. T. SURAI P.F. (2008): Producing specialist poultry products to meet human nutrition requirements: selenium enriched eggs. World's Poultry Science Journal 64, 85-98.

FREWER L. J., KOLE A., VAN DE KROON S. M. A., DE LAUWERE C. (2005): Consumer Attitudes Towards the Development of Animal-Friendly Husbandry Systems. Journal of Agricultural and Environmental Ethics 18, 345-367.

GOODROW E. F., WILSON T. A., HOUDE S. C., VISHWANATHAN R., SCOLLIN P. A., HANDELMAN G., NICOLOSI R. J. (2006): Consumption of one egg per day increases serum lutein and zeaxanthin concentrations in older adults without altering serum lipid and lipoprotein cholesterol concentrations. The Journal of Nutrition 136, 2519–2524.

GRASHORN M.A. (2005): Enrichment of eggs and poultry meat with biologically active substances by feed modifications and effects on the final quality of the product. Polish Journal of Food and Nutritional Science 14/55, 1, 15-20.

GRAY J., GRIFFIN B. (2009): Eggs and dietary cholesterol – dispelling the myth. Nutrition Bulletin, 34, 66-70.

GRUNERT K.G., BECH-LARSEN T., BREDAL L. (2000): Three issues in consumer quality perception and acceptance of dairy products. International Dairy Journal 10, 575-584.

GUO J., HOBBS D. A., COCKCROFT J. R., ELWOOD P. C., PICKERING J. E., LOVEGROVE J. A., GIVENS D. I. (2018): Association between egg consumption and cardiovascular disease events, diabetes and all-cause mortality. European Journal of Nutrition 57, 2943–2952.

GUYONNET V. (2012): Eggs and egg products: Consumers' attitudes, perceptions and behaviours. Proceedings of the XXIV World´s Poultry Congress, August 5 –

9, Salvador, Brazil, retrieved from http://www.facta.org.br/wpc2012-cd/pdfs/plenary/Vincent_Guyonnet.pdf, on 01.05.2021.
HANSSTEIN F. (2011): Profiling the egg consumer: attitudes, perceptions and behaviours. In: Woodhead Publishing Series in Food Science, Technology and Nutrition, Improving the Safety and Quality of Eggs and Egg Products. Eds Y. Nys, M. Bain and F. Van Immerseel, Woodhead Publishing, Cambridge, UK, 39-61.
HENG Y., PETERSON H. H., LI X. (2013): Consumer Attitudes toward Farm-Animal Welfare: The Case of Laying Hens. Journal of Agricultural and Resource. Economics 38, 418–434.
HERNANDEZ H. M., BEARDSWORTH P., WEBER G. (2005): Egg quality – meeting consumer expectations. International Poultry Production 13, 3, 20-23.
HERNANDEZ J. M. (2006). European consumer perspectives on egg quality. Proceedings of the 18th Australian Poultry Science Symposium, February 20-22, Sydney, 261-268.
INTERNATIONAL EGG COMMISSION – IEC (2018). Annual Review, retrieved from https://www.internationalegg.com/resource/global-egg-production-continues-to-grow/ on 01.07.2021.
IPARD PROGRAMME, SERBIA (2021): Measure 1 - Investments in physical assets of agricultural holdings, retrieved from https://ipard.co.rs/, on 23.06.2021.
JIN Y. H., LEE K. T., LEE W. I., HAN Y. K. (2011): Effects of storage temperature and time on the quality of eggs from laying hens at peak production. Asian-Australasian Journal of Animal Sciences 24, 2, 279-284.
KEHLBACHER A., BENNETT R., BALCOMBE K. (2012): Measuring the consumer benefits of improving farm animal welfare to inform welfare labelling. Food Policy 37, 627–633
KOLLENDA E., BALDOCK D., HILLER N., LORANT A. (2020): Transitioning towards cage-free farming in the EU: Assessment of environmental and socio-economic impacts of increased animal welfare standards. Policy report by the Institute for European Environmental Policy, Brussels & London.
KOSMAS C. E., MARTINEZ I., SOURLAS A., BOUZA K. V., CAMPOS F. N., TORRES V., MONTAN P. D, GUZMAN E. (2018): High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. Drugs Context 7, 212525.
KRALIK I., KRALIK Z., ZELIĆ S. (2014): Preferencije potrošača konzumnih jaja. Proceedings of the 49th Croatian & 9th International Symposium on Agriculture, February 16 – 21, Dubrovnik, Croatia, 156-160.
KRNJAIĆ S. (2019): Egg production and processing sector analysis in the Republic of Serbia Sektorska analiza proizvodnje i prerade jaja u Republici Srbiji (in Serbian), retrieved from http://www.minpolj.gov.rs/wp-

content/uploads/datoteke/IPARD/01%2004%202019%20Sektorska%20analiza%20proizvodnje%20i%20prerade%20jaja%20u%20Srbiji.pdf, on 28.06.2021.

KUANG H., YANG F., ZHANG Y., WANG T., CHEN G. (2018): The Impact of Egg Nutrient Composition and Its Consumption on Cholesterol Homeostasis. Cholesterol, Article ID 6303810

LAING P. M. (1988): Diseases of free range birds. World's Poultry Science Journal 44, 72-75.

LESNIEROWSKI G., STANGIERSKI J. (2018): What's new in chicken egg research and technology for human health promotion? - A review. Trends in Food Science and Technology 71, 46-51.

LOPÉZ-FANDINO R. (2007): Use of egg compounds for human nutrition. In: Bioactive Egg Compounds, Eds Huopalahti R., Lopéz-Fandino R., Anton M. and Schade R. Springer, Berlin, Heidelberg, Germany, 117-190.

LÓPEZ-SOBALER A. M., GONZÁLEZ-RODRÍGUEZ L. G. (2015): Role of eggs consumption in women at different life stages. Nutrición Hospitalaria 1, 35-40.

MAGDELAINE P. (2011): Egg and egg product production and consumption in Europe and the rest of the world. In: Improving the Safety and Quality of Eggs and Egg Products-Egg Chemistry, Production and Consumption Eds Y. Nys, M. Bain and F. Van Immerseel, Woodhead Publishing, Cambridge, UK, 3–16.

MARTELLI G. (2009): Consumers' perception of farm animal welfare: An Italian and European perspective. Italian Journal of Animal Science 8, 1, 31-41.

McNAMARA D.J. (2000): Dietary cholesterol and atherosclerosis. Biochimica et Biophysica Acta 1529, 310–320.

MENDONÇA JR. C. X., ALMEIDA C. R. M., MORI A. V., WATANABE C. (2002): Effect of dietary vitamin A on egg yolk retinol and tocopherol levels. Journal of Applied Poultry Research, 11, 373-378.

MILOŠEVIĆ N., PERIĆ L. (2011): Technology of poultry production / Tehnologija živinarske proizvodnje (in Serbian). University of Novi Sad, Faculty of Agriculture, Novi Sad.

MIRANDA J. M., ANTON X., REDONDO-VALBUENA C., ROCA-SAAVEDRA P., RODRIGUEZ J. A., LAMAS A., FRANCO C. M., CEPEDA A. (2015): Egg and Egg-Derived Foods: Effects on Human Health and Use as Functional Foods. Nutrients, 7, 706-729.

MIZRAK C., DURMUS I., KAMANLI S., ERDOGAN DEMIRTAS S. KALEBASI S., KARADEMIR E., DOGU M. (2012): Determination of egg consumption and consumer habits in Turkey. Turkish Journal of Veterinary and Animal Sciences 36, 6, 592-601.

MULDER M., ZOMER S. (2017): Dutch consumers‟ willingness to pay for broiler welfare. Journal of Applied Animal Welfare Science 20, 2, 1-18.

NOCELLA G., HUBBARD L., SCARPA R. (2010): Farm Animal Welfare, Consumer Willingness to Pay, and Trust: Results of a Cross-National Survey. Applied Economic Perspectives and Policy 32, 2, 275-297.

OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA NO. 41/2009. (2009): Law on animal welfare / Zakon o dobrobiti životinja (in Serbian).

OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA NO. 6/10; OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA NO. 57/2014-27 (2010, 2014): Rulebook on conditions for animal welfare in terms of animal housing, rooms and equipment in facilities where animals for production purposes are kept, reared and placed on the market, the manner of keeping, breeding and trade of certain species and categories of animals, as well as the content and management of animal records' keeping / Pravilnik o uslovima za dobrobit životinja u pogledu prostora za životinje, prostorija i opreme u objektima u kojima se drže, uzgajaju i stavljaju u promet životinje u proizvodne svrhe, naĉinu držanja, uzgajanja i prometa pojedinih vrsta i kategorija životinja, kao i sadržini i naĉinu voĊenja evidencije o životinjama (in Serbian).

PAL M., MOLNÁR J. (2021): The role of eggs as an important source of nutrition in human health. International Journal of Food Science and Agriculture, 5, 1, 180-182.

PARROTT P., WALLEY K., CUSTANCE P. (2013): Consumer defined dimensions of egg quality. EggMeat Symposia 2013, September 15-19, Bergamo; World’s Poultry Science Journal, 69 Supplement.

PAVLOVSKI Z., MAŠIĆ B. (1994): Odnos potrošača prema živinskim proizvodima. Živinarstvo 7-9, 77-82. (in Serbian)

PAVLOVSKI L., ŠKRBIĆ Z., LUKIĆ M. (2011): Free systems of laying of chickens and layer hens: quality of meat and eggs. Tehnologija mesa 52 1, 160-166.

PENRY J., MANORE M. (2008): Choline: an important micronutirent for maximal endurance-exercise performance? International Journal of Sport Nutrition and Exercise Metabolism 18, 191-203.

PERIĆ L., MILOŠEVIĆ N., ŽIKIĆ D., KANAČKI, Z., DŽINIĆ N., NOLLET L., SPRING P. (2009): Effect of selenium sources on performance and meat characteristics of broiler chickens. Journal of Applied Poultry Research 18, 403-409.

PERIĆ L., RODIĆ V., MILOŠEVIĆ N. (2011): Production of poultry meat and eggs as functional food - challenges and opportunities. Biotechnology in Animal Husbandry 27 3, 511-520.

QURESHI A. I., SURI F. K., AHMED S. NASAR A., DIVANI A. A., KIRMANI J. F. (2007): Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. Medical Science Monitor, 13, CR1-CR18.

RADER L., FAS Europe Specialists (2018): EU Organic Boom Brings Opportunities for U.S. Exporters, retrieved from https://globalorganictrade.com/sites/default/files/general_file/file/eu_organic_boom_brings_opportunities_for_u.s._exporters_berlin_germany_2-6-2018.pdf, on 20.06.2021.
RAKONJAC S. (2016): Production and quality of products from laying hens in alternative rearing systems / Proizvodnja i kvalitet proizvoda kokoši nosilja iz alternativnih sistema gajenja (in Serbian) Ph.D. Dissertation, Faculty of Agronomy Čacak, University of Kragujevac.
RODIĆ V., PERIĆ L., PAVLOVSKI Z., MILOŠEVIĆ N. (2010): Competitiveness of table eggs from non-cage housing systems. Biotechnology in Animal Husbandry, 26, 1-2, 117-128.
RODIĆ V., PERIĆ L., PAVLOVSKI, Z. (2014): Stavovi proizvođača konzumnih jaja prema regulativi za obezbeđenje dobrobiti nosilja (in Serbian) / Attitudes of table eggs producers towards regulations for ensuring the welfare of laying hens. Agroekonomika 43, 63-64, 125-135.
RUXTON C. H. S, DERBZSHIRE E., GIBSON S. (2010): The nutritional properties and health benefits of eggs. Nutrition & Food Science, 40, 3, 263-279.
SAKANAKA S., KITAHATA K., MITSUYA T., GUTIERREZ M. A., JUNEJA L. R. (2000): Protein Quality Determination of Delipidated Egg-yolk. Journal of Food Composition and Analysis, 13, 5, 773-781.
SCÄNOHR P., THOMSEN O. O, HANSEN R., BOBERG-ANS G., LAWAETZ H., WEEKE T. (1994): Egg consumption and high-density-lipoprotein cholesterol. Journal of Internal Medicine 235, 3, 249-51.
SHAHBANDEH M. (2021): Per capita consumption of eggs in the U.S. 2000-2021. Food & Nutrition, Consumer Goods and FMCG, retrieved from https://www.statista.com/statistics/183678/per-capita-consumption-of-eggs-in-the-us-since-2000/ on 28.06.2021.
SHAKOOR H., KHAN M. I., SAHAR A., KHAN M.K. I., FAIZ F., BASHEER AHMAD H. (2020): Development of omega-3 rich eggs through dietary flaxseed and bio-evaluation in metabolic syndrome. Food Science Nutrition 8, 6, 2619-2626.
ŠKRBIĆ Z., PAVLOVSKI Z., MITROVIĆ S., LUKIĆ M., TOMAŠEVIĆ D. (2006): Variability of certain table egg quality traits depending on the producer and investigation year. Biotechnology in Animal Husbandry 22, 5-6, 21-31.
STADIG L. M., AMPE B. A., VAN GANSBEKE S., VAN DEN BOGAERT T., D'HAENENS E., HEERKENS J. L. T., TUYTTENS F. A. M. (2016): Survey of egg farmers regarding the ban on conventional cages in the EU and their opinion of alternative layer housing systems in Flanders, Belgium. Poultry Science 95, 3, 715-725.

STATISTICAL OFFICE OF THE REPUBLIC OF SERBIA (2015, 2016, 2017, 2018, 2019): Household budget survey. Statistical Office of the Republic of Serbia, Belgrade.

STATISTICAL OFFICE OF THE REPUBLIC OF SERBIA (2020): Statistical Yearbook of the Republic of Serbia, Belgrade.

STOJANOVIĆ Ţ., GLIGORIJEVIĆ M., OGNJANOV G., VELJKOVIĆ S., MITIĆ S., FILIPOVIĆ J., ILIĆ J., BURAZEROVIĆ J., BURAZEROVIĆ E. (2014): Attitudes of consumers in Serbia on animal welfare / Stavovi potrošaĉa u Srbiji o dobrobiti ţivotinja (in Serbian), Organizacija za poštovanje i brigu o ţivotinjama – ORCA, Belgrade.

SURAI P.F. (2002): Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity. Worlds Poultry Science Journal 58, 333-347.

TACTACAN G. B., GUENTER W., LEWIS N. J., RODRIGUEZ-LECOMPTE J. C., HOUSE J. D. (2009): Performance and welfare of laying hens in conventional and enriched cages. Poultry Science 88, 698-707.

TOLIMIR N., JOVANOVIĆ R., MASLOVARIĆ M., VUKIĆ- VRANJEŠ M., DRINIĆ M. (2012): Testing concentrate feed mixtures with increased selenium concentration in order to produce eggs as functional food. Proceedings of the XV International Feed Technology Symposium Feed-to Food / Cost Feed for Health Joint Workshop, October 3 – 5, Institute of Food Technology, Novi Sad (Serbia), pp: 217-222.

TOLIMIR N., MASLOVARIĆ M., ŠKRBIĆ Z., LUKIĆ M., BUDIMOVIĆ N., MILIĆ D., RADIŠIĆ R. (2020): Attitudes of Serbian producers and consumers of table eggs on the ban on conventional batteries and the transition to enriched cages and alternative production systems. Biotechnology in Animal Husbandry 36, 4, 463-476.

TOLIMIR N., MASLOVARIĆ M., ŠKRBIĆ Z., LUKIĆ M., RAJKOVIĆ B., RADIŠIĆ R. (2017): Consumer criteria for purchasing eggs and the quality of eggs in the markets of the city of Belgrade. Biotechnology in Animal Husbandry 33, 4, 425-437.

TOLIMIR N., MASLOVARIĆ M., ŠKRBIĆ Z., RADIŠIĆ R., LUKIĆ M., RAJKOVIĆ B. (2019a): The attitudes of table egg consumers in Serbia on the welfare of laying hens. Biotechnology in Animal Husbandry 35 4, 387-398.

TOLIMIR N., MASLOVARIĆ M., ŠKRBIĆ Z., RAJKOVIĆ B., RADIŠIĆ R., LUKIĆ M. (2019b): Preferences of consumers/customers from Serbia toward organic eggs. Proceedings of the 12th International symposium „Modern trends in Livestock Production", October 9 – 11, Belgrade, 633-642.

TOLIMIR N., ŠKRBIĆ Z., RAJKOVIĆ B., TRAILOVIĆ J., MASLOVARIĆ M. (2016). Attitudes of consumers in Serbia towards the importance of a balanced diet and table eggs as foodstuff. Biotechnology in Animal Husbandry 32, 2, 205-218.

TUYTTENS F. A. M., SONCK B., STAES M., VAN GANSBEKE S., VAN DEN BOGAERT T., AMPE B. (2011): Survey of egg producers on the introduction of alternative housing systems for laying hens in Flanders, Belgium. Poultry Science 90, 941-950.

VAN HORNE P.L.M., BONDT N. (2017): Competitiveness of the EU egg sector, base year 2015. Wageningen Economic Research. Wageningen, Netherlands, pp 46.

VECCHIO R., ANNUNZIATA A. (2011): Italian consumer awareness of layer hens' welfare standards: a cluster analysis. International Journal of Consumer Studies 36, 6, 647-655.

VELJKOVIĆ S., STOJANOVIĆ Ț., FILIPOVIĆ J. (2015): Attitudes toward farm animals' welfare and consumers' buying intentions - case of Serbia. Economics of Agriculture 62, 1, 53-71.

VERBEKE W. (2009): Stakeholder, citizen and consumer interests in farm animal welfare. Animal Welfare 18, 325-333.

WANG J., CHI Y., CHENG Y., ZHAO Y. (2018): Physicochemical properties, *in vitro* digestibility and antioxidant activity of dry-heated egg white protein. Food Chemistry 246, 18-25.

WHO (2009). Vitamin A deficiency, retrieved from https://www.who.int/data/nutrition/nlis/info/vitamin-a-deficiency, on 25.06.2021.

YANNAKOPOULOS A. L., TSERVENI-GOUSI A. S., CRISTAKI E., FORTMARIS P., AVRAMIDIS A., DELAROUDIS E. (2005): Enhanced egg production in practice: the case of bio-omega-3 egg. International Journal of Poultry Science 4, 8, 531-535.

YAROSHENKO F. O., SURAI P. F., YAROSHENKO Y. F., KARADAS F., SPARKS N. H. C. (2004): Theoretical background and commercial application of production of Se-enriched chicken. Proceedings of the XXII WPC, June 8-13, Istanbul, Turkey.

ZELIĆ A., KRALIK Z., KRALIK I., MAHMUTOVIĆ H. (2016): Consumer preferences when purchasing table eggs in the area of Tuzla city in Bosnia and Herzegovina. Krmiva 57, 2, 74-79.

ZHUANG P., WU F., MAO L., ZHU F., ZHANG Y. (2021): Egg and cholesterol consumption and mortality from cardiovascular and different causes in the United States: A population-based cohort study. PLOS Medicine 18, 2, e1003508.

ZLATANOVIĆ V. (2015): Porodični budžet prosečnog domaćinstva u Republici Srbiji / Family budget of the average household in the Republic of Serbia. Ekonomija-teorija i praksa, 8 (3): 78-101.

ASSESSMENT OF FERTILITY OF BOARS – DIFFERENT APPROACHES

Radomir Savić, Vesna Davidović, Ivana Božičković

University of Belgrade, Faculty of Agriculture, 11080 Belgrade - Zemun, Republic of Serbia
Corresponding author: Radomir Savić, savic@agrif.bg.ac.rs
Invited paper

Abstract: Well timed identification and ranking of boars is an indispensable part of reproductive management. Fertility can be observed *in vitro* (sperm quality) or *in vivo* (insemination success and litter size). There are different approaches to assessing and opportunities to improve boar fertility. Some of the techniques for assessing the fertilization ability of sperm that enable the ranking of boars are based on individual characteristics (motility, morphological characteristics or chromatin structure) or are a combination of several traits (sperm binding ability or penetration). Sperm production is sensitive to various influences, which can lead to an increase in the share of pathological forms in the ejaculate. Many studies indicate great variability between boars in terms of farrowing rate and litter size at birth. The qualitative properties of sperm affect *in vivo* fertility, which obliges the evaluation of each boar ejaculate used for artificial insemination. Results of recent research indicate seminal plasma proteins as possible indicators of boar fertility, which could lead to the development of screening tests to assess boar fertility before introduction into reproduction.

Key words: boar, fertility, sperm, farrowing rate, litter size, seminal plasma proteins

Introduction

Productivity control and assessment of boar fertility is an indispensable part of reproductive management. Monitoring reproductive efficiency and ranking of boars implies timely identification of subfertile boars *(Savić et al., 2017)*. It is necessary to develop tests that will rank the relative fertility of boars and procedures to check their reproductive abilities *(Flowers, 2009)*. The most common reasons for exclusion of boars from reproduction according to *Robinson and Buhr (2005)* are: lower breeding value (20-45%), poor sperm quality (10-30%), weakening of sex drive (1-21%), impaired health status (13-60%) and others (10-20%).

Fertility can be observed *in vitro* (sperm quality) or *in vivo* (insemination success and litter size). Three key steps in assessing boar fertility are: sperm quality assessment based on *in vivo* reproductive data, boar ranking based on estimated relative fertility, and identification of subfertile ejaculates *(Flowers, 2009).* According to this author, the first two are carried out immediately before or after the introduction into reproduction, and the third during the entire productive life of the boar. The introduction of an *in vitro* fertility index increases the ability to select and rank boars used for artificial insemination, thus improving the control of boar productivity *(Jung et al., 2015).* Recent studies of boar fertility show a correlation between *in vivo* fertility with seminal plasma proteins, and the results of *Novak et al. (2010)* indicate several seminal plasma proteins and their importance in future research as markers of ejaculate quality and boar fertility.

Boar fertility is influenced by a large number of genetic and paragenetic factors: breed, breeding method, season, intensity of use, diet, accommodation, etc. Therefore, it is important to take into account the genetic structure of pig populations, to conduct continuous productivity control, to respect breeding plans and to use boars optimally. It is very important to opportunely identify and exclude animals with fertility below or around average. Boar greatly affects the productivity of the entire herd/population given the significantly higher number of offspring per year compared to sows. Therefore, the aim of this paper is to present different approaches in the assessment and possibilities of improving boar fertility.

Evaluation of growth and physical characteristics

In order for the testes to function properly, they must be maintained at lower temperatures than the rest of the body, with the difference between scrotal and rectal temperatures being about 3.2ºC *(Kyriazakis and Whittemore, 2006).* The process of spermatogenesis in boars begins at the age of 120 to 180 days in most breeds, with the exception of Chinese fertile breeds in which it begins earlier. The first ejaculation in boars occurs from 5 to 8 months of age, with sperm count and ejaculate volume continuously increasing during the first 18 months of life *(Kyriazakis and Whittemore, 2006).*

After the completion of the performance test and before the introduction of the young boar into reproduction, a preparatory period is required, since the premature introduction has a negative effect on boars' later sexual activity (*Savić et al., 2014).* Boars of fertile, meat breeds complete the performance test at the age of about 150-160 days. This is followed by a preparation period, which varies from 30 to 90 days, during which young boars get used to the phantom and collecting of ejaculate *(Flowers, 2009).* After that, boars are introduced into reproduction and their productive life begins. Boars aged 8 and 9 months at the time of entering reproduction have a higher number of functional spermatozoa than those aged 5, 6, 7 and 12 months in the moment of entering reproduction *(Wang et al., 2017).*

Selection towards improvement of carcass quality may result in reduced boar fertility *(Oh et al., 2006).* In the research of *Savić et al. (2014)* and *Savić et al. (2020)* a slight to weak correlation between traits from the performance test and ejaculate traits were found, leading to conclusion that selection for carcass characteristics improvement did not have a siginificant negative impact on later reproductive performance of boars. The reason for the weak correlation between fertility and production traits may lie in the different ages of animals at the moment of measuring the phenotypic values of these traits *(Savić et al., 2014).* In fact, phenotypic values of production traits were determined in boars of around 6 months of age, and fertility traits later, during reproductive life.

***In vitro* fertility**

In vitro male fertility implies different characteristics: ejaculate volume, sperm concentration, total number and number of functional spermatozoa, motility, percentage of live/dead spermatozoa, percentage of pathological forms (head and/or tail anomalies, presence of proximal or distal cytoplasmic droplets, etc.), morphological characteristics, viability and others. There are various techniques for assessing the fertilization ability of sperm, enabling the identification of relative fertility and the determination of differences between boars *(Flowers, 2009).* Some of these techniques are based on individual features such as motility, morphological characteristics or chromatin structure, while others are a combination of several properties (ability of sperm to bind or penetrate the egg cell).

Fluorescent staining can categorize live and dead sperm in the ejaculate and identify sperm with active mitochondria *(Jung et al., 2015).* Fertility of extended boar semen declines within the first 72h of storage *in vitro* and standard semen assessment (motility and membrane integrity) allows detection of lethal damage of spermatozoa *(Waberski et al., 2011).*

The share of pathological forms should not exceed 20%. The ejaculate of healthy boars always contains a certain percentage of morphologically abnormal sperm *(Jung et al., 2015).* Immature sperm with proximal cytoplasmic droplets originate from the testicles, and during their journey to the epididymis, the cytoplasmic droplet moves towards the so-called Jensen's ring on the sperm *(Savić and Petrović, 2019).* These authors further state that when sperm with distal cytoplasmic droplets reaches the tail of the epididymis, it loses the cytoplasmic droplet and gets the appearance of a mature sperm. Therefore, the presence of certain share of different types of immature sperm in the ejaculate makes it possible to determine the extent to which the epididymal sperm has matured. Distal drops give a better prognosis for the vitality and fertility of sperm than the proximal type, because sperm with distal drops are "more mature". The origin of abnormal forms, especially of the secondary type, and lack of sperm maturation, may be due to a decrease in testosterone flow at the testicular level. This is often a consequence of stressful situations or irrational use of boars.

Agglutination is the occurrence of sperm sticking, occuring as a consequence of the change in the polarity of the cell membrane of sperm, when instead of repelling each other, sperm are attracted to each other due to the opposite charge *(Savić and Petrović, 2019).* The most common type of false agglutination is when the sperm are dead, and it occurs as a consequence of the presence of toxic agents. Also, there is an actual agglutination which is a consequence of abnormalities in the composition of proteins and minerals of seminal plasma (due to inflammatory and/or infectious processes in the glands) or a loss of certain proteins that cover the surface of sperm. The cause of this type of agglutination can also be the appearance of autoimmune diseases, when the body produces antibodies against its own spermatozoa.

Sperm characteristics vary under the influence of various genetic and non-genetic factors. Various studies indicate the influence of: boar *(Savić et al., 2013),* breed *(Okere et al., 2005; Wolf and Smital, 2009),* season *(Kondracki et al., 2009; Kunowska-Slósarz and Makowska, 2011),* intensity of use *(Frangež et al., 2005; Wolf and Smital, 2009; Smital, 2010),* age *(Savić and Petrović, 2015),* photoperiod *(Petrocelli et al., 2015).* There are differences in testicular size, sperm count per ejaculate, ejaculate volume, sperm concentration and motility between breeds *(Caisin and Snitco, 2016).*

Sperm production is sensitive to various agents, so high body or ambient temperature can lead to lower ejaculate quality, or complete absence of live sperm in the ejaculate. Regarding high temperatures, the consequence of exposing pigs to temperatures above 27 ºC for several days can cause thermal stress that leads to an increase in abnormal forms of sperm, which can last for one month *(Savić and Petrović, 2019).* Seasonal differences in the qualitative properties of sperm may be associated with changes in the photoperiod and heat stress during the summer *(Lopez Rodriguez et al., 2017).* Season is a factor that affects viability, the share of total and primary pathological forms, sperm volume and concentration *(Petrocelli et al., 2015),* with the autumn period and the declining photoperiod having a negative impact on most sperm characteristics (except volume). The results of the research by *Tereszkiewicz and Pokrywka (2020)* indicate that seasonal changes in the quality of produced ejaculates of boars of meat breeds are similar to those that occur in wild boars, and the changes relate primarily to ejaculate volume, total sperm count and sperm concentration. Seasonal differences in sperm quality negatively affect the reproductive performance of sows *(Petrocelli et al., 2015).*

Diet can improve male sperm quality. Boars fed dietary flaxseed for 8 weeks displayed improved sperm morphology, increased membrane fluidity and better motility and viability during 5-7 day storage *(Radomil et al., 2011).*

In vivo fertility

Reproductive efficiency depends on a number of factors: boar, mating season, mating combination, weaning-estrus interval, insemination methods, sperm capacity and others *(Steverink, 1999; Luković et al., 2004; Savić, 2014).* It can be evaluated on the basis of the return and farrowing rate. *In vivo* fertility assessment is most often performed based on the farrowing rate and the number of live piglets at birth *(Flowers, 2013).*

According to research by *Ruiz-Sánchez et al. (2006)* there was a variation in the percentage of conception (73-98%) between boars, the farrowing rate (71-98%) and the litter size at birth (8.8-12.0 piglets). Identification of boars that have larger litters at birth is necessary for improving pig production. In the study of *Broekhuijse (2012),* a ranking and division into two groups (high and low fertile individuals) was performed, with the maximum difference between the groups 0.8 and the minimum 0.4 piglets. *Kovač and Malovrh (2005)* state that the difference in insemination success and litter size between the best and the worst boar can be up to 20%, i.e. 2.5 live-born piglets. If these values are applied to a large population of animals, the unquestionable influence of boar on reproductive parameters could be clearly seen. The study by *Broekhuijse et al. (2012a)* showed that only 5.9% of the total variations in the farrowing rate were caused by boar, while the variability of sperm traits was explained with 21% by genetic lineage, 11% by laboratory technique, and 7% by center for artificial insemination.

The study of *Young et al. (2010)* showed that there are differences between 30 analyzed herds in the farrowing rate (54.7-92.4%), and the cause of such a wide range lies in different procedures and technological operations performed in herd. In a study by *Savić et al. (2015)* boars were ranked according to the farrowing rate during reproductive exploitation, and the interval of variation between boars was from 59.31 to 85.37%. Although the differences in the farrowing rate between boars in aforementioned study were not statistically significant, the necessity to rank and timely identify boars with farrowing rate below the average remains undoubtful. Similar intervals of variation in the farrowing rate were found in other studies: 71-98% *(Ruiz-Sánchez et al., 2006),* 38.9-82.7% *(Didion et al., 2009),* 63.8-91.6% *(Park, 2013).*

Effect of *in vitro* on *in vivo* fertility

Early assessment of the correlation between different qualitative characteristics of sperm and reproductive performance of boars is the key for succesful production *(Flowers, 2009)*. It is a common practice that ejaculates are not used for insemination during the preparation period (after the end of the performance test, and before introduction into reproduction). However, the recommendation of *Flowers (2009)* is to use a certain number of ejaculates from that period for the so-called mating test, with mandatory feedback on the mating result (insemination success and litter size). This author further states that when *in vivo* data for a boar become available, an assessment of the relative fertility of the boar can be made. In the last step, when starting with intensive use of boars, it is necessary to identify subfertile ejaculates that should not be used for insemination.

The direct effect of boar explains 5.3% of the total variability in the farrowing rate *(Broekhuijse et al., 2012b)*. The direct influence of boar comes from: breed (22%), individual (29%), age of boars (0.3%), progressive sperm motility (9%), while about 40% of variability is not determined. Evaluation of standard sperm characteristics (sperm morphology, motility, concentration and ejaculate volume) enables the identification of ejaculates that are potentially poorly fertile, but the efficiency of predicting the fertility of boars based on these characteristics is not high enough *(Jung et al., 2015)*.

In a review paper, *Flowers (2003)* presented the results of several studies that indicate differences in the farrowing rate (from 65.8 to 92.5%) depending on the method of insemination, number of spermatozoa in the dose, dose volume and number of doses per sow. Results of *Tsakmakidis et al. (2010)* showed that the farrowing rate, in relation to differences in boar sperm, varied from 59.3 to 88.9%. The basic assumption for each type of sperm trait assessment is that sperm characteristics measured *in vitro* are also reflected in *in vivo* conditions *(Flowers, 2009)*. However, it is necessary to take into account the interaction of sperm with the female reproductive tract (fallopian tube).

The study of *Jung et al. (2015)* showed that boars with low fertility performance (low farrowing rate) had a significantly lower percentage of normal spermatozoa, a higher percentage of spermatozoa with cytoplasmic droplets and a higher proportion of morphologically altered spermatozoa compared to highly fertile boars. One of the possible explanations for the negative influence of the increased proportion of sperm with cytoplasmic droplets on the fertility of boars may stem from the correlation with the integrity of sperm DNA. Anomalies in the head or tail can affect sperm motility and their fertilizing ability in the reproductive tract of females. The study of *McPherson et al. (2014)* showed a correlation between the share of pathological forms of spermatozoa and spermatozoa with distal cytoplasmic droplets with the litter size. In their review, *Jung et al. (2015)* cite studies that found a negative correlation between the proportion of distal cytoplasmic droplets in diluted sperm and *in vivo* fertility (conception rate and litter size) when sperm doses were stored for two days, and the conception rate when doses were stored for four days. Sperm with proximal cytoplasmic droplets was negatively correlated with conception rate and litter size only when sperm doses were stored for four days in this research.

Motility is one of the most important traits that affects the *in vivo* fertility of boars (sperm penetration, farrowing rate and litter size). The average speed (µm/s) of sperm movement was significantly lower in boars with good fertilization performance, with boars with high non-return rate having a higher proportion of sperm moving linearly *(*Hirai et al., 2001 - cited *Jung et al., 2015)*. It is common not to use ejaculates with less than 60% motility of sperm for insemination. Some studies cited by *Flowers (2009)* have shown that if insemination doses of three and more billion sperm are used, the correlation between motility and reproductive performance of sows is asymptotic. The point at which fertility no longer increases at noticeable rates with increasing motility is between 60 and 70%.

Challenges and possibilities of improvement

Fifty percent of infertility in pigs is associated with male individuals, and molecular studies of sperm and sperm plasma may provide new insights into male infertility *(Ashrafzadeh et al., 2013)*. Causes of infertility or poor fertility of boars can be different: morpho-anatomical abnormalities of the reproductive tract, mechanical injuries, stress, neuro-endocrine disorders in sperm production, increased percentage of pathological sperm, irrational use of boars or infections caused by bacterial (brucellosis, leptospirosis), viral (PRRS) or fungal agents. Some of these causes are the result of technological errors, failure to implement appropriate preventive and biosecurity measures. Characteristics of the testicles influence the sperm production. Early detection of boars no longer suitable for sperm production could be possibly performed by testicular ultrasonography *(Lopez Rodriguez et al., 2017)*.

Continuous systematic analysis of the qualitative characteristics of ejaculate with the help of various modern methods and periodic microbiological analysis provides an objective insight into the quality of boar sperm *(Milovanović et al., 2013)*. If ejaculate collecting is not carried out in compliance with hygienic measures, bacterial contamination of ejaculate can occur, which can have a negative impact on sperm quality, and has been found to lead to antimicrobial resistance in isolates obtained from diluted semen *(Lopez Rodriguez et al., 2017)*. Therefore, it is recommended to use two pairs of hygienic gloves on hands when collecting sperm (so-called double glove technique), and to remove the first pair of gloves that are contaminated with the contents of the prepuce after emptying its' contents, and further use the clean gloves to fix the glans of the penis in order to reduce the risk of contamination *(Savić and Petrović, 2019).*

There has been increasing interest in proteins that are related with the sperm and their role in fertility *(Dyck et al., 2011).* Recent research indicate seminal plasma proteins as possible indicators of boar fertility, and according to *Novak et al. (2008)* identification of seminal plasma fertility markers could lead to the development of screening tests to assess boar fertility before introduction into reproduction. There are several seminal plasma proteins (20 kDA, pl 6.0; 25-29 kDA, pl 5.9-6.2; 55 kDA, pl 4.5-5.1; 60 klDA, pl 5.9) that are strongly correlated with boar fertility and have the potential to develop tests which can be used to assess the fertility of boars (*Flowers, 2009)*. Cryopreservation and sex-sorting have a detrimental impact on sperm quality, causing a destabilization of sperm membrane *(Leahy and Gadella, 2011). Barranco et al. (2015)* showed that there is variability in the total antioxidant capacity of seminal plasma (SP-TAC) between and to a lesser extent within boars. Although boar sperm is not suitable for deep freezing, the results of this research have shown that SP-TAC contributes to the ability of sperm to survive cryopreservation. Authors also indicate that this trait of seminal plasma can be considered a potential biomarker of fertility, since boars with higher levels of SP-TAC had better fertility results. Study of *Barranco et al. (2020)* showed that the concentration of SP-AMH (Anti-Mullerian hormone - a glycoprotein secreted by Sertoli cells), which is present in seminal plasma, varied between boar ejaculates, without differences between the examined breeds. Moreover, SP-AMH concentration proved to be a good predictive biomarker for sperm concentration, but poor biomarker for other sperm quality traits, functionality, and *in vivo* fertility parameters of diluted doses. When sperm plasma protein data were combined with other sperm quality assessments, the accuracy of prediction of boar and sperm fertility was significantly improved *(Flowers, 2009).*

Proteomic and genomic assessments of male fertility is limited by the avalilabity of appropriate numbers of boars with complete relative fertility data *(Dyck et al., 2011).* Authors also indicate that through working within existing populations of boars and accurate evaluation of their reproductive performances, it will be possible to identify, validate and practically apply reliable biomarkers of fertility in boars. Traits that are taken into account in the genomic selection of pigs range from traditionally selected traits, i. e. production or fertility traits, to new traits, such as boar taint, longevity or health traits *(Samorè and Fontanesi, 2016).* They cite research that indicates that male-based selection, as it is possible with genetic tools by exploiting the genomic selection traits wich are sex-limited, genetic gain resulted in an additional value of 20.5% compared to those on females. Effective predictors of fertility-related traits are essential to increase the efficiency of artificial insemination in pig production *(Tremoen et al., 2019).* They found few SNPs wich are significantly associated with the total number of piglets born, and these detected SNPs explained between 0.27% and 1.18% of the genetic variance. They point that these effects are too low for being used directly for selection, but can be of interest in SNP-panels used for genomic selection.

Standard laboratory practice includes measuring the motility and morphological characteristics of sperm, but flow cytometry is also used to assess sperm quality today *(Jung et al., 2015).* The use of flow cytometry can determine the integrity of the sperm membrane, damage to the acrosome and the percentage of sperm with damaged chromatin. *Jung et al. (2015)* state that sperm metabolism and function primarily depend on the integrity of the plasma membrane, whereby various techniques for determining this integrity exist, such as the eosin-nigrosine staining technique and the use of different fluorescent stainings. For the last 20 years, complex CASA (Computer-Assisted Semen Analysis) systems have been in use, which enable an objective assessment of multiple sperm kinetic parameters. This computerized system assesses sperm motility, concentration, morphology, and vitality in an objective, standardized, precise and much more complex procedure. When used properly, the CASA system should be able to assess sperm quality with high repeatability, which depends on the number of spermatozoa analyzed, and the results of the analysis become more reliable with the increase of the number of spermatozoa that are counted *(Feitsma et al., 2011).*

Procena plodnosti nerasta – različiti pristupi

Radomir Savić, Vesna Davidović, Ivana Božičković

Rezime

Pravovremena identifikacija i rangiranje nerasta nezaobilazni su deo reproduktivnog menadžmenta. Plodnost se može posmatrati *in vitro* (kvalitet sperme) ili *in vivo* (uspešnost osemenjavanja i veličina legla). Postoje različiti pristupi u oceni i mogućnosti unapređenja plodnosti nerasta. Neke od tehnika za ocenu fertilizacione sposobnosti spermatozoida koje omogućavaju rangiranje nerasta, bazirane su na pojedinačnim osobinama (pokretljivost, morfološke karakteristike ili hromatinska struktura) ili su kombinacija više osobina (sposobnost vezivanja spermatozoida ili penetracije). Produkcija sperme je osetljiva na različite uticaje, što može dovesti do povećanja udela patoloških formi u ejakulatu. Mnoga istraživanja ukazuju na veliku varijabilnost između nerasta u pogledu procenta prašenja i veličine legla pri rođenju. Kvalitativna svojstva sperme utiču na *in vivo* plodnost, što obavezuje ocenu svakog ejakulata nerasta koji se koristi za veštačko osemenjavanje. Novija istraživanja ukazuju na proteine seminalne plazme kao moguće indikatore plodnosti nerasta, što bi moglo da dovede do razvoja skrining testova procene plodnosti nerasta pre uvođenja u reprodukciju.

Ključne reči: nerast, plodnost, sperma, procenat prašenja, veličina legla, proteini seminalne plazme

Acknowledgements

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200116.

References

ASHRAFZADEH A., KARSANI S. A., NATHAN S. (2013): Mammalian Sperm Fertility Related Proteins. International Journal of Medical Sciences, 10, 2, 1649-1657.

BARRANCO I., TVARIJONAVICIUTE A., PEREZ-PATIÑO C., PARRILLA I., CERON J.J., MARTINEZ E.A., RODRIGUEZ-MARTINEZ H., ROCA J. (2015): High total antioxidant capacity of the porcine seminal plasma (SP-TAC) relates to sperm survival and fertility. Scientific Reports, 5, 18538.

BARRANCO I., FERNANDEZ-FUERTES B., PADILLA L., DELGADO-BERMÚDEZ A., TVARIJONAVICIUTE A., YESTE M. (2020): Seminal Plasma Anti-Müllerian Hormone: A Potential AI-Boar Fertility Biomarker? Biology, 9, 4, 78.

BROEKHUIJSE M.L.W.J. (2012): Prediction of porcine male fertility. PhD thesis, Dissertation Faculty of Veterinary Medicine, Utrecht University, the Netherlands. (Chapter 6. BROEKHUIJSE M.L.W.J., ŠOŠTARIĆ E., SCHOEVERS E.J., FEITSMA H., GADELLA B.M.: *In vitro* fertilisation competence of semen from AI boars has no predictive value for its fertilising capacity under commercial insemination conditions).

BROEKHUIJSE M.L.W.J., ŠOŠTARIĆ E., FEITSMA H., GADELLA B.M. (2012a): The value of microscopic semen motility assessment at collection for a commercial artificial insemination center, a retrospective study on factors explaining variation in pig fertility. Theriogenology, 77, 1466-1479.

BROEKHUIJSE M.L.W.J., ŠOŠTARIĆ E., FEITSMA H., GADELLA B.M. (2012b): Application of computer assisted semen analysis to explain variations in pig fertility. Journal of Animal Science, 90, 3, 779-789.

CAISIN L., SNITCO T. (2016): The influence of the seasonality on boars sperm production - breeder of different species. Bulgarian Journal of Agricultural Science, 22, Suppl. 1, 118–122.

DIDION B., KASPERSON M.K., WIXON L.R., EVENSON P.D. (2009): Boar Fertility and Sperm Chromatin Structure Status: A Retrospective Report. Journal of Andrology, 30, 6, pp. 7.

DYCK M.K., FOXCROFT G.R., NOVAK S., RUIZ-SANCHEZ A., PATTERSON J., DIXON W.T. (2011): Biological markers of boar fertility. Reproduction in Domestic Animals, 46, Suppl. 2, 55-58.

FEITSMA H., BROEKHUIJSE M.L., GADELLA B.M. (2011): Do CASA systems satisfy consumers demands? A critical analysis. Reproduction in Domestic Animals, 46, Suppl. 2, 49-51.

FLOWERS W.L. (2003): Future reproductive technologies- Applied results of trans cervical insemination and other studies related to artuficila insemination. Forty-seventh annual North Carolina Pork Conference, February 19 and 20, 2003, Greenville Convention Center, Greenville, NC State University.

FLOWERS W.L. (2009): Selection for boar fertility and semen quality--the way ahead. Society of Reproduction and Fertility Supplement, 66, 67-78.

FLOWERS W.L. (2013): Triennial Reproduction Symposium: Sperm characteristics that limit success of fertilization. Journal of Animal Science, 91, 3022-3029.

FRANGEŽ R., GIDER T., KOSEC M. (2005): Frequency of boar ejaculate collection and its influence on semen quality, pregnancy rate and litter size. Acta Veterinaria Brno, 74, 265-273.

JUNG M., RÜDIGER K., SCHULZE M. (2015): *In Vitro* Measures for Assessing Boar Semen Fertility. Reproduction in Domestic Animals, 50, Suppl 2, 20-24.

KONDRACKI S., WYSOKIŃSKA A., KOWALEWSKI D., MUCZYŃSKA E., ADAMIAK A. (2009): Season’s influence on the properties of male domestic pig semen. Rozprawy naukowePope John Paul II State School of Higher Vocational Education in Biała Podlaska, Vol. III, 177-187.

KOVAČ M., MALOVRH Š. (2005): Prednosti in slabosti osemenjevanja. Spremljanje proizvodnosti prašičev, IV. del. Urednici: Kovač M., Malovrh Š. Izdavač: Domžale, Univerza v Ljubljani, Biotehniška fakulteta, Enota za prašičerejo, biometrijo in selekcijo, 5-18.

KUNOWSKA-SLÓSARZ M., MAKOWSKA A. (2011): Effect of breed and season on the boar’s semen characteristics. Annals of Warsaw University of life sciences- SGGW, Animal Science, 49, 77-86.

KYRIAZAKIS I., WHITTEMORE T.C. (2006): Whittemore's science and practice of pig production. Blackwel Publishing, third edition, 1-685.

LEAHY T., GADELLA B.M. (2011): Capacitation and capacitation-like sperm surface changes induced by handling boar semen. Reproduction in Domestic Animals, 46, Suppl. 2, 7-13.

LOPEZ RODRIGUEZ A., VAN SOOM A., ARSENAKIS I., MAES D. (2017): Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Management, 3, 1, 1-12.

LUKOVIĆ Z., VINČEK D., GORJANC G., MALOVRH Š., KOVAČ M. (2004): Interval od odbića do koncepcije i veličina legla u svinja. XXXIX znanstveni skup hrvatskih agronoma s međunarodnim sudjelovanjem: priopćenja. Zagreb, Agronomski fakultet, 667-670.

MCPHERSON F.J., NIELSEN S.G., CHENOWETH P.J. (2014): Semen effects on insemination outcomes in sows. Animal Reproduction Science, 151, 28–33.

MILOVANOVIĆ A., BARNA T., MILANOV D., LAZAREVIĆ M. (2013): Model saradnje repro-centara i laboratorije za reprodukciju u kontroli kvaliteta semena nerastova. Arhiv veterinarske medicine, 6, 1, 57-70.

NOVAK S., RUIZ-SÁNCHEZ A., DIXON T. W., FOXCROFT R.G., DYCK K. M. (2010): Seminal Plasma Proteins as Potential Markers of Relative Fertility in Boars. Journal of Andrology, 31, 2, 188-200.

NOVAK S.,ODONOGHUE R.O', LEBOWA G., DIXON W., FOXCROFT G., DYCK M. (2008): Fertility Markers in Boar Semen. Advances in Pork Production, Volume 19, Abstract #28.

OH S. H., SEE M. T., LONG T. E., GALVIN J. M. (2006): Estimates of Genetic Correlations between Production and Semen Traits in Boar. Asian-Australian Journal of Animal Science, 19, 2, 160-164.

OKERE C., JOSEPH A., EZEKWE M. (2005): Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. Journal of Animal and Veterinary Advances, 4, 10, 885-888.

PARK S. (2013): Effects of sow, boar and semen traits on sow reproduction. University of Nebraska- Lincoln DigitalCommons@University of Nebraska-Lincoln, Animal Science Department, Theses and Dissertations in Animal Science, pp. 67.

PETROCELLI H., BATISTA C., GOSÁLVEZ J. (2015): Seasonal variation in sperm characteristics of boars in southern Uruguay. Revista Brasileira de Zootecnia, 44, 1, 1–7.

RADOMIL L., PETTITT M.J., MERKIES K.M., HICKEY K.D., BUHR M.M. (2011): Stress and dietary factors modify boar sperm for processing. Reproduction in Domestic Animals, 46, Suppl. 2, 39-44.

ROBINSON J.A.B., BUHR M.M. (2005): Impact of genetic selection on managment of boar replacement. Theriogenology 63, 668-678.

RUIZ-SÁNCHEZ A.L., O'DONOGHUE R., NOVAK S., DYCK M.K., COSGROVE J.R., DIXON W.T., FOXCROFT G.R. (2006): The predictive value of routine semen evaluation and IVF technology for determining realtive boar fertility. Theriogenology, 66, 736-748.

SAMORÈ A.B., FONTANESI L. (2016): Genomic selection in pigs: state of the art and perspective. Italian Journal of Animal Science, 15, 2, 211-232.

SAVIĆ R. (2014): Fenotipska i genetska varijabilnost plodnosti nerasta. Doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu.

SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N. (2013): The effect of breed, boar and season on some properties of sperm. Biotechnology in Animal Husbandry, 29, 2, 299-310.

SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N. (2014): Libido and ejaculate traits of performance tested boars. Journal of Animal and Plant Sciences, 24, 6, 1649-1654.

SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N., POPOVAC M. M., GOGIĆ M. (2015): Ejaculate properties and reproductive efficiency of large white boars duringexploitation. Biotechnology in Animal Husbandry, 31, 3, 397-405.

SAVIĆ R., PETROVIĆ M. (2015): Variability in ejaculation rate and libido of boars during reproductive exploitation. South African Journal of Animal Science, 45, 4, 355-361.

SAVIĆ R., AUSEJO M.R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., GOGIĆ M. (2017): Fertility of boars – what is important to know. Biotechnology in Animal Husbandry, 33, 2, 135-149.

SAVIĆ R., PETROVIĆ M. (2019): Praktikum iz svinjarstva. Univerzitet u Beogradu, Poljoprivredni fakultet, 1-182.

SAVIĆ R., RADOJKOVIĆ D., STOJILJKOVIĆ N., PARUNOVIĆ N., GOGIĆ M., RADOVIĆ Č. (2020): Effect of breed of performance tested boars on ejaculate traits. Biotechnology in Animal Husbandry, 36, 3, 309-316.

SMITAL J. (2010): Comparasion of environmental variations in boar semen characteristics of six breeds and their crossbreds over an eight-year period. Research in Pig Breeding, 4, 1, 26-32.

STEVERINK D.W.B. (1999): Optimising insemination strategies in pigs. Ph.D. thesis, Department of Animal Sciences (Animal Health and Reproduction Group), Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

TERESZKIEWICZ K., POKRYWKA K. (2020): Results of breeding use of insemination boars of selected breeds in the seasons of the reproductive cycle of the European wild boar (*Sus scrofa* L.). Acta Sci. Pol. Zootechnica, 19, 2, 37–46.

TREMOEN N.H., VAN SON M., ANDERSEN-RANBERG I., GRINDFLEK E., MYROMSLIEN F.D., GAUSTAD A.H, VÅGE D.I. (2019): Association between single-nucleotide polymorphisms within candidate genes and fertility in Landrace and Duroc pigs. Acta Veterinaria Scandinavica, 61, 58, 1-5.

TSAKMAKIDIS I.A., LYMBEROPOULOS A.G., KHALIFA T.A.A. (2010): Relationship between sperm quality traits and field-fertility of porcine semen. Journal of Veterinary Science, 11, 2, 151-154.

WABERSKI D., HENNING H., PETRUNKINA A.M. (2011): Assessment of storage effects in liquid preserved boar semen. Reproduction in Domestic Animals, 46, Suppl. 2, 45-48.

WANG C., LI J.L., WEI H.K., ZHOU Y.F., TAN J.J., SUN H.Q., JIANG S.W., PENG J. (2017): Linear growth model analysis of factors affecting boar semen characteristics in Southern China. Journal of Animal Science, 95, 12, 5339–5346.

WOLF J., SMITAL J. (2009): Effects in genetic evaluation for semen traits in Czech Large White and Czech Landrace Boars. Czech Journal of Animal Science, 54, 8, 349-358.

YOUNG B., DEWEY E.C., FRIENDSHIP M.R. (2010): Management factors associated with farrowing rate in commercial sow herds in Ontario. Canadian Veterinary Journal, 51, 185–189.

EFFECT OF BIRTH WEIGHT ON SURVIVAL AND GROWTH PERFORMANCE OF PIGLETS FROM LARGE LITTERS

Zoran Luković[1], Danijel Karolyi[1], Sven Menčik[2], Dubravko Škorput[1]

[1]University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia
[2]University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia
Corresponding author: Zoran Luković, lukovic@agr.hr
Invited paper

Abstract: The birth weight of piglets had a significant influence on pre-weaning mortality and subsequent growth performances. In birth weight class below 1.000 kg, almost 44% of piglets died during the lactation period, mainly in the first two weeks. The highest number of piglets belongs to the BW class between 1.201 and 1.400 kg. The effect of birth weight on piglet survival is not so obvious, because there is a tendency of decreasing of a number of stillborn piglets with increasing birth weight, but these differences were not significant among birth weight classes. Similar conclusions could be drawn for the number of piglets weaned (P>0.05). Pearson correlation coefficients among body weight traits ranged between 0.48 and 0.93. The lowest value of correlation coefficients was observed between birth weight and weight of piglets at weaning. In all observed periods, a significant effect of birth weight on daily gains was determined (P<0.05). Piglets with larger birth weight had larger daily gains. The difference in average daily gain between piglets with birth weight less than 1.000 kg and piglets with birth weight more than 1.600 kg was around 110 grams.

Key words: birth weight, litter size, growth, hyperprolific sows, survival

Introduction

Selection for improved litter size has led to an increase in the number of piglets born alive and the creation of highly productive sow lines *(Wolf et al., 2008; Luković and Škorput, 2015)*. However, the improvement at birth is not fully realized due to higher postnatal mortality at weaning *(Quiniou et al., 2002)*. The number of weaned piglets per sow is a trait of great economic importance in pig production *(Lund et al., 2002)*. At least in part, mortality during lactation is related to the decrease in average birth weight of piglets. Birth weight is the result of intrauterine growth of piglets and is considered one of the most important factors affecting pig survival *(Wolter et al., 2002; Deen and Bilkei, 2004)*. However, the distribution of birth weight within the litter (mean birth weight and within-litter variability) is also important for overall sow productivity *(Bee, 2007; Wolf et al., 2008)*. It is argued that litters with high birth weight variability have lower survival rates because direct competition excludes lighter littermates from access to functional and productive teats *(Milligan et al., 2001)*. As birth weight decreases, pigs are more likely to have higher mortality prior to weaning and during the nursery phase *(Fix et al., 2010)*. Not only survival rate, but also postnatal growth performance can be affected by low birth weight *(Bee, 2007; Vaclavkova et al., 2012)*. The aim of this study was to determine the effect of birth weight on survival and growth of piglets from a hyperprolific sow line in one of the leading commercial pig farms in Croatia.

Materials and Methods

The experimental herd consisted of 50 Pen Ar Lan Naima sows. The pregnant sows were brought to farrowing rooms one week before the expected farrowing. The sows were treated with d-cloprostenol on day 112 of the gestation *(Jančo et al., 2016)*. Each pen was equipped with a commercial crate. An infrared heat lamp was placed in each farrowing pen to provide additional heat to the piglets. On average, piglets were weaned at 28 days of age. From day 5 after farrowing until weaning, sows were fed *ad libitum*. Within the first 18 hours of life, piglets were individually weighed, teeth clipped and tails docked, and individually identified and sexed. On the third day after farrowing, piglets were injected with iron. Litter size was equalized within two days after farrowing by transferring piglets with lower birth weight to another sow. Male piglets were surgically castrated on the third day of life. Creep feeding of piglets started at 7 to10 days of age. During the study, 720 piglets (Naima sows x P76 Pen Ar Lan Hybrid boar) from 50 litters were weighed five times: on the first day after farrowing (BW), on the 14th day of life (W2), at weaning on the 28th day (W3), on the 30th day of the rearing period (W4) and at the end of the rearing period when piglets were 83 days old (W5). Normality of birth weight (BW) was tested using PROC UNIVARIATE *(SAS, 2013)*. Analysis of variance and main effects were performed using PROC GLM *(SAS, 2013)*. The statistical model included effects with levels: birth weight (BW), litter size as number of piglets born alive (NBA), and sex of piglets. Birth weight was divided into five classes: ≤1.000 kg, 1.001 – 1.200 kg, 1.201 – 1.400 kg, 1.401 – 1.600 kg and ≥1.601 kg. Number of piglets born alive (NBA) was divided into three groups: <14 NBA, 14 - 16 NBA, >16 NBA.

Results and Discussion

Piglets with a birth weight below 1.000 kg had the lowest survival rate presented as number of piglets weaned (Table 1), just above 56 %. As the birth weight increases, the survival rate also increases, so that piglets with a birth weight over 1.000 kg have a survival rate of over 80% and piglets with a birth weight over 1.400 kg have a survival rate of over 92%. The largest number of piglets had a birth weight between 1.201 and 1.400 kg.

Table 1. Number of piglets and piglets weaned (%) by birth weight class

Birth weight, kg	Number of piglets	Piglets weaned, %
≤1.000	114	56.1
1.001 – 1.200	118	81.4
1.201 – 1.400	192	84.4
1.401 – 1.600	158	92.4
≥1.601	138	92.7

The number of stillborn piglets did not differ between the defined classes of birth weight (Table 2). Although no statistically significant difference was found in the number of stillborn piglets between the observed groups according to the birth weight of piglets, there is a decreasing trend in the number of stillborn piglets with increasing birth weight, from 2.68 stillborn piglets in piglets with birth weight less than 1.0 kg to 2.05 stillborn piglets in the category of piglets with birth weight greater than 1.6 kg. The number of piglets died during lactation is highest in the group of piglets with birth weight less than 1.0 kg, although no statistical difference was found compared to the other birth weight categories (P>0.05). It is therefore obvious that birth weight is not the only factor affects piglet mortality during lactation.

Table 2. The effect of birth weight on number of stillborn piglets and number of piglets died in lactation (LSMEAN ± SE)

Birth weight, kg	Survival trait	
	Number of stillborn piglets	Number of piglets died in lactation
<1.000	2.68 ± 0.35	3.21 ± 0.17
1.001 – 1.200	2.50 ± 0.35	2.82 ± 0.18
1.201 – 1.400	2.27 ± 0.27	3.00 ± 0.14
1.401 – 1.600	2.24 ± 0.29	2.96 ± 0.15
≥ 1.601	2.05 ± 0.31	2.95 ± 0.16

* The values in the column marked with a different letter are significantly different P<0.01

Knol and Bergsma (2004) find that increasing birth weight does not reduce piglet mortality. The conclusions of *Knol and Bergsma (2004)* are in agreement with the results of this study where the effect of birth weight on the number of dead piglets was not found, i.e. the number of dead piglets did not decrease significantly with increasing birth weight, although a decreasing trend in the number of dead piglets was observed. Selection for better piglet survival favours piglets that have a better ability to emerge from the uterus to the outside world. In addition, maternal ability plays an important role in piglet survival. There are differences between pig genotypes, and they mainly relate to maternal behaviour towards piglets and the transfer of nutrients from mother to piglets. *Roehe (1999)* notes that mortality during lactation decreases rapidly with increasing birth weight, from 40% for piglets with a birth weight below 1 kg to less than 7% for piglets with a birth weight above 1.6 kg. Although our study shows a decreasing trend in the number of piglets died during lactation, this decrease is not statistically significant ($P>0.05$). To some extent, this suggests that there are also deaths of piglets with average and above average birth weight, that require attention, as the causes of these deaths are often more complex. Low birth weight of piglets results in higher mortality of piglets in lactation and slower growth *(Quiniou et al., 2002)*, and especially small and avital piglets have a much lower chance of survival *(Fix et al., 2010)*. *Marcatti (1986)*, in a study on the influence of litter balance on production traits until weaning, gives a mortality of 60% for piglets born at less than 800 grams. *Pardo et al. (2013)* in their study conclude that low birth weight of piglets is associated with higher mortality of piglets in the lactation and even three times higher than piglets with average birth weight.

Table 3. Pearson correlation coefficients among body weights of piglets

	W2	W3	W4	W5
BW	0.60	0.48	0.57	0.62
W2		0.93	0.77	0.75
W3			0.78	0.73
W4				0.91

* BW – birth weight; W2 – weight of piglets on day 14; W3 – weight of piglets at weaning on day 28; W4 – weight of piglets on day 58; W5 – weight of piglets at the end of rearing stage on day 83

The values of Pearson correlation coefficients among body weight measurements were moderate to very high (Table 3). The lowest value of correlation was found between birth weight of piglets (BW) and weight of piglets at weaning (W3) at the level of 0.48, indicating that birth weight is not the exclusive and most important indicator of growth of piglets in lactation and consequently weight of piglets at the end of lactation. The highest values of correlations above 0.90 were found between the body weight of piglets at 14 days of age and the weight of piglets at weaning, and between the body weights of piglets in the rearing phase (W4 and W5).

Table 4. Effect of birth weight on weaning weight and piglet weight at the end of rearing period (LSMEAN ± SE)

Birth weight, kg	Piglet weight	
	Weaning weight, kg	Weight at the end of rearing stage, kg
<1.000	5.75 ± 0.24[a]	21.92 ± 0.76[a]
1.001 – 1.200	6.48 ± 0.20[b]	24.73 ± 0.63[b]
1.201 – 1.400	7.16 ± 0.16[c]	27.46 ± 0.48[c]
1.401 – 1.600	7.80 ± 0.16[d]	29.75 ± 0.50[d]
≥ 1.601	8.09 ± 0.17[d]	32.30 ± 0.53[e]

* The values in the column marked with a different letter are significantly different P<0. 05

The birth weight of piglets significantly affects the weight of piglets at weaning, i.e. as birth weight increases, the weight of piglets at weaning also increases (Table 4). Piglets with birth weight less than 1.0 kg have significantly lower weight at weaning than piglets of all other classes of birth weight (P<0.01). No differences were found in the weight of piglets at weaning for piglets with a birth weight of 1.4 - 1.6 kg and piglets with a birth weight above 1.6 kg (P=0.21). Piglets with birth weight less than 1.2 kg did not reach the target weight at weaning of at least 7 kg. As the birth weight of piglets increases, the weight of piglets at the end of rearing increases in addition to the weight at weaning. Significant differences in final piglet weight were found between all birth weight categories. Between piglets with a birth weight below 1.0 kg and piglets with a birth weight greater than 1.6 kg, the difference in weight at the end of rearing is more than 10 kg.

Piglet birth weight affects the ability of piglets to grow in the later stages of rearing. Piglets with low birth weight start life smaller, gain less growth at all stages of rearing and are lighter at the end of the fattening phase *(Vaclavkova et al., 2012)*. These observations are consistent with the research we have conducted. *Schinckel et al. (2007)* also find that low birth weight piglets have lower body weights throughout the production period. *Fix et al. (2010)* list several important prenatal and postnatal factors responsible for slower growth of piglets due to low birth weight. First, the authors note that low birth weight piglets have a lower number of muscle fibers compared to average birth weight piglets, which directly affects slower growth in further rearing periods. Although the number of muscle fibers is determined prenatally, increasing their size affects the growth rate. Colostrum intake is mentioned as another important factor in piglet growth *(Fix et al., 2010).* Indeed, piglets with higher birth weight are more vital and occupy the anterior teats, which contain more milk, and thus have higher daily gains during lactation, but also in the later stages of rearing. *Roehe (1999)* finds that piglets with higher birth weight (1.6 kg versus 1.0 kg) have a higher daily gain of 53 g during the lactation period up to 21 days of age, or a higher daily gain during the period from weaning up to a body weight of 25 kg. *Schinckel et al. (2004),* analysing pig growth from birth to 60 days of age, found a correlation of 0.54 between birth weight and weight of piglets at weaning, while the correlations between weight at weaning and body weight in the period of piglet rearing (day 19 after weaning and 42nd day after weaning) are even higher, ranging from 0.81 to 0.74. *Zotti et al. (2017)* found that piglet weight increased with higher birth weight in all observation periods until 7, 21 and 59 days of age, respectively.

A significant effect of piglet birth weight on daily gain was found in all observed periods of piglet growth (Table 5). Piglets with birth weights ≤ 1.000 kg had the lowest daily gains in all periods. Piglets over 1.400 kg had the highest daily gains during lactation. Further increase in birth weight of piglets above 1.600 kg did not significantly increase daily gains during lactation. During the period from farrowing to the second weighing of piglets at 14 days of age, piglets with birth weights less than or equal to 1.000 kg had significantly lower daily gains compared with piglets of other weight classes ($P<0.05$). No significant difference in daily gain was found between piglets with a birth weight of 1.001 to 1.200 kg and piglets with a birth weight of 1.201 to 1.400 kg, and between piglets with a birth weight of 1.401 to 1.600 kg and piglets with a birth weight greater than 1.600 kg ($P>0.05$). In the period from the second weighing at 14 days of age to weaning of piglets at 28 days of age, piglets with a birth weight less than 1.200 kg had significantly lower daily gains compared to piglets with a birth weight of 1.201 to 1.400 kg and piglets with a birth weight above 1.400 kg ($P<0.05$). Similarly, in the period up to 14 days and in the period from 14 to 28 days of lactation, no difference in daily gains was found between piglets in the categories with a birth weight of 1.401 to 1.600 kg and piglets with a birth weight above 1.600 kg ($P>0.05$). Comparing the differences between the smallest and largest daily gains in the first two lactation periods, it is found that piglets with the highest birth weight above 1.600 kg have almost 70 g higher daily gains compared to piglets with the lowest birth weight below 1.000 kg, while in the second period, from 14 to 28 days slightly less and is 50 g. After weaning the piglets in the rearing period, which lasted from 28 to 83 days, the intensive growth of piglets continued, with significant differences in the values of daily gain between all categories of birth weight ($P<0.01$). The difference in daily gains between piglets with birth weight below 1.000 kg and piglets with birth weight above 1.600 kg is slightly less than 150 g, indicating an intensification of the growth rate of piglets until weaning, and when comparing the values of daily growth in all observed periods, the highest daily gains are obtained until the end of the piglet rearing period. From farrowing to the end of the nursery phase with a duration of 83 days, significant differences were found in the level of daily gains between piglets in the observed categories of birth weight (P<0.01). The difference in the level of daily gains between piglets with birth weight below 1.000 kg and piglets with birth weight above 1.600 kg is slightly less than 115 g.

Table 5. The effect of birth weight on daily gains in different periods from farrowing to the end of rearing period (LSMEAN ± SE)

Birth weight, kg	Daily gain, kg			
	0 – 14 days	14 – 28 days	28 – 83 days	0 – 83 days
<1.000	0.131 ± 0.009[a]	0.175 ± 0.009[a]	0.293 ± 0.012[a]	0.254 ± 0.009[a]
1.001 – 1.200	0.161 ± 0.008[b]	0.191 ± 0.007[a]	0.332 ± 0.010[b]	0.284 ± 0.008[b]
1.201 – 1.400	0.179 ± 0.006[b]	0.209 ± 0.006[b]	0.369 ± 0.007[c]	0.315 ± 0.006[c]
1.401 – 1.600	0.202 ± 0.007[c]	0.224 ± 0.006[c]	0.399 ± 0.008[d]	0.340 ± 0.006[d]
≥ 1.600	0.198 ± 0.007[c]	0.225 ± 0.006[c]	0.440 ± 0.008[e]	0.368 ± 0.006 [e]

* The values in the column marked with a different letter are significantly different P<0.05

Gondret et al. (2005) found that low birth weight piglets (from 0.8 to 1.1 kg) had 31% lower average daily gains during lactation and 26% lower average daily gains after weaning compared to higher birth weight piglets (from 1.75 to 2.05 kg). The authors found that individual birth weight was positively correlated with average daily gain during lactation (r=0.53) and in the post-weaning period (r=0.76). Piglet weight at weaning was also positively correlated with average daily gain during lactation (r=0.97, P<0.001) and during the post-weaning period (r=0.42, P=0.05). It is interesting to note, according to the authors, that the effect of birth weight on average daily gain decreases with time, i.e., as pigs grow, differences in daily gain become smaller. Similar observations of the influence of birth weight on daily gains were found by *Zotti et al. (2017)*, who also confirmed that over time the influence of birth weight on daily gains decreases. *Lynch (2006)* compared piglets according to three categories of birth weight (light, medium and heavy). They found a significant difference in daily gain between heavy and light piglets up to 75 days of age, while no difference in daily gain was found from day 75 to slaughter. There was a difference between light and medium piglets up to 50 days of age ranging from 0.05 to 0.07, after which there was no difference in daily gain. The smallest differences in daily gain were found between the medium and heavy piglet groups.

Conclusions

Piglet birth weight had a significant effect on preweaning mortality and subsequent growth performance. In large litters produced by highly productive sows, variability in piglet birth weight, along with increased numbers of lighter piglets, becomes a new challenge for swine producers. Advantages in litter size from highly productive sows can only be meaningful if piglet mortality is reduced and adequate growth performance in piglets is ensured.

Uticaj težine na rođenju na preživljavanje i rast prasadi iz velikih legala

Zoran Luković, Danijel Karolyi, Sven Menčik, Dubravko Škorput

Rezime

Težina prasadi na rođenju imala je značajan uticaj na mortalitet pre odbijanja i naknadni rast. U težinskoj klasi ispod 1,000 kg, skoro 44% prasadi je umrlo tokom perioda laktacije, uglavnom u prve dve nedelje. Najveći broj prasadi pripada klasi TM između 1,201 i 1,400 kg. Uticaj težine na rođenju na preživljavanje prasadi nije toliko očigledan, jer postoji tendencija smanjenja broja mrtvorođenih prasadi sa povećanjem težine na rođenju, ali ove razlike nisu bile značajne među klasama težine. Slični zaključci mogli bi se izvući i za broj odbijene prasadi ($P>0,05$). Pearsonovi koeficijenti korelacije među osobinama telesne težine kretali su se između 0,48 i 0,93. Najniža vrednost koeficijenata korelacije zabeležena je između težine na rođenju i težine prasadi pri odbijanju. U svim posmatranim periodima utvrđen je značajan uticaj težine na rođenju na dnevne priraste ($P<0,05$). Prasad sa većom težinom na rođenju imali su veće dnevne priraste. Razlika u prosečnom dnevnom prirastu između prasadi sa težinom na rođenju manjom od 1,000 kg i prasadi čija je težina veća od 1,600 kg bila je oko 110 grama.

Ključne reči: težina na rođenju, veličina legla, rast, hiperplodne krmače, preživljavanje

References

BEE G. (2007): Birth weight of litters as a source of variation in postnatal growth and carcass and meat quality. Advances in Pork Production 18, 191-196.

DEEN M.G.H., BILKEI G. (2004): Cross fostering of low-birthweight piglets. Livestock Production Science, 90, 279-284.

FIX J.S., CASSADY J.P., HERRING W.O., HOLL J.W., CULBERTSON M.S., SEE M.T. (2010). Effect of piglet birth weight on body weight, growth, backfat, and longissimus muscle area of commercial market swine. Livestock Science, 127, 51-59.

GONDRET F., LEFAUCHEUR L., LOUVEAU I., LEBRET B., PICHODO X., LE COZLER Y. (2005): Influence of piglet birth weight on postnatal growth performance tissue, lipogenic capacity and muscle histological traits at market weight. Livestock Production Science 93, 137-146.

JANČO N., JANČO G., ŠKORPUT D., LUKOVIĆ Z. (2016): Utjecaj vremena tretiranja d-kloprostenola na početak partusa u krmača. Proceedings of 51st Croatian and 11th International Symposium on Agriculture. Sveučilište u Zagrebu, Agronomski fakultet, Opatija, Croatia, February 15-18, 347-350.

KNOL E.F., BERGSMA R. (2004): Piglet survival and sow efficiency. Proceedings of AGBU Pig Genetics Workshop,17.-18. November 2004.

LYNCH P.B., CAHILL A., LAWLOR P., BOYLE L., O´DOHERTY J.V., LE DIVIDICH J. (2006): Studies on growth rates in pigs and the effect of birth weight, End of Project Report RMIS No. 5220

LUKOVIĆ Z., ŠKORPUT D. (2015): Factors influencing litter size in pigs. CAB Reviews Perspectives in Agriculture Veterinary Science Nutritionand, Natural Resources 1, 006, 1-9.

LUND M., PUONTI M., RYDHMER L., JENSEN J. (2002): Relationship between litter size and perinatal and pre-weaning survival in pigs. Animal Science, 74, 217-222.

MARCATTI N.A. (1986): Effect of cross fostering on piglets pre-weaning performance. Arquivo Brasileiro de Medicina Veterinaria Zootecnia 38, 413-417.

MILLIGAN B.N., FRASER D., KRAMER D.L. (2001): Birth weight variation in the domestic pig: effects on offspring survival, weight gain and suckling behaviour. Applied Animal Behaviour Science, 73,179-191.

PARDO C., KREUZER M., BEE G. (2013): Effect of average litter weight in pigs on growth performance, carcass characteristics and meat quality of the offspring as depending on birth weight. Animal, 7, 11, 1884-1892.

QUINIOU N., DAGORN J., GAUDRE D. (2002): Variation of piglets' birth weight and consequences on subsequent growth performance. Livestock Production Science, 78, 63–70.

ROEHE R. (1999): Genetic determination of individual birth weight and its association with sow productivity traits using Bayesian analyses. Livestock Production Science, 77, 170–175.

SAS (2013): Statistical analysis systems user's guide, Version 9.4, SAS Institute Inc., North Carolina

SCHINCKEL A.P., CABRERA R., BOYD R.D., JUNGST S., BOOHER C., JOHNSTON M., EINSTEIN M.E. (2007): Impact of birth and body weight at twenty days on the postweaning growth of pigs with different weaning management. The Professional Animal Scientist, 23, 197-210.

WOLF J., ŽÁKOVÁ E., GROENEVELD E. (2008): Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and losses until weaning. Livestock Science, 115, 195-205.

WOLTER B.F., ELLIS M., CORRIGAN B.P., DEDECKER J.M. (2002): The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on preweaning and postweaning growth performance and carcass characteristics. Journal of Animal Science 80, 301–308.

VÁCLAVKOVÁ E., DANĚK P., ROZKOT M. (2012): The influence of piglet birth weight on growth performance. Research in pig breeding, 6,1.

ZOTTI E., RESMINI F., SCHUTZ L., VOLZ N., MILANI R., BRIDI A., ALFIERI A., SILVA C. (2017): Impact of piglet birthweight and sow parity on mortality rates, growth performance, and carcass traits in pigs. Revista Brasileira de Zootecnia, 46, 856-862.

PERFORMANCE, METABOLIC AND MEAT QUALITY IMPLICATIONS OF IMMUNOCASTRATION IN IBERIAN PIGS

Patricia Palma-Granados, Ignacio Fernández-Fígares, Isabel Seiquer, Manuel Lachica, Luis Lara, Ana Haro, Rosa Nieto

Department of Physiology and Biochemistry of Animal Nutrition, Estación Experimental del Zaidín, CSIC, Profesor Albareda, 1, 18008, Granada, Spain
Corresponding author: Rosa Nieto, rosa.nieto@eez.csic.es
Invited paper

Abstract: Immunocastration is an alternative to surgical castration of male pigs to prevent boar taint in meat products. The procedure is feasible for autochthonous breeds raised outdoors up to heavy weights to obtain high-quality products, in which entire male production is not an option. Immunocastrated pigs could benefit from a greater growth potential during the growing period in addition to considerable improvements of animal welfare. Further research is needed to design specific vaccination protocols for heavy pigs taking into account the relevance of maintaining the highest quality standard in meat products.

Key words: animal welfare, growth performance, Iberian pig, immunocastration, meat quality, protein retention

Iberian pig production

Spain in one of the most relevant pig producers worldwide. The EU is the second largest pork meat producer. After China, USA and Germany, Spain is the fourth producing country, while, at European level, Spain is in second place regarding production, with approximately 19% of total pig meat produced, and the first in census, with nearly 21% of total pigs (more than 32 million animals, *MAPA*, *2021*). The pig industry represents a meaningful sector for the country accounting for nearly 40% of the economic value derived from livestock production. In recent years the pig sector has grown significantly, both in terms of production and census, boosted by exports to foreign markets -esp. China and other Southeast

Asian countries- and the competitiveness of the sector in the global market (*MAPA, 2021*). In a national context dominated by cosmopolitan breeds, there are also some native pig breeds of which the Iberian breed is by far the most important -both in numbers and economically. This autochthonous breed is derived from ancestral domestic pig populations of the Iberian Peninsula widely spread for centuries all over the territory (*López-Bote, 1998*). Nowadays it can be found in the Southwest of the Peninsula where the Mediterranean-type forest (*dehesa*) still persists. The breed has undergone a remarkable recovery during the last decades due to the revalorization of its products, the increasing demand for traditional food products of high organoleptic quality, and the social awareness for preservation of the genetic heritage and the natural habitat associated with the breed (*Nieto et al. 2019*). Currently it represents 11% of the overall pig production in Spain, counting purebred and crossbred animals (50% Iberian: purebred Iberian dam × purebred Duroc sire; and 75% Iberian: purebred Iberian dam × (purebred Iberian dam × purebred Duroc sire). This proportion has been maintained or slightly increased over the last years (*MAPA, 2021*). The Iberian pig has a long productive cycle and a slow growing rate. It is characterized by a low capacity for protein deposition and high adiposity, which is accentuated as animals go further in their productive cycle (*Barea et al., 2007; Conde-Aguilera et al., 2011; Nieto et al., 2012*). Iberian meat products are very appreciated due to their outstanding high-quality (*Nieto et al., 2019*) and their consumption has increased more than 20 % in the lasts years (*MAPA, 2021*). Animals are slaughtered at 160 kg BW or more (*Serrano et al., 2009*) and surgical castration of males during the first days of life is a common practice to prevent boar taint -an unpleasant odour and flavour- in fresh meat and cured products.

Immunocastration, animal welfare and performance in the Iberian pig

The traditional solution to avoid boar taint and aggressive behavior in male pigs has been their surgical castration (*Needham and Hoffman, 2015; Čandek-Potokar et al., 2017*). Boar taint can appear in meat from mature male pigs due to accumulation of androstenone and skatole in adipose tissue. Androstenone, like other testicular steroids, is produced in the Leydig cells, and its production is under control of luteinizing hormone (LH). Synthesis and release of LH and follicle stimulating hormone (FSH) are controlled by gonadotropin-releasing hormone (GnRH). Skatole is produced by the microbial degradation of tryptophan in the large intestine of pigs (*Zamaratskaia et al. 2008*). Surgical castration has been also traditionally applied in females reared under extensive conditions to avoid unwanted mating and the risk of disease transmission through the contact with wild boars (*Martinez-Macipe et al. 2016*). Surgical castration prevents the accumulation of androstenone and skatole, however, it is associated with some productivity disadvantages, as it stops the synthesis of testicular steroids including testosterone and estrogens and thus adversely affects lean tissue growth (*Bauer et al. 2009*). In addition, this practice is frequently applied without anesthesia or analgesia which makes it an important animal welfare issue for many European citizens (*Kress et al. 2019*).

Production of entire males and immunocastration are currently available alternatives to surgical castration. Nevertheless, raising boars is not applicable to production systems based on heavy pig production and focused on high-quality products (*Bonneau et al., 2018*). Immunocastration was approved for the use in males in the EU in 2009 and for females in Spain in 2014. The product commonly used was developed in Australia and it is currently approved in more than 60 countries around the world (*Zamaratskaia and Rasmussen*, *2015*). The compound is a synthetic GnRF antigen that, together with a carrier protein, acquires the necessary immunogenicity to act as a vaccine. Its administration stimulates antibody production that neutralize GnRF, responsible for regulating testicular function at the hypothalamus (*Čandek-Potokar et al. 2017*). Vaccination inhibits sex hormones production by the testes in males (*Dunshea et al., 2001; Brunius et al., 2011; Batorek et al., 2012*) and suppresses the ovarian cycle in females (*Oliver et al., 2003; Bohrer et al., 2014*) without any intrinsic hormonal or chemical activity (*Dunshea et al., 2001*). Some metabolic changes are induced leading to alterations in behavior (reduced aggression, increased appetite and food consumption) and growth rate (*Čandek-Potokar et al., 2017*).

The GnRH vaccination protocol involves the subcutaneous administration of two doses at least 4 weeks apart, the last one usually 4 to 6 weeks prior to slaughter. Immunocastrated (IC) male pigs show higher performance than surgically castrated (SC) pigs before the second vaccination dose (*Millet et al., 2011; Batorek et al., 2012; Dunshea et al., 2013*). In addition to clear animal welfare advantages, this performance benefit could be relevant for Iberian and other autochthonous pig breeds characterised by reduced capacity for lean growth (*Nieto et al., 2012*).

Data available on the effects of immunocastration on the performance of the Iberian pigs are very scarce. Studies carried out in crossbred Iberian females (Duroc × Iberian) showed that after two vaccinations, IC females had greater feed intake and average daily gain compared to entire and SC females, but lower feed efficiency than the latter group. The weight of prime cuts was higher in entire and IC females, therefore, the authors considered SC the least interesting option (*Gómez-Fernandez et al., 2013*). In a recent study (*Palma-Granados et al. 2021*) we compared the performance in pure Iberian (Silvela strain) IC males and females with SC males, which are considered the standard for Iberian pig production. IC pigs were vaccinated at 18 and 25 weeks of age and all pigs were slaughtered at 105 kg body weight, 5 weeks after the second vaccination. IC males grew at higher rate and more efficiently than SC males before the second vaccination against GnRH. Iberian IC females showed similar growth performance to SC males. Although differences between sex groups disappeared after the second dose, daily gain and feed efficiency were still higher in IC males compared with the rest of groups considering the whole fattening period. Regarding carcass yield, it was lower in IC v. SC due to higher viscera proportions in IC, including sexual organs. Some lean carcass components as loin, sirloin and butt lean were greater in IC compared with SC males and IC females, with no differences between the last two groups. Kidney fat and backfat thickness at different locations decreased, and carcass length increased, in IC males compared with SC males and IC females (*Palma-Granados et al. 2021*). *Martínez-Macipe et al. (2016)* reported carcass traits in Iberian (Valdesequera line) IC females, IC males and SC males slaughtered at 150 kg BW. These authors found similar carcass traits of SC males and IC females, both of them were different from IC males, in line with our observations, despite differences in slaughter BW and vaccination protocols between studies. The responses found in growth performance and carcass traits are, in general, in agreement with the information available for conventional IC and SC pigs (*Millet et al., 2011; Batorek et al., 2012, Dunshea et al., 2013*).

Immunocastration and metabolic changes in Iberian pigs

Besides the animal welfare benefits of immunocastration, the increased performance observed between both vaccination doses could be an additional asset for Iberian -and native pig breeds in general- which show decreased rates of growth, particularly of lean tissue growth, compared with conventional pig breeds (*Nieto et al., 2012*). Few studies focused on the dynamics of nitrogen retention in IC pigs. *Huber et al. (2014)* found that protein deposition decreased in IC from levels observed in entire males to approach those of SC from 2-3 weeks after the second vaccination dose. Based on their metabolic characteristics, nutrition programmes specifically designed for IC pigs of conventional genotypes have been suggested (*Elsbernd et al., 2015, 2017; Moore et al., 2016*).

No information on the immunocastration influence on dynamics of nitrogen retention is available for autochthonous breeds, apart from the mentioned paper in Iberian pigs (*Palma-Granados et al, 2021*). In this study, performed with 54 Iberian males and gilts following identical nutritional and management protocols, two nutrient digestibility and nitrogen balance assays were performed, one before

and one after the second vaccination dose (at 60 and 95 kg BW, respectively). Total tract apparent digestibility of nutrients and nitrogen retention were determined to check the hypothesis of different potential for protein deposition between IC and SC Iberian pigs. Pigs were reared in intensive conditions until approximately 105 kg BW, resembling production conditions prior to outdoor finishing (*montanera*). Increased nitrogen retention was observed in Iberian IC males v. SC males before the second vaccination -when IC pigs are physiologically similar to entire males- which was in line with the greater growth rate and feed efficiency detected in IC males during this period (*Palma-Granados et al., 2021*). Efficiency of nitrogen retention was the highest in IC males while no changes in nitrogen digestibility were observed. Despite differences in protein deposition capacity and efficiency between genotypes – being lower in the Iberian breed- these results are in agreement with observations in leaner breeds described by *Elsbernd et al. (2015)* before the second vaccination dose, and *Huber et al. (2014)* from day 1 to 7 after de second dose. Before the second dose of the vaccine, IC males are metabolically similar to boars, with higher lean growth than SC due to the anabolic potential of circulating androgenic steroids (*Dubois et al., 2012*). Nevertheless, two weeks after the second vaccination, differences in protein deposition (13% higher in Iberian IC males before the second vaccination) and in efficiency of nitrogen retention between IC and SC males disappeared, in agreement with nitrogen retention dynamics described for leaner breeds after the second vaccination (*Metz et al., 2002; Huber et al, 2014; Elsbernd et al., 2015*). Regarding Iberian IC females, both protein deposition and efficiency of nitrogen retention were intermediate between IC and SC males before the second vaccination, and differences between all sex groups disappeared two to three weeks after (*Palma-Granados et al. 2021*). No information regarding protein deposition in IC females have been found in the literature. *Elsbernd et al. (2015)* reported similar protein retention and efficiency for protein retention in gilts and SC male pigs, that were both lower than values from IC males before the second vaccination.
The analysis of some plasma metabolites and hormones at slaughter support in part the increased performance and nitrogen retention capacity observed in IC male Iberian pigs compared to SC males and IC females, which can be attributed to increased anabolism in Iberian IC males (*Fernández-Fígares et al. 2019, 2020).*

Immunocastration and meat quality in Iberian pigs

Iberian pig meat and cured products are highly appreciated by consumers and achieve elevated market prices (*Mesias et al. 2009; García-Gudiño et al., 2021*). The amount and composition of intramuscular fat (IMF) and the concentration of heme pigments have been highlighted among important factors influencing the quality of Iberian meat (*Ventanas et al., 2006*). The concentration and fatty acid profile of IMF are considered major factors affecting meat organoleptic characteristics (*Wood et al., 2008*), and positive relationships have been established between acceptability or tenderness of pork and IMF content (*Font-i-Furnols et al., 2012*).

Most of the information regarding the effects of immunocastration on meat quality in conventional pig types indicates that meat quality attributes are essentially similar in IC and SC pigs. In addition, compared with meat from entire males, meat from IC presents better quality as, apart from no sexual odour, it has more IMF and it is more tender (*Batorek et al., 2012; Čandek-Potokar et al., 2017*). Nevertheless, some disadvantages have also been reported, such as greater drip loss in meat from IC than in entire males and in SC in particular (*Batorek et al., 2012*).

Iberian pigs are slaughtered at higher weight and age than conventional fatteners and it is essential that vaccination effects are maintained until slaughter. In crossbred Iberian × Duroc females slaughtered 14.5 weeks after the second vaccination, the quality of the fresh loin in terms of color, pH, drip loss and composition, were similar in IC compared to entire and SC females (*Gamero-Negrón et al., 2015a*). The same authors did not find differences in the physical-chemical composition of cured shoulders from IC, SC and entire females (*Gamero-Negrón et al., 2015b*), although cured loins from IC and SC had greater IMF compared to cured loins from entire females. Higher scores for sensory characteristics (brightness, marbling, chewing and juiciness) were obtained in cured loins from the IC group compared with SC and entire females (*Gamero-Negrón et al., 2018*).

In studies on heavy pigs of conventional breeds (*Pinna et al., 2015*) and on pure Iberian pigs (*Martinez-Macipe et al., 2016*) a three-dose protocol was also used to prevent the setback of the vaccination effects. In *Martinez-Macipe et al. (2016)*, carcass and meat quality traits in Iberian IC, SC and entire females and IC and SC males (Valdesequera line), reared in free-ranging conditions, were compared. Pigs were slaughtered at 16 months of age (156 kg BW) two months after the last vaccination (at 11, 12 and 14 months). Carcass and meat quality traits were very similar among all female groups, therefore, vaccination or surgical castration did not influence quality traits in this category. In IC males, final pH and IMF content were lower and shear force and rancidity higher than in SC males. IC males presented some quality attributes clearly different from the rest of sex groups, being SC males similar to female categories. These results agree with our observations from the aforementioned study (*Palma-Granados et al. 2021*) in which a two-dose protocol (at 4.5 and 6.3 months) was applied to younger pure Iberian (Silvela line) pigs -IC males and females and SC pigs- slaughtered 5 weeks after the second dose (105 kg BW). Muscles from IC males were less red and had lower IMF content than those from SC and IC females and IC loins presented the highest drip losses. Muscles from IC males also showed the highest proportions of polyunsaturated fatty acids (*Seiquer et al. 2019*). In a recent study, *Font-i-Furnols et al. (2021)* evaluated carcass fatness, ham tissue composition and meat quality in pure male Iberian pigs (Valdesequera line) either under early or late vaccination protocols (at 4.5, 5.5 and 9, v. 11, 12 and 14 months of age, respectively), slaughtered at 17 months of age. They concluded that although the effects of IC protocol should be further confirmed, early vaccinated pigs had greater carcass weight, fatter carcasses and a more marbled meat than late vaccinated pigs at the same slaughter age; nevertheless, these differences did not affect meat quality and acceptability by consumers.

Although more research is needed to further define vaccination protocols for heavy pigs (time frame and dose number), the available data indicates that regarding product quality, immunocastration is a good choice for females pigs raised in outdoor conditions. For IC male pigs the product obtained seems to have somewhat lower quality standards than in SC pigs.

In summary, for Iberian pigs -and autochthonous breeds in general- in addition to the unquestionable advantages for animal welfare and the elimination of boar taint in meat products, the practice of immunocastration opens the possibility of a window of transient increased growth performance and potential for lean growth. These traits are relevant for pig breeds with limited capacity to deposit protein. Through nutritional manipulation it could be possible to increase the lean tissue mass deposited during the growing phase in which feeding can be controlled, before finishing outdoors. On the other hand, during the outdoor period feeding is not controlled and growth is based mainly on lipid deposition. However, effects of immunocastration on the quality of the meat from males need further research and development of appropriate vaccination strategies, since high quality standards and customer acceptance are key issues in Iberian pig production.

Uticaj imunokastracije iberijskih svinja na proizvodne performanse, metabolizam i kvalitet mesa

Patricia Palma-Granados, Ignacio Fernández-Fígares, Isabel Seiquer, Manuel Lachica, Luis Lara, Ana Haro, Rosa Nieto

Rezime

Imunokastracija je alternativa hirurškoj kastraciji muških svinja radi sprečavanja mirisa nerastova u mesnim proizvodima. Postupak je izvodljiv za autohtone rase uzgajane na otvorenom do velikih težina radi dobijanja visokokvalitetnih proizvoda, u kojima proizvodnja sa nekastriranim nerastovima nije opcija. Imunokastrirane svinje bi mogle imati koristi od većeg potencijala rasta tokom perioda porasta, pored značajnog poboljšanja dobrobiti životinja. Potrebna su dalja istraživanja kako bi se osmislili posebni protokoli vakcinacije za teške svinje uzimajući u obzir važnost očuvanja najvišeg standarda kvaliteta u mesnim proizvodima.

Ključne reči: dobrobit životinja, performanse rasta, iberijska svinja, imunokastracija, kvalitet mesa, zadržavanje proteina

Acknowledgements

Supported by European project H2020 TREASURE (GA No. 634476). The text reflects only the opinion of the authors. The European Union is not responsible for any use that may be made of the information it contains. We are also grateful to Sánchez Romero Carvajal Jabugo S.A. (El Puerto de Santa María, Cádiz, Spain) for providing purebred Iberian pigs, and to Miguel Liñán Fernández and Alfonso García Écija for their skillful animal and laboratory work.

References

BAREA R., NIETO R., AGUILERA J.F. (2007): Effects of the dietary protein content and the feeding level on protein and energy metabolism of Iberian pigs growing from 50 to 100 kg. Animal 1, 357-365.

BATOREK N., CANDEK-POTOKAR M., BONNEANU M., VAN MILGEN J. (2012): Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal 6, 1330-1338.

BAUER A., LACORN M., CLAUS R. (2009): Effects of two levels of feed allocation on IGF-I concentrations and metabolic parameters in GnRH-immunized boars. Journal of Animal Physiology and Animal Nutrition 93:744–753.

BOHRER B.M., FLOWERS W.L., KYLE J.M., JOHNSON S.S., KING V.L., SPRUILL J.L., THOMPSON D.P., SCHROEDER A.L., BOLER D.D. (2014): Effect of gonadotropin releasing factor suppression with an immunological on growth performance, estrus activity, carcass characteristics, and meat quality of market gilts. Journal of Animal Science 92, 4719–4724.

BONNEAU M., CANDEK-POTOKAR M., SKRLEP M., FONT-I-FURNOLS M., ALUWÉ M., THE CASTRUM NETWORK, FONTANES I L. (2018): Potential sensitivity of pork production situations aiming at high-quality products to the use of entire male pigs as an alternative to surgical castrates. Animal 12, 1287-1295.

BRUNIUS C., ZAMARATSKAIA G., ANDERSSON K., CHEN G., NORRBY M., MADEJ A., LUNSTROM K. (2011): Early immunocastration of male pigs with Improvac® . Effect on boar taint, hormones and reproductive organs. Vaccine 29, 9514–9520.

CANDEK–POTOKAR M., ŠKRLEP M., ZAMARATSKAIA G. (2017): Immunocastration as alternative to surgical castration in pigs, Theriogenology, Payan Carreira R. (Ed.), InTech, DOI: 10.5772/intechopen.68650.

CONDE-AGUILERA J.A., AGUINAGA M.A., AGUILERA J.F., NIETO R. (2011): Nutrient and energy retention in weaned Iberian piglets fed diets with different protein concentrations. Journal of Animal Science 89, 754-763.

DUBOIS V., LAURENT M., BOONEN S., VANDERSCHUEREN D., CLAESSENS F. (2012): Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions. Cellular and Molecular Life Sciences 69, 1651–1667.

DUNSHEA F.R., ALLISON J.R.D., BERTRAM M., BOLER D.D., BROSSARD L., CAMPBELL R., CRANE J.P., HENNESSY D.P., HUBER L., DE LANGE C., FERGUSON N., MATZAT P., MCKEITH F., MORAES P.J., MULLAN B.P., NOBLET J., QUINIOU N., TOKACH M. (2013): The effect of immunization against GnRF on nutrient requirements of male pigs: a review. Animal 7, 1769-1778.

DUNSHEA F.R., COLANTONI C., HOWARD K., MCCAULEY I., JACKSON P., LONG K.A., HENNESSY D.P. (2001): Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. Journal of Animal Science 79, 2524–2535.

ELSBERND A.J., DE LANGE C.F.M., STALDER K.J., KARRIKER L.A., PATIENCE J.F. (2017): SID lysine requirement of immunologically and physically castrated male pigs during the grower, early and late finisher periods. Journal of Animal Science 95, 1253-1263.

ELSBERND A.J., STALDER K.J., KARRIKER L.A., PATIENCE J.F. (2015): Comparison among gilts, physical castrates, entire males, and immunological castrates in terms of growth performance, nitrogen and phosphorous retention, and carcass fat iodine value. Journal of Animal Science 93, 5702-5710.

FERNANDEZ-FIGARES I., LACHICA M., SEIQUER I., LARA L., HARO A., NIETO R. (2019): Effect of immunocastration and dietary protein on plasma

metabolites and hormone concentrations of Iberian pigs. Energy and Protein Metabolism and Nutrition (Ed. Mario Luiz Chizzoti), Wageningen Academic Publishers, EAAP publication No. 138, 367-368.
FERNÁNDEZ-FÍGARES I., LARA L., LACHICA M., SEIQUER I., HARO A., NIETO R. (2020): Effects of immunocastration on plasma free amino acid concentration of Iberian pigs. 71st EAAP Virtual Meeting of the European Federation of Animal Science, Book of Abstracts nº 26, 131.
FONT-I-FURNOLS M., TOUS N., ESTEVE-GARCIA E., GISPERT M. (2012) : Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. Meat Science 91, 448-453.
FONT-I-FURNOLS M., GARCÍA-GUDIÑO J., IZQUIERDO M., BRUN A., GISPERT M., BLANCO-PENEDO I., HERNÁNDEZ-GARCÍA F.I. (2021): Non-destructive evaluation of carcass and ham traits and meat quality assessment applied to early and late immunocastrated Iberian pigs. Animal 15, 100189.
GAMERO-NEGRÓN R., GARCÍA C., REINA R., SÁNCHEZ DEL PULGAR J. (2018): Immune-spaying as an alternative to surgical spaying in Iberian x Duroc females: Effect on the sensory traits and volatile organic compound profile of dry-cured shoulders and dry-cured loins. Meat Science 143, 237-241.
GAMERO-NEGRÓN R., SÁNCHEZ DEL PULGAR J., GARCÍA C. (2015b): Immune-spaying as an alternative to surgical spaying in Iberian × Duroc females: Effect on quality characteristics and fatty acid profile in dry-cured shoulders and loins. Meat Science 104, 52–57.
GAMERO-NEGRÓN R., SÁNCHEZ DEL PULGAR J., VENTANAS J, GARCÍA C. (2015a): Immune-spaying as an alternative to surgical spaying in iberian × Duroc females: Effect on carcass traits and meat quality characteristics. Meat Science 99, 99-103.
GARCÍA-GUDIÑO J., BLANCO-PENEDO I., GISPERT M., BRUN A., PEREA J., FONT-I-FURNOLS M. (2021): Understanding consumers' perceptions towards Iberian pig production and animal welfare. Meat Science 172, 108317.
GÓMEZ-FERNÁNDEZ J., HORCAJADA S., TOMÁS C., GÓMEZ-IZQUIERDO E., DE MERCADO E. (2013): Efecto de la inmunocastración y de la castración quirúrgica sobre los rendimientos productivos y la calidad de la canal en cerdas ibéricas de cebo (The effect of immunocastration and surgically castration on growth performance and carcass quality in fattening period of Iberian female pigs). ITEA, 109, 33–48.
HUBER L., SQUIRES E.J., DE LANGE C.F.M. (2014) : Dynamics of nitrogen retention in entire male pigs immunized against gonodatropin-releasing hormone. Journal of Animal Science 91, 4817-4825.

KRESS K., MILLET S., LABUSSIÈRE E., WEILER U., STEFANSKI V. (2019): Sustainability of pork production with immunocastration in Europe. Sustainability 11, 3335.

LÓPEZ-BOTE C. (1998): Sustained utilization of the Iberian pig breed. Meat Science 49, S1, S17-S27.

MARTÍNEZ-MACIPE M., RODRÍGUEZ P., IZQUIERDO M., GISPERT M., MANTECA X., MAINAU E., DALMAU A. (2016): Comparison of meat quality parameters in surgical castrated versus vaccinated against gonadotrophin-releasing factor male and female Iberian pigs reared in free-ranging conditions. Meat Science, 111, 116–121.

MESÍAS F.J., GASPAR P., PULIDO A. F., ESCRIBANO,M., PULIDO F. (2009): Consumers' preferences for Iberian dry-cured ham and the influence of mastfeeding: An application of conjoint analysis in Spain. Meat Science 83 684–690.

METZ C., HOHL K., WAIDELICH S., DROCHNER W., CLAUS R. (2002): Active immunization of boars against GnRH at an early age: Consequences for testicular function, boar taint accumulation and N retention. Livestock Production Science 74, 147-157.

MILLET S., KWINTEN G., DE BRABANDER D., JANSSENS G.P.J. (2011): Considerations on the performance of immunocastrated male pigs. Animal 5, 1119-1123.

MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACION (MAPA) (2021): https://www.mapa.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/sectores-ganaderos/porcino/

MOORE K.L., MULLAN B.P., KIM J.C., DUNSHEA F.R. (2016): Standardized ileal digestible lysine requirements of male pigs immunized against gonadotrophin releasing factor. Journal of Animal Science 94, 1982-1992.

NEEDHAM T., HOFFMAN L.C. (2015): Physical meat quality and chemical composition of the Longissimus thoracis of entire and immunocastrated pigs fed varying dietary protein levels with and without ractopamine hydrochloride. Meat Science, 110, 101–108.

NIETO R. GARCÍA-CASCO J.M., LARA L., PALMA-GRANADOS P., IZQUIERDO M., HERNANDEZ F., DIEGUEZ E., DUARTE J.L., BATOREK-LUKAČ N. (2019): Ibérico (Iberian) Pig, In: European Local Pig Breeds - Diversity and Performance. A study of project TREASURE (Ed. M. Candek-Potokar and R. M. Nieto Linan), DOI: http://dx.doi.org/10.5772/intechopen.83765

NIETO R., LARA L., BAREA R., GARCÍA-VALVERDE R., CONDE-AGUILERA J.A., AGUILERA J. F. (2012): Response analysis of the Iberian pig growing from birth to 150 kg body weight to changes in protein and energy supply. Journal of Animal Science 90, 3809-3820.

OLIVER W.T., MCCAULEY I., HARRELL R.J., SUSTER D., DUNSHEA F.R. (2003): A GnFR vaccine (Improvac ®) and porcine somatotropin have synergistic and additive effects on growth performance in group-housed boars and gilts, respectively. Journal of Animal Science 81, 1959-1966.

PALMA-GRANADOS P., LARA L., SEIQUER I., LACHICA M., FERNÁNDEZ-FÍGARES I., HARO A., NIETO R. (2021): Protein retention, growth performance and carcass traits of individually housed immunocastrated male- and female- and surgically castrated male Iberian pigs fed diets of increasing amino acid concentration. Animal 15, 100187.

SEIQUER I., PALMA-GRANADOS P., HARO A., LARA L., LACHICA M., FERNANDEZ-FIGARES I., NIETO R. (2019): Meat quality traits in longissimus lumborum and gluteus medius muscles from immunocastrated and surgically castrated Iberian pigs. Meat Science 150, 77-84.

SERRANO M. P., VALENCIA D. G., FUENTETAJA A., LÁZARO R., MATEOS G. G. (2009): Effect of castration on productive performance, carcass characteristics and meat quality of Iberian pig females reared under intensive management systems. Livestock Science, 123, 147–153.

VENTANAS S., VENTANAS J., JURADO A., ESTÉVEZ M. (2006): Quality traits in muscle biceps femoris and back-fat from purebred Iberian and reciprocal Iberian×Duroc crossbred pigs. Meat Science, 73, 651-659.

WOOD J.D., ENSER M., FISHER A.V., NUTE G.R., SHEARD P.R., RICHARDSON R.I., HUGHES S.I., WHITTINGTON F.M. (2008): Fat deposition, fatty acid composition and meat quality: a review. Meat Science 78, 343–358.

ZAMARATSKAIA G., RYDHMER L., ANDERSSON K., CHEN G., LOWAGIE S., ANDERSSON K., LUNDSTROM K. (2008): Long-term effect of vaccination against gonadotropin-releasing hormone, using ImprovacTM, on hormonal profile and behaviour of male pigs. Animal Reproduction Science 108, 37–48.

ZAMARATSKAIA G., RASMUSSEN M.K. (2015): Immunocastration of male pigs: situation today. Procedia Food Science, 5, 324-327.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

RAISING MALE PIGS AS AN ALTERNATIVE TO SURGICAL CASTRATION

Galia Zamaratskaia[1], Andriy Getya[2]

[1]Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences,750 07 Uppsala, Sweden
[2]National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
Corresponding author: Galia Zamaratskaia; galia.zamaratskaia@slu.se
Invited paper

Abstract: Castration of male piglets is performed in many European countries to reduce boar taint. However, a more animal friendly alternatives are available. The present work aims to characterize current alternatives to reduce boar taint with special emphasis on nutrition factors. There have been numerous attempts to find an alternative diets for entire male pigs, which would fulfil nutritional requirements and reduce skatole and eventually androstenone levels without compromising animal welfare. The level of boar taint can be reduced by including non-digestible carbohydrates (to reduce skatole) and by including adsorbent materials (to reduce androstenone) into the pig's diet. Further research to optimize diet and evaluate the effect of dietary supplements on pig's health and welfare are required.

Key words: swine, castration, nutrition, animal welfare, consumers

Introduction

Animal welfare aspects and the question of whether the male piglets should be surgically castrated to eliminate boar taint have caused debate across Europe. Surgical castration without anesthesia and analgesia are considered unacceptable among many consumers *(Fredriksen et al., 2011, Sødring et al., 2020)*. Possible alternatives to surgical castration are widely discussed. The alternatives to surgical castration of pigs includes raising entire (uncastrated) male pigs or immunocastration, both being considered as the welfare-friendly alternatives for the pigs. Immunocastration is a technique based on the use of a vaccine, which is far less invasive than surgical castration and reduces the levels of both main boar taint compounds, skatole and androstenone *(Zamaratskaia et al., 2008a, Zamaratskaia et al., 2008b)*. Many consumers are, however, doubtful about immunocastration because of the fear of residuals in meat and unknown long-term effect of using the vaccine for the consumers *(Aluwé et al., 2020, Fredriksen et al., 2011)*. Moreover, production of entire male pigs can result in a positive effects on the production and carcass composition *(Lundström et al., 2009)*. However, to expand the production of entire male pigs for meat industry, the methods to minimize boar taint should be developed. These methods should be cost-efficient and reliable, and should not negatively affect animal health and welfare. Some progress has recently been made in development of the alternative methods to minimize boar taint without need of surgical and immunocastration.

The aim of the present review is to summarize, compare and discuss the methods to reduce the accumulation of boar taint compounds in pigs, address possible limitations of these methods and suggest directions of further research.

Boar taint compounds

The compounds androstenone, skatole and indole are mainly responsible for the risk of boar taint development in meat from some entire male pigs *(Zamaratskaia and Squires, 2009)*. Skatole and indole result from microbial degradation of the amino acid tryptophan in the pig intestine *(Wesoly and Weiler, 2012)* whereas androstenone is synthesised in the testes in parallel with anabolic hormones such as testosterone and oestrogens *(Zamaratskaia and Squires, 2009)*. Androstenone itself has no hormonal activity.

The ability to smell androstenone highly varies among consumers, ranging from no sensation to a description of a very unpleasant urine-like smell. Generally, women are more sensitive to androstenone than men, and androstenone perception is genetically regulated *(Lundström et al., 2009)*. Almost all consumers can detect the smell of skatole and describe it as an unpleasant faecal-like smell. Research on indole is currently limited, but the odour of indole is known to be weaker compared to skatole. High levels of boar taint compounds in meat from entire male pigs leads to lower acceptability ratings in meat, at least for sensitive consumers.

To satisfy the consumer’s needs, the levels of all boar taint compounds should be reduced. Nutrition, hormonal status and genetic background are important factors to determine the final concentrations of boar taint compounds.

Ability of different dietary supplements to reduce the levels of boar taint in pigs

Given that skatole and indole originate from tryptophan in the large intestine, it is not surprising that dietary composition is an important factor affecting skatole and indole production. Non-digestible carbohydrates are known to reduce intestinal production of skatole *(Øverland et al., 2011)*. These carbohydrates escape digestion in the small intestine and serve as an energy substrate for bacteria in the large intestine *(Pieper et al., 2014)*. Thus, high levels of non-digestible carbohydrates can lead to increased incorporation of skatole precursor tryptophan into the bacteria *(Wesoly and Weiler, 2012)*. Low levels of non-digestible carbohydrates can lead to use of tryptophan as energy source for proteolytic bacteria and skatole formation. Additionally, non-digestible carbohydrates increase faecal weight and decrease intestinal transit time leading to a reduction of the absorption rate of skatole from the gut. Importantly, those carbohydrates have also potential implications for animal health with regard to intestine conditions *(Peled and Livney, 2021)*.

Dietary supplement of raw potato starch repeatable reduced skatole and indole levels in porcine tissues in castrated and entire male pigs *(Lösel and Claus, 2005, Zamaratskaia et al., 2005)*. This reduction is explained by the inhibition of cell apoptosis in the colon and thus reduced tryptophan availability for skatole production *(Claus et al., 2003)*. Butyrate is formed in high quantities when the supply of resistant starch is high and causes a reduction in apoptosis of epithelial cells thus decreasing availability tryptophan for skatole and indole synthesis *(Claus et al., 2003)*. Feeding trials demonstrated that 20% for potato starch is needed to achieve a measurable skatole reduction in the adipose tissue and blood *(Zamaratskaia et al., 2005)*.

Many studies successfully attempted to reduce boar taint levels by inulin or fructooligosaccharides *(Aluwé et al., 2017, Rasmussen et al., 2012, Salmon and Edwards, 2015)*. For example, feeding pigs with Jerusalem artichoke (8.1 and 12.2%) as a source of inulin reduced the level of skatole in the adipose tissue without affecting indole *(Okrouhlá et al., 2020, Vhile et al., 2012)*. Addition of 1.0 and 1.5% fructooligosaccharides to the pig fecal slurries reduced the rate of tryptophan degradation *(Xu et al., 2002)*.

Another promising additive to reduce boar taint is hydrolysable tannins, the group of astringent polyphenolic compounds widely distributed in plants. Hydrolysable tannins were suggested to reduce skatole and indole production by an inhibition of the total activity of caecal bacteria *(Biagi et al., 2010)*. Several studies demonstrated the potential of tannin supplementation to reduce boar taint although the optimal dose is still to be established *(Bahelka et al., 2021, Čandek-Potokar et al., 2015, Tretola et al., 2019)*. Importantly, supplementing the diet of pigs with tannins had no adverse effects on animal growth and performance at 1% or 2% of feed, however higher dose of tannins might result in reduced feed intake *(Čandek-Potokar et al., 2015)*. This is not surprising because tannins have long been considered as antinutritrients and were shown to reduce feed intake and feed efficiency. Furthermore, supplementing the diet with tannins had no effect on amino acid composition and fatty acid composition in fat *(Bahelka et al., 2021)*. Hydrolysable tannins (1% chestnut-tannin extract) might also be a good candidate for decreasing the risk of development of postweaning diarrhea *(Girard et al., 2018)*.

Dietary protein with a low precaecal digestibility increased availability of tryptophan in the hindgut which leaded to high skatole formation *(Claus and Raab, 1999)*.

Thus, majority of studies were focused on the reduction of skatole intestinal production. Less is known about how the feed components can affect skatole metabolism. It is known that the activity of cytochrome P450, enzymes which mediate the metabolic transformation of skatole *(Rasmussen and Zamaratskaia, 2014)*, can be either inhibited or up-regulated by bioactive compounds in feed. For example, increased hepatic CYP2E1 activity or protein expression in pigs has been observed as a result of high-fat/high-cholesterol diets *(Puccinelli et al., 2013)*, dried chicory root *(Rasmussen et al., 2011)* and sugar beet supplementation *(Whittington et al., 2004)*. Furthermore, dietary supplement with hydrolysable tannins induced activities of CYP2A19 and CYP2E1 in entire male pigs *(Čandek-Potokar et al., 2015)*. However, more studies are needed to find a safe dietary supplement which can enhance skatole metabolism through induction of the activities of cytochrome P450.

The use of adsorbent materials to increase faecal excretion of boar taint compounds through the intestinal binding recently attracted research interest. Feeding 5.0% activated carbon or 5.0% tween-60 for 28 days reduced the levels of androstenone in both adipose tissue and plasma of the entire male pigs, whereas no obvious effects on skatole levels were observed probably because of overall low skatole levels in the investigated pigs *(Jen and Squires, 2011a)*. The observed reduction in androstenone levels was explain by the fact that androstenone can re-enter the blood from the intestines via enterohepatic circulation. Androstenone is removed through liver degradation, excreted into the bile and then can be recirculated back through the enterohepatic circulation. Absorbent material binds androstenone in the intestine and remove it with the faeces, thus preventing it to be recirculated. The authors concluded that addition of activated carbon or Tween to the diet can reduce androstenone levels of adipose tissue and plasma, but further research is required to investigate the effects of these absorbent materials on reducing skatole levels. Activated carbon were able to reduce skatole levels *in vitro*, where the pH, temperature and transit time were chosen to simulate physiological conditions *(Jen and Squires, 2011b)*. The use of biochar, which is more economical compared to activated carbon, as a dietary supplement did not decrease either skatole or indole concentrations either in faeces or plasma

(Schubert et al., 2021). In that study, skatole or indole concentrations were low also in the control group.

Ability of different dietary supplements to reduce the levels of skatole in other species

There is a number of species which do not produce traditional boar taint but might have high skatole levels. It is of importance to investigate scientific literature on the effects of diet on skatole levels in these species. This knowledge might provide useful information and suggest further research regarding reduction of boar taint.

Similar to pigs, skatole and indole are produced during the microbial degradation in the large intestine of chickens with the highest skatole levels observed in the cecum *(Yang et al., 2019)*. Dietary composition is one of the major factors responsible for skatole formation in chickens. Diet supplemented with soybean oligosaccharides (2.0, 3.5 and 5.0 g/kg) reduced the excreta skatole and indole concentrations in broilers *(Liu et al., 2021)*. Similarly, dietary supplementation with soybean oligosaccharide, stachyose, and raffinose reduced skatole and indole concentrations in broiler cecal digesta *(Zhu et al., 2020)*. Some studies demonstrated that the administration of tannins reduced the levels of skatole in chickens *(Choi and Kim, 2020)*.

It was shown that consumption of probiotics decreased skatole and other indolic compound concentrations in mice *(Zhang et al., 2021)*.

A feeding trial using Sprague Dawley rats demonstrated a reduction in caecal skatole and indole levels when including lablab bean husk or soya bean husk in the diet *(Myint et al., 2018)*, whereas animal protein source (the high-chicken-protein or high-pork-protein) diets increased skatole and indole levels in cecal and colonic contents, feces, and adipose tissue of rats *(Shi et al., 2020)*.

Conclusion

There have been numerous attempts to find an alternative diets for entire male pigs, which would fulfil nutritional requirements and reduce skatole and eventually androstenone levels without compromising animal welfare. The level of boar taint can be minimized by including non-digestible carbohydrates (to reduce skatole) and by including adsorbent materials (to reduce androstenone) into the pig’s diet. Further research to optimize diet and evaluate the effect of dietary supplements on pig’s health and welfare are required.

Uzgoj muških svinja kao alternativa hirurškoj kastraciji

Galia Zamaratskaia, Andriy Getya

Rezime

Kastracija muške prasadi vrši se u mnogim evropskim zemljama kako bi se smanjio miris nerasta. Međutim, dostupne su alternative prilagođenije životinjama. Ovaj rad ima za cilj da opiše postojeće alternative za smanjenje mirisa svinja sa posebnim naglaskom na faktore ishrane. Bilo je mnogo pokušaja da se pronađe alternativna ishrana za nekastrirane nerastove, koja bi ispunila nutritivne potrebe i smanjila nivo skatola i na kraju androstenona bez ugrožavanja dobrobiti životinja. Nivo mirisa može se smanjiti uključivanjem nesvarljivih ugljenih hidrata (za smanjenje skatola) i uključivanjem adsorbujućih materijala (za smanjenje androstenona) u ishranu svinja. Potrebna su dalja istraživanja radi optimizacije ishrane i procene uticaja dodataka ishrani na zdravlje i dobrobit svinja.

Ključne reči: svinje, kastracija, ishrana, dobrobit životinja, potrošači

References

ALUWÉ M., HEYRMAN E., ALMEIDA J. M., BABOL J., BATTACONE G., ČÍTEK J., FONT I. F. M., GETYA A., KAROLYI D., KOSTYRA E., KRESS K., KUŠEC G., MÖRLEIN D., SEMENOVA, A., ŠKRLEP M., STOYANCHEV T., TOMAŠEVIĆ I., TUDOREANU L., VAN SON M., ŻAKOWSKA-BIEMANS S., ZAMARATSKAIA G., VAN DEN BROEKE A., EGEA M. (2020): Exploratory Survey on European Consumer and Stakeholder Attitudes Towards Alternatives for Surgical Castration of Piglets. Animals (Basel), 10.

ALUWÉ M., HEYRMAN E., THEIS S., SIELAND C., THURMAN K., MILLET S. (2017): Chicory Fructans in Pig Diet Reduce Skatole in Back Fat of Entire Male Pigs. Research in Veterinary Science, 115**,** 340-344.

BAHELKA I., BUČKO O., FĽAK P. (2021): Can Hydrolysable Tannins in Diet of Entire Male Pigs Affect Carcass, Pork Quality Traits, Amino and Fatty Acid Profiles, and Boar Taint, Skatole and Androstenone Levels? Animals (Basel), 11.

BIAGI G., CIPOLLINI I., PAULICKS B. R., ROTH F. X. (2010): Effect of Tannins on Growth Performance and Intestinal Ecosystem in Weaned Piglets. Archives of Animal Nutrition, 64**,** 121-135.

ČANDEK-POTOKAR M., ŠKRLEP M., BATOREK LUKAČ N., ZAMARATSKAIA G., PREVOLNIK POVŠE M., VELIKONJA BOLTA Š., KUBALE V., BEE G. (2015): Hydrolysable Tannin Fed to Entire Male Pigs Affects Intestinal Production, Tissue Deposition and Hepatic Clearance of Skatole. The Veterinary Journal, 204**,** 162-167.

CHOI J., KIM W. K. (2020): Dietary Application of Tannins as a Potential Mitigation Strategy for Current Challenges in Poultry Production: A Review. Animals (Basel), 10.

CLAUS R., LÖSEL D., LACORN M., MENTSCHEL J., SCHENKEL H. (2003): Effects of Butyrate on Apoptosis in the Pig Colon and Its Consequences for Skatole Formation and Tissue Accumulation. Journal of Animal Science, 81**,** 239-48.

CLAUS R., RAAB S. (1999): Influences on Skatole Formation from Tryptophan in the Pig Colon. *In:* HUETHER, G., KOCHEN, W., SIMAT, T. J. & STEINHART, H. (eds.) *Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications.* Boston, MA: Springer US.

FREDRIKSEN B., JOHNSEN A. M., SKUTERUD E. (2011): Consumer Attitudes Towards Castration of Piglets and Alternatives to Surgical Castration. Research in Veterinary Science 90**,** 352-7.

GIRARD M., THANNER S., PRADERVAND N., HU D., OLLAGNIER C., BEE G. (2018): Hydrolysable Chestnut Tannins for Reduction of Postweaning Diarrhea: Efficacy on an Experimental Etec F4 Model. PloS one, 13**,** e0197878-e0197878.

JEN K., SQUIRES E. J. (2011a): Efficacy of Non-Nutritive Sorbent Materials as Intestinal-Binding Agents for the Control of Boar Taint. Animal, 5**,** 1814-20.

JEN K., SQUIRES E. J. (2011b): In Vitro Assessment of the Effectiveness of Non-Nutritive Sorbent Materials as Binding Agents for Boar Taint Compounds. Animal, 5**,** 1821-8.

LIU H. Y., LI X., ZHU X., DONG W. G., YANG G. Q. (2021): Soybean Oligosaccharides Attenuate Odour Compounds in Excreta by Modulating the Caecal Microbiota in Broilers. Animal, 15**,** 100159.

LUNDSTRÖM K., MATTHEWS K. R., HAUGEN J. E. (2009): Pig Meat Quality from Entire Males. Animal, 3**,** 1497-507.

LÖSEL D., CLAUS R. (2005): Dose-Dependent Effects of Resistant Potato Starch in the Diet on Intestinal Skatole Formation and Adipose Tissue Accumulation in the Pig. Journal of veterinary medicine. A, Physiology, pathology, clinical medicine 52**,** 209-12.

MYINT H., KISHI H., IWAHASHI Y., SABURI W., KOIKE S., KOBAYASHI Y. (2018): Functional Modulation of Caecal Fermentation and Microbiota in Rat by Feeding Bean Husk as a Dietary Fibre Supplement. Beneficial Microbes, 9**,** 963-974.

OKROUHLÁ M., ČÍTEK J., ŠVEJSTIL R., ZADINOVÁ K., POKORNÁ K., URBANOVÁ D., STUPKA R. (2020): The Effect of Dietary Helianthus Tuberosus L. On the Populations of Pig Faecal Bacteria and the Prevalence of Skatole. Animals : an open access journal from MDPI, 10**,** 693.

PELED S., LIVNEY Y. D. (2021): The Role of Dietary Proteins and Carbohydrates in Gut Microbiome Composition and Activity: A Review. Food Hydrocolloids, 120**,** 106911.

PIEPER R., BOUDRY C., BINDELLE J., VAHJEN W., ZENTEK J. (2014): Interaction between Dietary Protein Content and the Source of Carbohydrates Along the Gastrointestinal Tract of Weaned Piglets. Archives of Animal Nutrition, 68**,** 263-280.

PUCCINELLI E., GERVASI P. G., PELOSI G., PUNTONI M., LONGO V. (2013): Modulation of Cytochrome P450 Enzymes in Response to Continuous or Intermittent High-Fat Diet in Pigs. Xenobiotica, 43**,** 686-98.

RASMUSSEN M. K., BRUNIUS C., ZAMARATSKAIA G., EKSTRAND B. (2012): Feeding Dried Chicory Root to Pigs Decrease Androstenone Accumulation in Fat by Increasing Hepatic 3β Hydroxysteroid Dehydrogenase Expression. The Journal of Steroid Biochemistry and Molecular Biology, 130**,** 90-95.

RASMUSSEN M. K., ZAMARATSKAIA G. (2014): Regulation of Porcine Hepatic Cytochrome P450 - Implication for Boar Taint. Computational and structural biotechnology journal, 11**,** 106-112.

RASMUSSEN M. K., ZAMARATSKAIA G., EKSTRAND B. (2011): In Vivo Effect of Dried Chicory Root (Cichorium Intybus L.) on Xenobiotica Metabolising Cytochrome P450 Enzymes in Porcine Liver. Toxicology Letters, 200**,** 88-91.

SALMON L., EDWARDS S. A. (2015): The Effects of Dietary Fructo-Oligosaccharide Addition on Boar Taint Compounds and Performance in Heavy Slaughter Weight Boars and Gilts. Animal Feed Science and Technology, 207**,** 130-139.

SCHUBERT D. C., CHUPPAVA B., WITTE F., TERJUNG N., VISSCHER C. (2021): Evaluation of Coated Biochar as an Intestinal Binding Agent for Skatole and Indole in Male Intact Finishing Pigs. Animals (Basel), 11**,** 760.

SHI J., ZHAO D., SONG S., ZHANG M., ZAMARATSKAIA G., XU X., ZHOU G., LI C. (2020): High-Meat-Protein High-Fat Diet Induced Dysbiosis of Gut Microbiota and Tryptophan Metabolism in Wistar Rats. Journal of Agricultural and Food Chemistry, 68**,** 6333-6346.

SØDRING M., NAFSTAD O., HÅSETH T. T. (2020): Change in Norwegian Consumer Attitudes Towards Piglet Castration: Increased Emphasis on Animal Welfare. Acta veterinaria Scandinavica, 62, 22-22.
TRETOLA M., MAGHIN F., SILACCI P., AMPUERO S., BEE G. (2019): Effect of Supplementing Hydrolysable Tannins to a Grower-Finisher Diet Containing Divergent Pufa Levels on Growth Performance, Boar Taint Levels in Back Fat and Intestinal Microbiota of Entire Males. Animals (Basel), 9.
VHILE S. G., KJOS N. P., SØRUM H., OVERLAND M. (2012): Feeding Jerusalem Artichoke Reduced Skatole Level and Changed Intestinal Microbiota in the Gut of Entire Male Pigs. Animal, 6, 807-14.
WESOLY R., WEILER U. (2012): Nutritional Influences on Skatole Formation and Skatole Metabolism in the Pig. Animals (Basel), 2, 221-242.
WHITTINGTON F. M., NUTE G. R., HUGHES S. I., MCGIVAN J. D., LEAN I. J., WOOD J. D., DORAN E. (2004): Relationships between Skatole and Androstenone Accumulation, and Cytochrome P4502e1 Expression in Meishan×Large White Pigs. Meat Science, 67, 569-76.
XU Z. R., HU C. H., WANG M. Q. (2002): Effects of Fructooligosaccharide on Conversion of L-Tryptophan to Skatole and Indole by Mixed Populations of Pig Fecal Bacteria. The Journal of general and applied microbiology 48, 83-90.
YANG G., ZHANG P., LIU H., ZHU X., DONG W. (2019): Spatial Variations in Intestinal Skatole Production and Microbial Composition in Broilers. Animal Science Journal, 90, 412-422.
ZAMARATSKAIA G., ANDERSSON H. K., CHEN G., ANDERSSON K., MADEJ A., LUNDSTRÖM K. (2008a): Effect of a Gonadotropin-Releasing Hormone Vaccine (Improvac) on Steroid Hormones, Boar Taint Compounds and Performance in Entire Male Pigs. Reproduction in domestic animals, 43, 351-359.
ZAMARATSKAIA G., BABOL J., ANDERSSON H. K., ANDERSSON K., LUNDSTRÖM K. (2005): Effect of Live Weight and Dietary Supplement of Raw Potato Starch on the Levels of Skatole, Androstenone, Testosterone and Oestrone Sulphate in Entire Male Pigs. Livestock Production Science, 93, 235-243.
ZAMARATSKAIA G., RYDHMER L., ANDERSSON H. K., CHEN G., LOWAGIE S., ANDERSSON K., LUNDSTRÖM K. (2008b): Long-Term Effect of Vaccination against Gonadotropin-Releasing Hormone, Using Improvac, on Hormonal Profile and Behaviour of Male Pigs. Animal Reproduction Science, 108, 37-48.
ZAMARATSKAIA G., SQUIRES E. J. (2009): Biochemical, Nutritional and Genetic Effects on Boar Taint in Entire Male Pigs. Animal, 3, 1508-21.
ZHANG L., ZHANG X. T., JIN P., ZHAO H., LIU X., SHENG Q. (2021): Effects of Oral Administration of Spirulina Platensis and Probiotics on Serum

Immunity Indexes, Colonic Immune Factors, Fecal Odor, and Fecal Flora in Mice. Animal science journal, 92**,** e13593.

ZHU X., LIU J., LIU H., YANG G. (2020): Soybean Oligosaccharide, Stachyose, and Raffinose in Broilers Diets: Effects on Odor Compound Concentration and Microbiota in Cecal Digesta. Poultry Science, 99**,** 3532-3539.

ØVERLAND M., KJOS N. K., FAUSKE A. K., TEIGE J., SØRUM H. (2011): Easily Fermentable Carbohydrates Reduce Skatole Formation in the Distal Intestine of Entire Male Pigs. Livestock Science, 140**,** 206-217.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

SILESIAN PIGS' FATTENERS - THE IDEA FOR HAELTHY PIG AND HIGH QUALITY PORK MEAT

Władysław Migdał[1], Rafał Duś[2], Maria Walczycka[1], Łukasz Migdał[3]

[1]Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 31-149 Kraków, Poland
[2]Śląski tucznik - Spółdzielnia Opolskie Fermy Trzody, Kolnica 92, 49-200 Grodków, Poland
[3]Department of Animal Genetics, Breeding and Ethology, Faculty of Animal Science, University of Agriculture in Kraków, al. Mickiewicza 24/28, 30-059 Kraków; Poland;
Corresponding author:Władysław Migdał, wladyslaw.migdal@urk.edu.pl
Invited paper

Abstract:“Silesian fattener” is a technology of pigs breeding (sows, piglets, fatteners)is applied by the members of the “Opolskie Fermy Trzody” – Kolnica (Opole Fatteners Farms – Kolnica) members. The exception and innovation of the technology of breeding bases on application in swine’s fed of a few additives which cooperating together give different results changing the meat and fat composition and give to pork the positive physico-chemical, culinary and pro-health characteristics in the changed fatty acids profile as favorable to humans. Also such production is favorable for the environment. Beside the higher costs of production the Society members obtain higher, at about 10%, price for reared them pigs. The technology “Silesian Fattener” is an original idea for profitable, environment friendly production of high quality fatteners.

Key words: pigs, Silesian fatteners, breeding technology, effective microorganisms

Introduction

Animal production, especially production of pig fatteners should be recognized as the part of contemporary bio-economy. It should supply the fatteners with a high slaughter value with good quality meat characterized by a high consumption and food production suitability. The high quality of pork is the most important characteristics expected by consumers. The lowering of pigs fatness caused also the decrease in intramuscular fat and, as a consequence, the worsening of meat quality through flavor and tenderness diminishing. The meat without intramuscular fat, after the heat treatment, is dry and tough. The vector of flavor is the fat which forms so named “marbling” of meat. To obtain the high flavor traits the intramuscular fat has to be present. The opinions of dieticians concerning the harmfulness of fat caused consumers to search for lean pork meat so the meat industry forced the breeders to change fatteners standard and to increase their meatiness. The meatiness of fatteners above 60% caused worsening of quality and flavor traits of pork and pork meat products. Also the usage for crossbreeding of high meat content breeds such as Pietrain and Belgium Landrace with the higher stress susceptibility (the gene of stress susceptibility RYR1 causes fastening of glycolytic transformations in the carcass directly after slaughter) caused intensification of PSE meat presence with the high drip loss, pale colour and disturbances in meat tendering processes and as a result the worsening of quality *(Przybylski et al., 2012)*. The lowering of intramuscular and muscular fats caused worsening of meat quality through decrease in its flavor and tenderness. In pursuit of high meatiness of fatteners the carrier of taste and odour has been forgotten and the marbling of meat lost. The optimum level of meatiness which guaranties the good pork quality is 57-58%. Such assumptions were taken into account by Polish Pig Breeders and Producers Association „POLSUS" and „Polskie Mięso"("Polish Meat") Association in the Pork Quality System (PQS), which at 11th of December 2009 was recognized by the Ministry of Agriculture and Development as the country-wide system of food quality *(Blicharski et al., 2010; Krzyżanowski, 2017)*.

Demands and regulations concerning pigs fattening

The pigs breeding should be economically profitable and friendly for the environment. The concentrations of animals (large herds, large farms), especially present in pigs breeding increases nuisance of such production against environment, surrounding and the most of all is unpleasant for next to living inhabitants. The odor nuisance is connected with emission of odors originating from feces, urine of animals and their fed. The mixture of odor is formed from at about 160 identified gaseous compounds (mainly: ammonia, hydrogen sulfide, phenols, aromatic hydrocarbons etc.). It is estimated that the year emission of ammonia, in Poland, is at about 260 thousand tons, where 2/3 originates from animal production. The source of ammonia are animal feces where the decomposition of nitrogen compounds takes place as a result of bacteria and enzymes activity *(Ministerstwo Klimatu - Climate Ministry, 2020)* negative influence of ammonia on the environment caused, in 2001, the issue of EU Directive of national levels of pollutants (National Emissions reduction Commitments (NEC) Directive) and its amendment from 31st December 2016 (*Directive UE 2016/2284, 2016*), according to which each of EU countries has an obligation to diminish the emission of pollutants of established percentage as a ratio obtained (calculated) in 2005 (for example the emission of nitrogen oxides, in

Poland, has to be diminished of 30%). To obtain above aims there is proposed, among others, replacement of fertilizers based on urea with fertilizers based on ammonium nitrate and popularizing of replacement of mineral fertilizers with organic fertilizers – for example with manure *(Directive UE 2016/2284, 2016)*. The animal production and the pigs breeding were imposed on with many regulations to protect the natural environment: Directive 91/676/EEC *(Council Directive 91/676/EEC, 1991)* concerning protection of waters against pollution caused by nitrates of agricultural origin; Directive of the European Parliament and of the Council 2010/75/ UE *(Directive 2010/75/EU, 2010)* issued at 24th November 2010, concerning the industrial emission – IED combined with UE Commission executive decision 2017/302 issued at 15th February 2017 laying down the conclusions concerning the best available techniques (BAT) in reference to intensive breeding of poultry and/or pigs. (BAT) *(Commission Implementing Decision (EU) 2017/302, 2017)*. The Best Available Technique - BAT was defined in art. 2 p. 11 of Council Directive 96/61/CE issued at 24th September 1996 concerning integrated preventing of pollution and of its control *(Council Directive 96/61/EC, 1996)*. Best available techniques' shall mean the most effective and advanced stage in the development of activities and their methods of operation which indicate the practical suitability of particular techniques for providing in principle the basis for emission limit values designed to prevent and, where that is not practicable, generally to reduce emissions and the impact on the environment as a whole.

The regulations concern all installations used in intensive breeding of poultry and pigs where the amount of quarters is above 2000 for fatteners (above 30 kg of weight) or 750 for sows. Such buildings, in Polish conditions, have to obtain the integrated permission for the activity and BAT imposes the necessity of application of environmentally friendly breeding technology and also the fertilizing plans and activities reducing emission of gases and odors. For monogastric animals 65-70% of nitrogen consumed with fed goes back to the environment with manure and urea, so to diminish emission of odors the optimization of animals feeding is undertaken (through diminishing the amount of protein in fed dose of 15-20%; addition of synthetic amino acids; multiphase feeding), there are added to fed some substances which modify the digestion processes (phytobiotic mixtures, alluminosilicates, probiotic formulations); to the manure, slurry are added blends restricting fermentation processes (acidification of slurry; technology of effective microorganisms – EM) *(Ministerstwo Rolnictwa i Rozwoju Wsi - Ministry of Agriculture and Rural Development, 2019)*. The founder of EM technology is Mr Teruo Higa from Japan *(Higa, 1991; 1993; 2001)*. The effective microorganisms (EM) is the composition of at about 80 bacteria cultures (lactic acid bacteria, photosynthetic bacteria and yeasts) introduced to agro- or eco-system to fasten of its biological regeneration and forming of favorable microbiological environment. The usage of EM helps in applying of the best methods of soil disposal such as crops rotation, usage of organic adjuvants, conservation tillage, recycling of the rest of crops and biocontrol of pests. Through proper application of EM formulations the effects of above pro-ecological ways of soil treatment can be strengthen and at the same time are growing the yield and quality of crops, the fertility and productivity of soil and the usage of artificial fertilizers and pesticides is lowered *(Higa and Parr, 1994; Joshi et al., 2019).*

The prohibition of application, in swine breeding, antibiotic growth stimulators (from 1st January 2006) caused introduction of herbs and medicinal plants or formulations obtained from above. The substances contained in these plants have the bacteriostatic and germicidal properties, they stimulate digestion and act as immunoregulators. The herbs have an stimulating influence on immunomodulation functions of the body through, for example, rising of the concentration of immunoglobulins in the blood stream. They have the antivirus, anti-inflammatory, bettering of metabolism properties; rise the excretion of digestion enzymes to the blood stream, have a diastolic effect on smooth muscles of digestive system, neutralize some toxins of bacterial and/or fungi origin, and are also natural antioxidants. The herbal aroma substances acting on taste receptors cause the better imbibition of feds, which are rather not eagerly consumed by animals (post-extraction rapeseed meal, legumes), cause the greater excretion of digestive juices and better usage of fed components for the growth of body mass. The substances contained in herbs and medicinal plants present in fed migrate to sow's milk what is important for the most critical period of piglets rearing – during exchanging of mothers milk into the solid fed. The application of 2% additive to the fed of fatteners influences favorably on some production characteristics and on carcasses musculature and also on dietetic and taste characteristics of pork meat causing the bettering of taste , aroma, tenderness and juiciness of meat. Thanks to application of herbs the animals gain higher body daily weights what, in practice, gives the shortening of breeding period and betters fed usage. The most frequently applied herbs and medical plants are: garlic, oregano, peppermint, sage, lemon balm, camomile, willow bark, nettle, St. John's wort (*Hypericum perforatum*), evening primrose, *Plantago lanceolata*, couch, milfoil, calendula, thyme *(Wenk, 2003; Karwowska et al., 2007; Karwowska et al., 2008; Grela and Kowalczuk-Vasilev, 2010; Semeniuk and Grela, 2011; Radkowska, 2013).*

Silesian pigs fatteners way of breeding

"Silesian fattener" is a technology of pigs breeding (sows, piglets, fatteners)is applied by the members of the "Opolskie Fermy Trzody – Kolnica

(Opole Fatteners Farms – Kolnica) members. The exception and innovation of the technology of breeding bases on application in swine's fed of a few additives (extracts from herbs, cold pressed rape-seed oil, rape-seed oil obtained lecithin, alive probiotic bacteria cultures, some, alive favorable fungi species, low protein feeding based on components without GMO plants – mostly of home country origin) which cooperating together give different results changing the meat and fat composition and give to pork the positive physico-chemical, culinary and pro-health characteristics in the changed fatty acids profile as favorable to humans. Also such production is favorable for the environment. All listed functional additives are introduced to the animal organism with full dose fed mixture with balanced composition during the whole period of fattening or rearing. The parameters of full dose mixture are not a standard – they meaningfully differ from levels proposed by Polish Norms of Feeding of Reared Animals. The innovation lies in creation of mixture which has the very low level of total protein but the proportions and levels of essential amino-acids are very high. The amount of the most important amino-acid – lysine is calculated based on energy level in fed expressed in MJ. The feeding in this technology is based on leading to the superata of energy in animals breeding through application of energy from fat – the cold pressed rape-seed oil in amounts from 0.5 to 4.0% of mixture (Table 1and 2).
The applied herbal mixture contains the dry extracts from origano, sage, garlic, rosemary, chili, thyme and imbir.

Table 1.The additives applied in full dose feed mixtures for pigs in individual periods of breeding and fattening

Silesian fatteners	Rapeseed cold pressed oil kg\1 ton of fed	Lecithin kg\1 ton of fed	Herbal mixture extracts 40 :1 kg\1 ton of fed	Living cultures of probiotic bacteria and yeasts kg\1 ton of fed	Period of feeding
Piglets body weight 7-15 kg	10	1	0,5	3	from 10 kg per month
Weaner body weight16-50 kg	15	2	1	5	whole
Fatteners from 51kg till end of breeding	20	3	1,3	7	whole

Table 2. The nutritional value of concentrated compound feed for individual swine groups

	Energy MJ	MJ : Lis	Total protein	Lysine	Methionine + Cysteine	Threonine	Tryptophan
Piglets body weight 7-15 kg	15	1:1,1	160 g	16,5 g	10,4 g	11,05 g	3,8 g
Weaner body weight16-50 kg	14,5	1:0,97	150 g	14 g	8,82 g	9,38 g	0,22 g
Fatteners from 51kg till end of breeding	14	1:0,9	140 g	12,6 g	7,94 g	8,44 g	2,9 g

The role of individual functional fed components:

- the mixture with low total protein level – the application of low protein feeding causes lower emission of sulphur hydrogen and ammonium; the health of animals is raised up –less of diarrhea with piglets (*E.coli* elimination); less antibiotics used for healing of gastrointestinal problems
- the addition of cold pressed rape-seed oil – causes the surplus of energy in fed and in animal organism what results in formation of intramuscular fat at the planned level 3-4%; the fat is a carrier of odor and taste and the herbal aroma is cumulated in it – during the thermal treatment the aroma compounds present in meat (originating from herbal fed extracts) are developed and give the desirable aroma to meat
- lecithin cumulates in meat and fat and serves as a natural supplement to the human diet
- the herbal mixture applied as extracts beside the aroma transmission to meat and fat also stabilizes the pork; the proper proportions of intently applied herbal composition the meat and fat aroma can be influenced on starting from very early stages of animal rearing; usage of herbs with germicidal properties, for example of garlic, chili pepper, the pork meat

keeps its freshness without preservatives addition;

- the application of the alive bacteria and fungi cultures bettered the digestion process of animals: strengthened the health, lowered emission of sulphur hydrogen and ammonium, raised up piglets health through diminishing diarrhea problems

The addition of plant extracts improves pig growth performance, and carcass weight by reducing the negative effects of heat stress, without markedly modifying blood constituents, meat quality, and sensory attributes of the pork *(Dávila-Ramírez et al., 2020)*. On the other hand, the research by *Janz et al. (2007)* and *Simitzis et al. (2010)* indicate that the addition of plant extracts to the diet of pigs does not modify the sensory characteristics of the meat.

All above factors interacting together give as the effect the functional pork –"Silesian Fattener". After the initial research it was stated that the fatty acids profile of that pork has been changed. The result of modification is the growth of polyunsaturated fatty acids (PUFA) – especially of PUFA n-3 with pro-health activity, in the advantage of saturated fatty acids (SFA) (Table 3). It bettered the pro-health value of pork meat, especially broadened the ratio of PUFA from Ω 6 (n-6) family to PUFA from Ω 3 (n-3) family to at about 12. In the meat of "Silesian fattener" the trace amounts of conjugated linoleic acid (CLA) was present. The modification of fatty acids profile did not influenced on lowering its oxidative stability. *Lei et al. (2018)* showed that natural or fermented herbs improved growth performance and nutrient digestibility in growing-finishing pigs. Additionally, fermented herbs supplementation positively changed fatty acid profiles in *Longissimus dorsi* muscle. *Cao et al. (2012)* and *Ahmed et al. (2016)* suggest that dietary phenolic compounds can positively modify the fatty acid composition via preventing the oxidation of unsaturated fatty acids.

Table 3. The profile of fatty acids of loin of Silesian Fatteners and of industrial fatteners (own research data)

Fatty acids	Pork - loin	
	Silesian fattener	Industrial fattener
C-10:0	0.07	0.06
C-12:0	0.08	0.09
C-14:0	1.06	1.56
C-14:1	0.02	0.08
C-15:0	0.04	0.05
C-16:0	2051	26.75
C-16:1n9	0.23	0.75
C-16:1n7	3.41	2.88
C-17:0	0.14	0.18
C-17:1	0.19	0.10
C-18:0	10.60	13.64
C-18:1n-9	46.89	42.88
C-18:1n-7	4.79	4.85
C-18:2n-6	7.79	4.60
C-18:3n-6	0.08	0.04
C-18:3n-3	0.43	0.10
CLA	0.09	0.06
C-20:0	0.14	0.25
C-20:1	0.73	0.69
C-20:2	0.30	0.04
C-20:3n-6	0.21	0.09
C-20:4n-6	1.24	0.08
C-20:4n-3	-	0.01
C-20:5n-3	0.05	0.02
C-22:4n-6	0.26	0.03
C-22:5n-3	0.26	0.02
C-22:5n-6	-	0.03
C-22:6n-3	0.01	0.02
SFA	32.64	42.58
PUFA	10.72	5.14
PUFA n-3	0.75	0.18
PUFA n-6	9.58	4.87
PUFA n6/n3	12.77	27.06
PUFA/SFA	0.328	0.12

SFA—sum of saturated fatty acids: PUFA—sum of polyunsaturated fatty acids

The members of Society “Opolskie Fermy Trzody – Kolnica” perform the breeding and rearing of swines both in closed and open systems (produce own piglets and also buy for fattening). They kept animals in different bedding systems: thin bedding, self-cleaning floors, deep bedding, rearing on grate floors. After a series of training concerning the application of pro-health EM systems they decided to widen the range of mixtures fed with the product of Greenland Technology EM, which is the admixture supplying the basic fed, containing the Effective Microorganisms (EM) Carbon Bokashi. Effective Microorganisms® are the composition of advantageous microorganisms with probiotic and regeneration abilities made by Terugo Hira *(Higa, 1991; 1993; 2001)*. The producers group applied similar probiotics and yeasts, in a liquid state, to manure, slurry pipes, slurry and liquid manure tanks etc. EM Carbon Bokashi additionally contains pre-fermented wooden carbon with the against diarrhea, absorbing bacteria- and myco-toxins and metabolism gases. Also the product contains calcium carbonate, nettle and linen flax. EM Carbon Bokashi is applied in amounts:

- sows: 5 kg-10 kg /1 ton of fed
- piglets: 5 kg-15 kg /1 ton of fed
- fatteners: 3 kg-5 kg /1 ton of fed.

This mixture is a symbiotic preparation because it contains both prebiotics and probiotics.

The prebiotic modulates activity of probiotic bacteria and can have a significant influence on end results of supplementation. After application of *L. paracasei* with the prebiotic additional growth of bacteria of *Lactobacillus* sp. *and Bifidobacterium* and in manure of weaned piglets obtaining *L. paracasei* the lower amounts of *Clostridium* sp. and *Enterobacteriaceae* sp. were detected *(Nemcová et al., 1999)*. Effective Microorganisms (EM) positively influences the morphological characteristics of the porcine jejunum and increases the expression of genes related to the metabolism and functioning of the gastrointestinal tract *(Reszka et al., 2020b; Laskowska et al., 2017)*. Meat from pigs fed the effective microorganism additive was much harder and was characterized by less thermal drip loss *(Reszka et al., 2020a)*.

Conclusion

The members of the Society produce a few thousands of fatteners yearly, which according to initial deal are bought by the meat factories specialized in production of traditional meat products. Beside the higher costs of production the Society members obtain higher, at about 10%, price for reared them pigs. The technology "Silesian Fattener" is an original idea for profitable, environment friendly production of high quality fatteners.

„Šlezijski tovljenik" - koncept zdrave svinje i visokokvalitetnog svinjskog mesa

Władysław Migdał, Rafał Duś, Maria Walczycka, Łukasz Migdał

Rezime

„Šlezijski tovljenik" je tehnologija uzgoja svinja (krmače, odojci, tovljenici) koju primenjuju članovi „Opolskie Fermi Trzody" - Kolnica (Opole farma tovljenika - Kolnica). Izuzetak i inovacija tehnologije uzgoja se zasniva na primeni u ishrani svinja nekoliko aditiva koji zajedno daju različite rezultate menjajući sastav mesa i masti i daju svinjetini pozitivne fizičko-hemijske, kulinarske i pro-zdravstvene karakteristike u promenjenom profilu masnih kiselina koji je povoljan za ljude. Takođe, takva proizvodnja je povoljna za životnu sredinu. Osim većih troškova proizvodnje, članovi Društva dobijaju i veću cenu od oko 10% za uzgojene svinje. Tehnologija „Šlezijski tovljenik" originalna je ideja za profitabilnu, ekološki prihvatljivu proizvodnju visokokvalitetnih tovljenika.

Ključne reči: svinje, šlezijski tovljenici, tehnologija uzgoja, efikasni mikroorganizmi

References

AHMED S.T., MUN H.S., ISLAM M.M., KO S.Y., YANG C.J. (2016): Effects of dietary natural and fermented herb combination on growth performance, carcass traits and meat quality in grower-finisher pigs. Meat Science, 122, 7–15.

BICHARSKI T., HAMMERMEISTER A., WARDA A. (2010): Kompleksowy system wytwarzania wysokiej jakości wieprzowiny − PQS (Pork Quality System) − współpraca hodowców, producentów i przetwórców. Przegląd Hodowlany (in Polish), 11, 11-13.

CAO F.L., ZHANG X.H., YU W.W., ZHAO L.G., WANG T. (2012): Effect of feeding fermented Ginkgo biloba leaves on growth performance, meat quality, and lipid metabolism in broilers. Poult Science, 91, 1210–1221.

COMMISSION IMPLEMENTING DECISION (EU) 2017/302 of 15 February 2017 establishing best available techniques (BAT) conclusions, under Directive 2010/75/EU of the European Parliament and of the Council, for the intensive rearing of poultry or pigs (notified under document C(2017) 688).

COUNCIL DIRECTIVE 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources

COUNCIL DIRECTIVE 96/61/EC of 24 September 1996 concerning integrated pollution prevention and control.

DÁVILA-RAMÍREZ J. L., MUNGUÍA-ACOSTA L. L., MORALES-CORONADO J. G., GARCÍA-SALINAS A. D., GONZÁLEZ-RÍOS H., CELAYA-MICHEL H., SOSA-CASTAÑEDA J., SÁNCHEZ-VILLALBA E., ANAYA-ISLAS J., BARRERA-SILVA M. A. (2020): Addition of a Mixture of Plant Extracts to Diets for Growing-Finishing Pigs on Growth Performance, Blood Metabolites, Carcass Traits, Organ Weight as a Percentage of Live Weight, Quality and Sensorial Analysis of Meat. Animals, *an open access journal from MDPI*, *10*(7), 1229. https://doi.org/10.3390/ani10071229.

DIRECTIVE 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control.

DIRECTIVE (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC.

GRELA E. R., KOWALCZUK-VASILEV E. (2010): Influence of digestible crude protein reduction and amino acids additive in growing pigs' diets on their performance and nitrogen balance. Annales Universitatis Mariae Curie-Skłodowska. Sectio EE Zootechnica, 28(1), 1-8.

HIGA T. (1991): Effective microorganisms: a biotechnology for mankind. First International Conference on Kyusei Nature Farming. Washington, DC, USA, 8-14.

HIGA T. (1993): An Earth Saving Revolution: A Means to Resolve Our World's Problems Through Effective Microorganisms (EM), Sunmark Publishing Inc., Tokyo, Japan, 335.

HIGA T. (2001): Effective Microorganisms in the context of Kyusei Nature Farming: a technology for the future. In: Senanayake, Y.D.A., Sangakkara, U.R. (Eds.), Sixth International Conference on Kyusei Nature Farming. Pretoria, South Africa, 40-43.

HIGA T., PARR J. (1994): Beneficial and effective microorganisms for a sustainable agriculture and environment. International Nature Farming Research Center, Atami, Japan, 1-16.

JANZ J.A.M., MOREL P.C.H., WILKINSON B.H.P, PURCHAS R.W. (2007): Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. Meat Science, 75, 350–355.

JOSHI H., SOMDUTTAND, CHOUDHARY P., MUNDRA S.L. (2019): Role of Effective Microorganisms (EM) in Sustainable Agriculture. International Journal of Current Microbiology and Applied Sciences, 8(03), 172-181 doi.org/10.20546/ijcmas.2019.803.024.

KARWOWSKA M., DOLATOWSKI Z. J., GRELA E. R. (2007): The effect of dietary supplementation with extracted alfalfa meal on oxidation stability of cooked ham oxidation. Polish Journal of Food and Nutrition Sciences, 57(4 B), 271-274.

KARWOWSKA M., DOLATOWSKI Z. J., GRELA E. R. (2008): Wpływ dodatku preparatu z lucerny do paszy na właściwości fizykochemiczne mięsa wieprzowego i wyrobów. Roczniki Naukowe Zootechniki (in Polish), 35(2), 101-107.

KRZYŻANOWSKI J.T. (2017): Krajowe systemy zapewnienia jakości żywności w wybranych krajach Unii Europejskiej. Zeszyty Naukowe Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie Problemy Rolnictwa Światowego (in Polish)., 17 (XXXII), 2, 136–143 doi: 10.22630/PRS.2017.17.2.33

LASKOWSKA E., JAROSZ Ł., GRĄDZKI Z. (2017): The effect of feed supplementation with effective microorganisms (EM) on pro- and anti-inflammatory cytokine concentrations in pigs. Research in Veterinary Science, 115, 244-249. doi: 10.1016/j.rvsc.2017.03.008.

LEI X.J., YUN H.M., KIM I.H. (2018): Effects of dietary supplementation of natural and fermented herbs on growth performance, nutrient digestibility, blood parameters, meat quality and fatty acid composition in growing-finishing pigs. Italian Journal of Animal Science, 17, 984–993 doi:10.1080/1828051X.2018.1429955

MINISTERSTWO ROLNICTWA I ROZWOJU WSI (2019): Kodeks doradczy dobrej praktyki rolniczej dotyczący ograniczenia emisji amoniaku. Ministry of Agriculture and Rural Development: Codex of Good Ammonium Practices. Warszawa (in Polish).

MINISTERSTWO KLIMATU - CLIMATE MINISTRY (2020): Krajowy bilans emisji SO2, NOx, CO, NH3, NMLZO, pyłów, metali ciężkich i TZO za lata1990 – 2018, Warszawa (in Polish).

NEMCOVÁ R., BOMBA A., GANCARCIKOVÁ S., HERICH R., GUBA P. (1999): Study of the effect of *Lactobacillus paracasei* and fructooligosaccharides on the faecal microflora in weanling piglets. Berliner und Münchener Tierärztliche Wochenschrift, 112, 225–228.

PRZYBYLSKI W., JAWORSKA D., BORUSZEWSKA K., BOREJKO M., PODSIADŁY W. (2012): Jakość technologiczna i sensoryczna wadliwego mięsa wieprzowego. Żywność. Nauka. Technologia. Jakość, 80, 1, 116-127.

RADKOWSKA I. (2013): Wykorzystanie ziół i fitogenicznych dodatków paszowych w żywieniu zwierząt gospodarskich. Use of herbs and phytogenic feed additives in livestock feeding. Wiadomości Zootechniczne, R. LI, 4: 117–124 (in Polish).

RESZKA P., CYGAN-SZCZEGIELNIAK D., JANKOWIAK H., CEBULSKA A., MIKOŁAJCZAK B., BOGUCKA J. (2020a): Effects of Effective Microorganisms on Meat Quality, Microstructure of the *Longissimus Lumborum* Muscle, and Electrophoretic Protein Separation in Pigs Fed on Different Diets. Animals, 10(10), 1755, 1-16, doi.org/10.3390/ani10101755.

RESZKA P., DUNISLAWSKA A., SLAWINSKA A., SIWEK M., KAPELANSKI W., BOGUCKA J. (2020b): Influence of the effective microorganisms (em) on performance, intestinal morphology, and gene expression in the jejunal mucosa of pigs fed different diets. Animal Physiology and Animal Nutrition, 104, 1–10.

SEMANIUK W., GRELA E.R. (2011): Wpływ obniżonego poziomu białka w żywieniu tuczników w systemie dawkowanym lub ad libitum na poziom wskaźników azotowych w krwi i moczu. Effect of a reduced protein content in the nutrition of growing-finishing pigs fed a restricted or ad libitum diet on nitrogen parameters in their blood and urine. Medycyna Weterynaryjna (in Polish), 67, 5, 339-342.

SIMITZIS P.E., SYMEON G.K., CHARISMIADOU M.A., BIZELIS J.A., DELIGEORGIS S.G. (2010): The effects of dietary oregano oil supplementation on pig meat characteristics. Meat Science, 84, 670–676.

WENK C. (2003): Herbs and botanicals as feed additives in monogastric animals. Asian-Australasian Journal of Animal Sciences, 16, 282-298.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

LIFE DAILY GAIN OF INDIGENOUS PIG BREEDS IN SERBIA

Marija Gogić[1], Nenad Katanić[2], Vladimir Živković[1], Nenad Stojiljković[1], Violeta Mandić[1], Maja Petričević[1], Radomir Savić[3]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Ministry of Agriculture, Forestry and Water Management, Nemanjina 22-24, 11000, Belgrade, Republic of Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: Marija Gogić, gogic.marija@gmail.com
Invited paper

Abstract: Indigenous breeds of pigs, in addition to representing genetic heritage, have great scientific, cultural and economic significance for every country. The Serbian indigenous breeds of pigs Mangalitsa, Moravka and Resavka are endangered breeds with little research interest, which resulted in insufficient data on their production results. The aim of this study was to determine the differences in the life daily gain (LDG) of male heads of Swallow Belly Mangalitsa breed of different body weights (20 kg - I group, 45 kg - II group and 100 kg - III group), as well as between males of Swallow Belly Mangalitsa, Moravka and Resavka in seven consecutive measurements, once a month, during the experiment. Within the Swallow Belly Mangalitsa breed, the third group (280 g/day) had a statistically significantly higher LDG compared to the first (110 g/day) and the second (200 g/day). In all measurements, Moravka had the highest LDG, and Mangalitsa the lowest, but the differences were not significant. It was to be expected that the Mangalitsa would have the lowest LDG because it is a fatty breed, unlike Moravka and Resavka, which are breeds of combined production abilities (meat and fat). By researching the production performance of indigenous pig breeds, it is possible to predict and improve production possibilities by selection measures while preserving the desired genetic structure.

Key words: life daily gain, indigenous breeds, Mangalitsa, Moravka, Resavka

Introduction

Pig farming in the Republic of Serbia has long been of great importance and represents a very important branch of agricultural production (*Radović et al.,* *2019a*). Pig farming is a significant branch of animal husbandry that is mainly based on the breeding of highly productive breeds of pigs. This has led to a decrease in the size of the population of indigenous breeds of pigs that today belong to endangered species. Currently, three autochthonous pig breeds (Mangalitsa, Moravka and Resavka) are bred in the Republic of Serbia, while within the Mangalitsa breed, the Swallow Belly strain is the most represented, compared to the red and white strains. In the last few years, there has been a growing interest in the farming of autochthonous breeds of pigs, both for the preservation of genes and for the production of cured meat products produced in the traditional way (*Petrović et al., 2010*). Autochthonous breeds of pigs in Serbia can be divided into fatty breeds, such as Mangalitsa and breeds of combined production abilities (Moravka and Resavka), *Petrović et al. (2007).* Life daily gain is one of the key traits in pig breeding because it contributes to its efficiency (*Nielsen et al., 2018*). Otkrivanje i uklanjanje ograničavajućih faktora u svinjarskoj proizvodnji predstavlja važan segment za efikasno iskorišćavanje resursa, promovisanje održivog pristupa uzgoja svinja i poboljšanja dobiti farmera *(Carter et al., 2013).* The profitability and economy of pig production is largely conditioned by the growth traits. The profitability and economy of pig production is largely conditioned by the growth traits. Growth is an important property of all living organisms (*Lawrence and Fowler, 1997*), and represents an increase in cell number and body size over a period of time (*Schulze et al., 2001*). Potential growth is also defined as the highest level at which an animal can grow in non-restrictive conditions (*Emmans and Kyriazakis, 1999; 2000*). It is conditioned by genetic parameters and the current state of the individual animal. Non-restrictive conditions are following: 1. the diet must be *ad libitum*, 2. the nutrient content must at least provide the required energy level, 3. food consumption must not be limited due to inaccessibility of food or the presence of toxins, and 4. environmental factors (temperature and diseases) must not restrict food consumption. The growth rate is therefore influenced by various genetic and non-genetic factors. The increase can be expressed in absolute or average daily gain over a period of time. The average daily gain is a value that shows how much the body weight of the animal has increased daily, i.e. the ratio between the increase in body weight in a certain period and the duration of the period (*Marin et al., 2013*). It has long been known that the difference between individuals in the conversion of ingested food into body weight is an important determinant of profit in pork production (*MacNeil and Kemp, 2014*) and is also an indicator of how well an individual uses ingested food into the body. The increase depends on two factors: food intake and food efficiency (*Patience et al., 2001*).

The aim of this study was to determine variation in the average life daily gain of Swallow Belly Mangalitsa males, within the breed under the effect of body weight of animals, and between and between three indigenous breeds in seven consecutive measurements, until slaughter.

Materials and Methods

The trial was conducted on the experimental pig farm of the Institute of Animal Husbandry, Belgrade-Zemun, where the conditions on the farm enabled a semi-intensive system of farming. The animals used in the experiment come from the herd of several breeders, and after weaning they were brought to the said farm. Only male heads that fully phenotypically corresponded to their breed were used in the experiment. The adaptation of the animals to the new breeding conditions lasted until they reached about 20-25 kg of body weight, when the experimental period began. Males gradually were accustomed to complete feed mixtures used on the farm in accordance with the age category. During the entire examination, all animals were kept in the same conditions of accommodation, nutrition and care. The animals were kept in groups, in a semi-intensive system, with each box having a range (each box had an open and a covered part). The total area of the boxes with the range was 150 m^2. The trial lasted 28 weeks.

The first part of the experiment included male heads of the Swallow Belly Mangalitsa breed. The animals were divided into three groups: I group - 11 animals aged 24 weeks, average body weight of 20 kg; II group - 9 heads, aged 33 weeks and weighing 45 kg; III group of animals - 13 animals, about one year old and weighing an average of 100 kg. The second part of the experiment included male heads of all three indigenous breeds of pigs - 9 boars of Mangalitsa, 8 Moravka males and 7 heads of Resavka breed. Statistical data processing was performed using the software package *SAS Institute Inc (2002-2010*). Basic descriptive statistical parameters are presented: average value and standard deviation. The assessment of the effect of body weight and breed was performed using the GLM procedure (General Linear Model) in the mentioned software package. Determination of the statistical significance of the differences between the obtained mean values (Mean) was carried out using the t-test, at the level of significance of $P<0.05$.

The following models were used to assess the influence of body weight (1) and breed (2):

$y_{ij} = \mu + T_i + \varepsilon_{ij}$ (1);
$y_{ij} = \mu + R_i + b(x_{ij} - \bar{x}) + \varepsilon_{ij}$ (2),

where: y_{ij} - investigated trait, μ- general population average, T_i – the effect of body weight groups (i=1,2,3), R_i – the effect of breed (i=1,2,3), $b(x_{ij} - \bar{x})$ – linear regression effect of body weight and ε_{ij} – random error.

Results and Discussion

The average values and standard deviation of daily life gain in different phases of postnatal life of Swallow Belly Mangalitsa pigs, as well as differences between groups of animals are shown in Table 1.

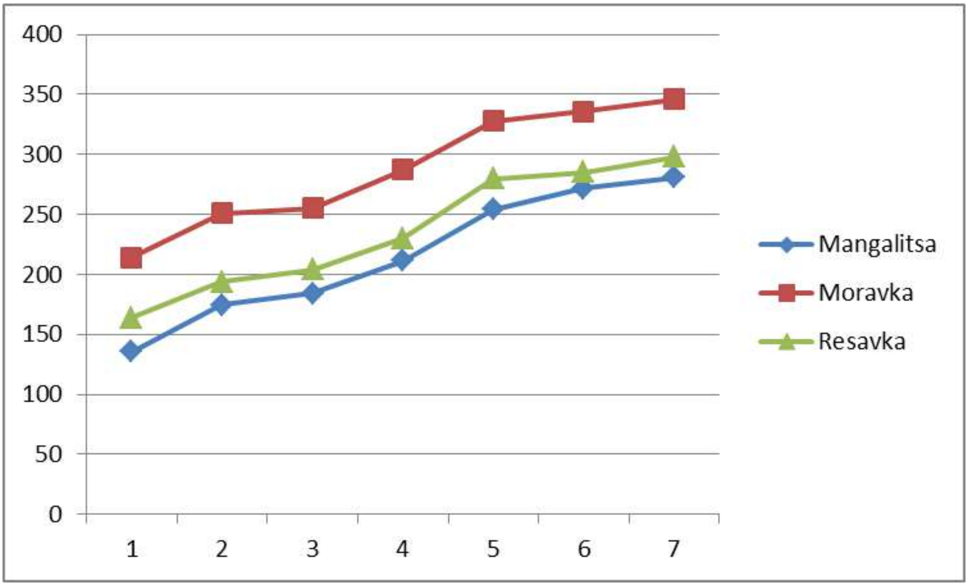
The differences in growth rate from birth to reaching the slaughter weight of 100 kg, were established during different production phases of indigenous pig breeds. These differences depended on the anatomical-histological-physiological principles of postnatal development of pigs. Mangalitsa piglets were born with a body weight of about 1 kg and during the first 5-6 months (24 weeks) piglets showed the lowest average life gain (110 g) with a larger relative deviation from the average (CV = 18%). In group II there is a statistically significant increase in LDG compared to group I, but also in group III compared to group I. Also, a statistically highly significant difference (P <0.001) in LDG was found between the second and third groups. During phases II and III, the uniformity of animals is greater, with a relative deviation of 5% or less. The reason for that is a more stable defence system of animals older than 24 weeks, so that they show better resistance to various environmental agents, with good potential for food use.

Table 1. Average life daily gain (g) at different phases of postnatal life

Phases	N	$\bar{x}$	SD	SE	t - test		
					I-II	I-III	II-III
I	11	110	20	10	P<0.001	P<0.001	P<0.001
II	9	200	10	0			
III	13	280	10	0			

Phases: I - $\bar{x}$ = 20 kg (age 24 weeks), II - $\bar{x}$ = 45 kg (age 33 weeks), III - $\bar{x}$ = 100 kg (age 52 weeks); $\bar{x}$ - average value, N – number of animals, SD – standard deviation, SE – standard error, P – statistical significance.

Graph 1 shows the daily life gain (g/day) of three indigenous breeds of pigs in 7 phases of measurement at different body weights (kg).



Graph 1. Trend of life daily gain in 7 consecutive measurements

The highest absolute gain between the two measurements was shown by the heads of the Moravka breed for all measurements, except for the third and second measurements, where the heads of the Resavka breed achieved the highest absolute gain, because they also had the highest average daily gain between the two measurements (323 g/day).

In the first measurement, Moravka pigs were the youngest (144 days), and Resavka pigs were the oldest (174 days). At the end of the experimental period, the heads of the Mangalitsa breed were the oldest (351 days), and the heads of the Moravka breed were the youngest (314 days). By comparing breeds, it was determined that there are differences for the LDG trait, but these differences were not statistically significant. Observing all examined traits in the experiment (animal age, absolute gain as well as average daily gain between two consecutive measurements and average life daily gain), there was only a statistically significant difference (P <0.01) at the end of the experiment between Mangalitsa and Moravka. The average life gain of Moravka was 347 g/day, Resavka 298 g/day and Mangalitsa 281 g/day. The obtained results were also expected, considering that Moravka and Resavka are breeds of combined production abilities, while Mangalitsa is a fatty breed. Also, with the increase in the age of the animals, there was a decrease in the life daily gain.

Contrary to our research related to monitoring the average life gain in different phases of postnatal life, *Radović et al. (2019b)* report a better increaee of

LDG in Swallow Belly Mangalitsa. The authors report an LDG of 136 g/day in the early phase of growth, corresponding to the suckling period, 434 g/day for the whole phase of fattening (430 in the early phase of fattening, 519 g in the mid phase and 405 g/day in the final phase of fattening), with animals gaining on average 307 per day in the period from birth to slaughter. In the early phase of growth, the daily gain was 310 g/day. A partial similarity exists with the research of *Savić et al. (2019)* who divides the growth levels for the breed as the growth phase (from weaning to 30 kg body weight) and the phases of early, mid and final fattening with body weights of between 30 and 60 kg, 60 and 100 kg and above 100 kg body weight, respectively. The authors show that the daily gain in the growth phase is extremely low (192 g/day), which corresponds to the second phase of postnatal development from our experiment, and increases to 477, 521 and 478 g/day in the early, mid and final phase of fattening, respectively, while the average daily gain for the phase of total fattening is 508 g/day, and only 285 g/day for the period from birth to slaughter. LDG in our study was much lower than in the research of *Pietrol et al. (2006)* who report LDG of 467 g/day in the Italian indigenous Casertana breed grown in the open system of a body weight of 35 to 60 kg, 491 g/day in animals of body weight of 60 to 100 kg and 361 g/day for heads with a body weight above 100 kg, until the moment of slaughter. All these differences in growth are a consequence of different breeds, different systems of farming and conditions, but also experimental design. Also, in our study LDG is lower than in research by *Brunius (2011)* who established LDG 854 g/day. Differences in LDG between studies can be explained in differences in breeds, housing systems, and conditions, as well as in experimental design. In all measurements during the experiment, the heads of the Moravka breed had higher LDG in relation to the heads of the Mangalitsa breed, similarly to *Radović et al. (2017a; 2017b).* However, as noted, the differences were not statistically significant, similarly to *Petrović et al. (2011).*

Conclusion

The results of the research show that there are highly significant (P<0.001) differences in LDG between different weight groups of boars of the Swallow Belly Mangalitsa, which is primarily conditioned by the anatomical-histological-physiological lprinciples of postnatal development of pigs. The heads of the Moravka breed had the highest absolute gain between the two measurements, except between the third and second measurements, where the heads of the Resavka breed had the highest absolute gain. The highest values for LDG in the measurement phases were observed in the heads of the Moravka breed.

This research should be expanded and improved in order to increase LDG and other production traits of indigenous breeds, all in the function of encouraging their breeding and preservation. It is necessary to carry out selection measures in order to prevent their extinction, such as the forever lost breeds of pigs of Šiška and Šumadinka.

Životni dnevni prirast autohtonih rasa svinja u Srbiji

Marija Gogić, Nenad Katanić, Vladimir Živković, Nenad Stojiljković, Violeta Mandić, Maja Petričević, Radomir Savić

Rezime

Autohtone rase svinja, osim što predstavljaju genetičko nasleđe, imaju veliki naučni, kulturni i ekonomski značaj za svaku državu. Srpske autohtone rase svinja mangulica, moravka i resavka su rizično ugrožene rase sa malim istraživačkim interesom, što je rezultiralo nedovojnim podacima o njihovim proizvodnim rezultatima. Cilj ovog rada bio je da se utvrde razlike u životnom dnevnom prirastu (ŽDP) muških grla rase lasaste mangulice različitih telesnih masa (20 kg – I grupa, 45 kg – II grupa i 100 kg – III grupa), kao i između muških grla lasaste mangulice, moravke i resavke u sedam uzastopnih merenja, jednom mesečno, tokom trajanja ogleda. Unutar rase lasasta manuglica, statistički značajno veći ŽDP imala je treća grupa (280 g/day) u poređenju sa prvom (110 g/day) i drugom (200 g/day). U svim merenjima moravka je imala najveći ŽDP, a mangulica najmanji, ali razlike nisu bile značajne. Bilo je za očekivati da će mangulica imati najmanji ŽDP jer je masna rasa, za razliku od moravke i resavke koje su rase kombinovanih proizvodnih sposobnosti (meso i mast). Istraživanjem proizvodnih osobina autohtonih rasa svinja moguće je predvideti i unaprediti proizvodne mogućnosti selekcijskim merama uz očuvanje poželjne genetičke strukture.

Ključne reči: životni dnevni prirast, autohtone rase, mangulica, moravka, resavka

Acknowledgment

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

References

BRUNIUS C. (2011): Early Immunocastration of Male Pigs-Effects on Physiology, Performance and Behaviour. PhD thesis. Swedish University of Agricultural Sciences, Faculty of Natural Resources, Department of Food Science, Uppsala, Sweden.

CARTER N., DEWEY C., MUTUA F., DE LANGE C., GRACE D. (2013): Average daily gain of local pigs on rural and peri-urban smallholder farms in two districts of Western Kenya. Trop Anim Health Prod 45, 1533–1538.

EMMANS G. C., KYRIAZAKIS I. (1999): Growth and body composition. In A quantitative biology of the pig (ed. I. Kyriazakis), 181-197. CAB International, Wallingford.

EMMANS G. C., KYRIAZAKIS I. (2000): Issues arising from genetic selection for growth and body composition characteristics in poultry and pigs. In The challenge of genetic change in animal production (ed. W. G. Hill, S. C. Bishop, B. McGuirk, J. C. McKay, G. Simm and A. J. Webb), British Society of Animal Science occasional publication, 27, 39-53.

LAWRENCE T.L.J., FOWLER V.R. (1997): Growth of farm animals. CAB International, Wallingford.

MACNEIL M.D., KEMP R.A. (2014): Genetic parameter estimation and evaluation of Duroc boars for feed efficiency and component traits. Canadian Journal of Animal Science, 95, 155-159.

MARIN D., PĂCALĂ N., PETROMAN I., PETROMAN C., CIOLAC R., VĂDUVA L., LOZICI A. (2013): Values of Average Daily Gain of Swine Posted to Commercial Hybrids on Pork in Youth Phase Depending on the Type and Size of Stalls. Animal Science and Biotechnologies, 46, 2, 30-32.

NIELSEN H.M., ASK B., MADSEN P. (2018): Social genetic efects for growth in pigs difer between boars and gilts. Genetics Selection Evolution, 50, 4.

PATIENCE J.F., COOPER D.R., SHAW M.I., LEVESQUE C.L., GILLIS D.A. (2001): Factors Driving the Improvement of Average Daily Gain. Focus on the Future Conference, February 20-21, 2001 Red Deer, Alberta, Canada.

PETROVIĆ M., MIJATOVIĆ M., RADOVIĆ Č., RADOJKOVIĆ D., JOSIPOVIĆ S. (2007): Genetic resources in pig breeding – carcass quality traits of breeds moravka and mangalitsa. Biotechnology in Animal Husbandry 23, 5-6, 421 – 428.

PETROVIĆ M., RADOVIĆ Č., PARUNOVIĆ N., MIJATOVIĆ M., RADOJKOVIĆ D., ALEKSIĆ S., STANIŠIĆ N., POPOVAC M. (2010): Quality traits of carcass sides and meat of moravka and mangalitsa pig breeds. Biotechnology in Animal Husbandry 26, 1-2, 21-27.

PETROVIĆ M., RADOVIĆ Č., MIJATOVIĆ M., RADOJKOVIĆ D., STANIŠIĆ N., PARUNOVIĆ N. (2011): The share of tissues in pig carcass sides of autochthonous breeds depending on the body mass and sex. Biotechnology in Animal Husbandry, 27, 3, 561-569.

PIETROLÀ E., PILLA F., MAIORANO G., MATASSINO D. (2006): Morphological traits, reproductive and productive performances of Casertana pigs reared outdoors. Italian Journal of Animal Science, 5, 2, 139-146, DOI: 10.4081/ijas.2006.139

RADOVIĆ Č., PETROVIĆ M., SAVIĆ R., GOGIĆ M., LUKIĆ M., STANIŠIĆ N., ČANDEK-POTOKAR M. (2017a): Growth potential of serbian local pig breeds Mangalitsa and Moravka. Agriculturae Conspectus Scientificus, 82, 3, 217-220.

RADOVIĆ Č., PETROVIĆ M., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R., STANIŠIĆ N., GOGIĆ M. (2017b): Carcass and pork quality traits of indigenous pure breeds (Mangalitsa, Moravka) and their crossbreads. Indian J. Anim. Res., 51, 2, 371-376.

RADOVIĆ Č., PETROVIĆ M., GOGIĆ M., RADOJKOVIĆ D., ŽIVKOVIĆ V., STOJILJKOVIĆ N., SAVIĆ R. (2019a): Autochthonous Breeds of Republic of Serbia and Valuation in Food Industry: Opportunities and challenges, Food Processing, Food Processing, 1 - 19, http://dx.doi.org/10.5772/intechopen.88900.

RADOVIĆ Č., SAVIĆ R., PETROVIĆ M., GOGIĆ M., LUKIĆ M., RADOJKOVIĆ D., BATOREK-LUKAČ N. (2019b): Mangalitsa (Swallow-Belly Mangalitsa) Pig, European Local Pig Breeds - Diversity and Performance. A study of project TREASURE, Marjeta Candek-Potokar and Rosa M. Nieto Linan, IntechOpen, DOI:10.5772/intechopen.83773. https://www.intechopen.com/books/european-local-pig-breeds-diversity-and-performance-a-study-of-project-treasure/mangalitsa-swallow-belly-mangalitsa-pig

SAVIĆ R., RADOVIĆ Č., PETROVIĆ M., GOGIĆ M., RADOJKOVIĆ D. BATOREK-LUKAČ N. (2019): Moravka Pig, European Local Pig Breeds - Diversity and Performance. A study of project TREASURE, Marjeta Candek-Potokar and Rosa M. Nieto Linan, IntechOpen, DOI: 10.5772/intechopen.83777. https://www.intechopen.com/books/european-local-pig-breeds-diversity-and-performance-a-study-of-project-treasure/moravka-pig

SAS INSTITUTE INC (2002-2010). The SAS System for Windows, Cary, NC.

SCHULZE V., ROHE R., LOOFT H., KALM E. (2001): Genetic analysis of the course of individual growth and feed intake of group-penned performance tested boars. Arch. Tierz. Dummerstorf, 44, 139-156.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

THE EFFECT OF PHYTOGENIC ADDITIVES ON THE DEGREE OF BACTERIAL INFECTION *B. hyodysenteriae* IN WEANED PIGLETS

Nikola Delić, Dragan Nikšić, Maja Petričević, Aleksandar Stanojković, Vladimir Živković, Marina Lazarević, Nevena Maksimović

Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: Nikola Delić, delicnikola68@yahoo.com
Invited paper

Abstract: In this study, the average Ct values in 4 groups of piglets in a three - week time interval were examined. The groups consisted of 16 male piglets each. All four groups of piglets received a complete feed mixture that fully met the needs for a given category of animals (NRC, 2012). In addition to the mentioned mixture, one group was given the additive Patente Herba® (PH), the second group was given the food additive Patente Herba® Plus (PHP), the third group received the antibiotic thiamulin (Tiamulin - P - 10%) and presented a positive control (TK +), while the fourth group received only complete feed and represented a negative control (K-). The lowest average Ct value (i.e. the highest amount of DNA of the bacterium B. hyodysenteriae) in all four observed periods (first, second and third week of the experiment and in the entire, three-week experimental period) was recorded in the K-group. The highest mean Ct value (i.e. the lowest amount of B. hyodysenteriae DNA) on days 0 and 7 was in the PHP group, and on days 14 and 21 in the TK + group. The results of the analysis of variance indicated that the average Ct values between the groups did not differ statistically significantly on day 0 ($F=2.48$; $p=0.070$) or on day 7 ($F=1.88$; $p=0.143$), day14 ($F=0.92$; $p=0.439$) and 21 ($F=0.64$; $p=0.593$). Since for the 14th and 21st day the values of F statistics were less than 1, it means that the data in the groups differed more than between the groups, and in the groups themselves the variability was not manifested ($cv \leq 30\%$).

Key words: adittives, infection rate, piglets, B. hyodysenteriae, nutrition

Introduction

Swine dysentery is a disease characterized by muco-hemorrhagic inflammation of the mucous membrane of the large intestine, with the formation of fibrin-necrotic pseudomembranes. The cause of this disease is the bacterium *Brachyspira hyodysenteriae*, an anaerobic spirochete belonging to the family *Brachyspiracae* of the order *Spirohaetales* within the class *Spirohaetaes*.

Six species of *Brachyspira* can be found in the large intestine of pigs. Two species are pathogenic, *B. hyodysenteriae*, the causative agent of swine dysentery, and *B. pilisocoli*, the causative agent of swine spirochetal colitis. *B. suanatina*, some strains of *B. murdochii* and *B. intermedia* can sporadically cause colitis, while *B. innocens* is considered an apathogenic species.

Brachyspira species that can colonize the mucosa of the large intestine of pigs are 5–11 µm long, 0.2–0.4 µm in diameter. Like other spirochetes, they have two sets of periplasmic flagella located on the opposite poles. Each set of flagella is wrapped along the body of the bacterium, and is located under the outer bacterial sheath, the free ends of which overlap in half of the cell. Flagella have the ability of spiral motility, which enables the motility of bacteria and their penetration through the dense viscous content of mucus on the surface of the epithelium of the colon (*Hampson, 2012*).

The metabolic activity of *B. hyodysenteriae* and other intestinal *Brachyspira* has been described by *Stanton et al. (2006).* The metabolic characteristics of the three completely sequenced species are fully determined with only small differences found to separate the species (*Bellgard et al., 2009; Wanchanthuek et al., 2010).* Properties that can be used to differentiate species include the size and intensity of the hemolysis zone, the indole production ability, and the enzyme profile contained in commercial API-ZYM kits (*bioMérieux, Marcy l'Etoile, France; Fellström et al., 1997*).

Analysis of the population of *B. hyodysenteriae* using MLEE proved a significant genetic diversity of this bacterium, which includes a large number of genetically different strains (*Lee et al., 1993*). Also, the species has been found to encompass a highly recombinant population of *B. hyodysenteriae* strains, but still has clonal epidemic potential (*Trott et al., 1997b*). Also, recent research using MLST (*La et al., 2009b; Råsbäck et al., 2007, Savic et al., 2017*) and MLVA (*Hidalgo et al., 2010a*) have established significant genetic diversity of the species, however based on the established index genetic differences of the population (index of association) it has been determined that *B. hyodysenteriae* is nevertheless a non-recombinant or clonal species.

Brachyspira hyodysenteriae possesses numerous genes responsible for bacterial motility and chemotaxis (*Bellgard et al., 2009*). It demonstrates chemotoxic and viscotoxic activity against mucin (*Milner and Sellwood 1994; Naresh and Hampson, 2010*). This activity allows *B. hyodysenteriae* to adhere to the colonic mucosa (*Kennedy et al., 1988*). Inactivation or damage to flagellar genes (flaA and

flaB) results in reduced bacterial motility and mucosal colonization ability (*Kennedy et al., 1997; Rosey et al., 1996*).
B. hyodysenteriae possesses pronounced hemolytic activity (*Bellgard et al., 2009*). A significant factor in the virulence of *B. hyodisenteriae* is its pronounced motility in the contents and mucus of the colon (*Kennedy et al., 1997*), as well as the presence of a gene for the synthesis of the enzyme NADH oxidase that ensures its survival in microaerophilic conditions (*Stanton et al., 1999*). The activity of the enzyme NADH oxidase can facilitate the colonization of the intestinal mucosa by *B. hyodysenteriae,* by protecting it from the toxic activity of oxygen. In this regard, strains of *B. hyodysenteriae* with an inactivated NADH oxidase (nox) gene show a far lower ability to colonize the colonic mucosa and cause disease (*Stanton et al., 1999*).
Brachyspira hyodysenteriae grows in an anaerobic environment at temperatures of 37–42°C on tryptose strain (TS) agar or similar agar containing 5-10% defibrinated blood. After 3-5 days of incubation on nutrient media, low, flat foggy growth of bacteria with an intense zone of beta-hemolysis without the formation of colonies is observed, and its growth is facilitated by agar digestion during seeding. The concentration of bacteria from 108 to 109 per milliliter is reached after 2-3 days of anaerobic incubation in TS liquid media or brain heart infusion (BHA) media with the addition of 10 (v/v) calf serum. The addition of 1% O2 to the atmosphere significantly facilitates the growth of B. *hyodysenteriae* (*Hampson, 2012).*

Material and Method

The experiment included examining the effects of two commercially available phytogenic additives, Patente Herba® and Patente Herba® Plus (Patent Co. DOO, Mišićevo, Serbia), on the degree of infection with *B. hyodysenteriae* in piglets. Phytogenic additive Patente Herba® consists of plant extracts (*Castanea sativa* - chestnut, *Rosmarinus officinalis* - rosemary, *Thymus vulgaris* - thyme, *Origanum vulgare* - oregano, *Allium sativum* - garlic, *Eucalyptus globules* - eucalyptus), essential oils (thymol, carvacrol, eucalyptol, para-cimen, menthol and eugenol) and clinoptilolite, while the phytogenic additive Patente Herba® Plus has all the ingredients as Patente Herba® with the addition of lysozyme and nicotinamide. Both phytogen additives were added to animal feed in accordance with the manufacturer's recommendations, i.e. Patente Herba® in the amount of 2 kg/t of complete feed, and Patente Herba® Plus in the amount of 1 kg/t of complete feed daily during the three-week experiment, that is, starting from the 1st (day after day 0) to the 21st day of treatment. The antibiotic thiamulin (Tiamulin - P - 10%, Vetmedic d.o.o., Vršac, Serbia), which was used in the treatment and control of

swine dysentery, was selected for the treatment of a group of piglets in a positive control. The antibiotic was administered per/os via food, in doses recommended by the manufacturer.
In the period between 2016 and 2018, on the pig farm of the Institute of Animal Husbandry Belgrade - Zemun, an experiment was conducted on clinically healthy weaned piglets aged seven weeks. The research included the formation of four groups of animals on the first day of the experiment (which is marked as day 0, because it is the day before the start of administration of the phytogenic additives). Each group consisted of 16 male piglets, so that the total number of piglets in the experiment was 64. The piglets were taken from 16 litters and after marking and measuring, they were assigned to four experimental groups, respecting the principle that one piglet from each litter is assigned to each group. In this way, four groups of 16 piglets were formed, each group consisting of piglets of 16 sows.
The experimental groups of piglets were housed in boxes that were physically separated so that there was one empty between each of the two experimental boxes. All four groups of piglets received a complete feed mixture that fully met the needs for a given category of animals (*NRC, 2012*). In addition to the mentioned mixture, one group was given the food additive Patente Herba® (PH), the other was given the food additive Patente Herba® Plus (PHP), the third group received the antibiotic thiamulin (Tiamulin - P - 10%) and presented a positive control. (TK +), while the fourth group received only complete feed mixture and represented a negative control (K-).

Molecular genetic identification of B. hyodysenteriae

Isolation of *B. hyodysenteriae* DNA from the feces of experimental animals was performed using a commercial kit "QIAamp® DNA Stool Mini Kit" (Cat. No. 51504, QIAGEN, Germany) according to a specific protocol.
Real-time PCR (qPCR) was used to quantify the DNA of this bacterium in order to analyze the possibility of using this method in assessing the degree of infection (Ct) with *B. hyodysenteriae*, for which a commercial set of chemicals "KAPA SYBR® FAST Master Mix (2X) Universal" was used (KAPA Biosystems, MA, USA). Amplification reactions were performed on a “Rotor-Gene Q 5plex apparatus” (QIAGEN, Germany). For PCR identification of *B. hyodysenteriae,* a pair of oligo-nucleotide primers were used that allow amplification of a specific segment of the mcpA gene of *B. hyodysenteriae* (*Davis et al., 2005*):
• RT1 (5’-GCAGAAATTTTATGGAACTTAGACCAG-3’) and
• RT2 (5’-TCCTGTAGCTGCCGATTCTTTAA-3’)

Statistical analysis of the results was performed using the software STATISTICA v6.0 (StatSoft, Inc., Tulsa, USA) and GraphPad Prism version 6.0 (GraphPad, San Diego, CA, USA).

Results and Discussion

Analysis of the presence of *B. hyodysenteriae* in fecal samples was performed by isolation of the pathogen (microbiological method) and identification by molecular genetic method by real-time PCR. By using the real-time PCR method, quantitative data were obtained that indicate the amount of DNA of the monitored pathogen in the sample, i.e. Ct values (Ct value is inversely proportional to the initial amount of target DNA). The lowest average Ct value (i.e. the highest amount of DNA of the bacterium *B. hyodysenteriae*) in all four observed periods (first, second and third week of the experiment and in the entire, three-week experimental period) was recorded in the K-group.

Table 1 shows that in the first week of the study the highest degree of infection or the lowest average Ct value (i.e. the highest amount of DNA of the bacterium *B. hyodysenteriae*) was recorded in piglets of the K-group (30.50) which was a negative control group, while the piglets from the PHP group had the highest average Ct value (i.e. the lowest amount of DNA of the bacterium *B. hyodysenteriae*) (32.90). Piglets from the TK + group had an average Ct value of 31.51, while the PH group of piglets had slightly lower value, 30.99.

In the second week, piglets from the K-group had the lowest average Ct value (30.55), followed by heads from the PH group (32.30). Piglets from the TK + group had an average Ct value of 33.66, while piglets from the PHP group had the highest Ct value (33.93).

Slightly different results were obtained in the third week of the trial. Piglets of TK + group had the highest average Ct value 34.35, and piglets from K-group had the lowest 31.41. Piglets fed diet supplemented with Patente Herba® (PH) and Patente Herba® Plus (PHP) additives showed 32.97 and 33.59 mean Ct values in the third week of the experiment, respectively.

If we observe the entire three-week experimental period, we see that the lowest average Ct value (i.e. the highest amount of DNA of the bacterium *B. hyodysenteriae*) was in the K-group of piglets 33.25, followed by the PH group of piglets (34.11), then the group of piglets fed diet to which the Patente Herba® Plus (PHP) additive was added (34.47), while the highest average Ct value was in the group of piglets that received the antibiotic thiamulin (Tiamulin - P - 10%) where the Ct was 35.85.

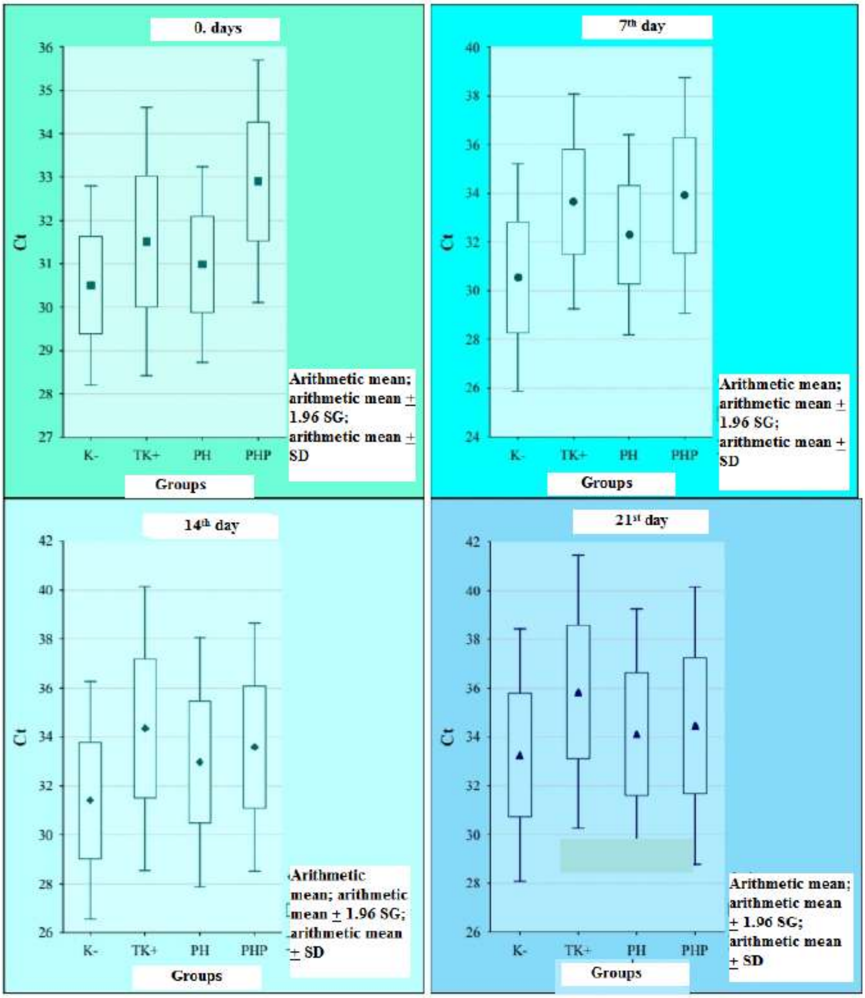
Table 1. Basic statistical indicators of Ct values by observed periods and groups

Observed period (days)	Group	Arithmetic mean	Minimum	Maximum	Coefficient of variation (%)
First week (1-7)	K-	30.50	26.44	34.61	7.52
	TK+	31.51	26.78	38.99	9.81
	PH	30.99	27.44	34.51	7.28
	PHP	32.90	28.98	38.11	8.49
0 day		F=2.48		p=0.07	
Second week (2-14)	K-	30.55	22.01	44.21	15.27
	TK+	33.66	27.21	43.49	13.11
	PH	32.30	26.99	43.01	12.75
	PHP	33.93	28.54	44.34	14.29
7 days		F=1.88		p=0.143	
Third week (15-21)	K-	31.41	25.22	42.22	15.47
	TK+	34.35	28.12	44.66	16.89
	PH	32.97	28.09	44.06	15.45
	PHP	33.59	27.07	44.51	15.09
14 days		F=0.92		p=0.439	
Complete, three-week experimental period (1-21)	K-	33.25	25.55	43.76	15.56
	TK+	35.85	27.85	42.91	15.61
	PH	34.11	27.96	43.84	15.07
	PHP	34.47	25.93	44.91	16.51
21 days		F=0.64		p=0.593	

Comparing the obtained results of the average degree of infection and its influence on the growth of piglets obtained by *Delić et al. (2018),* a positive effect of phytogenic additives on the degree of infection with the bacterium *B. hyodysenteriae* and thus a direct effect on the growth of piglets can be observed.

Based on the coefficient of variation in all four observed periods (i.e. in the first, second and third weeks of the experiment separately, as well as in the entire, three-week experimental period), it can be concluded that all groups were homogeneous (Table 1 and Graph 1).

Methodological limitations and inconsistency in the existing susceptibility testing procedures are the reason why the current diagnostic methods cannot reliably examine the effect of drugs in the treatment of swine dysentery. Our results of the analysis of variance on the absence of statistically significant differences in the average Ct value between the groups on day 0 (p=0.070) and on day 7 (p=0.143), day 14 (p=0.439) and day 21 (p=0.593), and findings F<1 for days 14 and 21, indicated that real-time PCR is not adequate for quantitative evaluation of the efficacy of the preparation in the control of swine dysentery, which means that based on the obtained Ct values the amount of DNA *B. hyodysenteriae* in the sample cannot be validly estimated, and therefore that real-time PCR is not an adequate method for assessing the efficacy of the preparation against *B. hyodysenteriae.*



Graph 1. The trend of Ct values by measurement time and groups

Conclusion

Based on the obtained Ct values, it can be concluded that the highest amounts of DNA of B. hyodysenteriae were in negative control, and the lowest amounts in the groups treated with Patente-Herba® Plus on days 0 and 7, but also in the tiamulin-treated group on days 14 and 21. Real-time PCR can be used to quantify the DNA of *B. hyodysenteriae*, however, to assess the effectiveness of the preparation in controlling swine dysentery in relation to the quantification of the total number of *B. hyodysenteriae*, it is necessary to optimize the method.

Uticaj fitogenih aditiva na stepen infekcije bakterijom *B. hyodysenteriae* kod zalučene prasadi

Nikola Delić, Dragan Nikšić, Maja Petričević, Aleksandar Stanojković, Vladimir Živković, Marina Lazarević, Nevena Maksimović

Rezime

U ovom radu ispitivane su prosečne Ct vrednosti kod 4 grupe prasadi u tronedeljnom vremenskom intervalu. Grupe su imale po 16 prasadi muškog pola. Sve četiri grupe prasadi dobijale su potpunu krmnu smešu koja je u potpunosti zadovoljavala potrebe za datu kategoriju životinja (NRC, 2012). Pored pomenute smeše, jednoj grupi u hranu je dodat aditiv Patente Herba® (PH), drugoj je davan hranom aditiv Patente Herba® Plus (PHP), treća grupa je hranom dobijala antibiotik tiamulin (Tiamulin - P - 10%) i predstavljala je pozitivnu kontrolu (TK+), dok je četvrta grupa dobijala samo potpunu krmnu smešu i predstavljala je negativnu kontrolu (K-). Najmanja prosečna Ct vrednost (odnosno najveća količina DNK bakterije B. hyodysenteriae) u sva četiri posmatrana perioda (prvoj, drugoj i trećoj nedelji eksperimenta i u celokupnom, tronedeljnom eksperimentalnom periodu) zabeležena je u K- grupi. Najveća prosečna Ct vrednost (odnosno najmanja količina DNK bakterije B. hyodysenteriae) 0. i 7. dana je bila u PHP grupi, a 14. i 21. dana u TK+ grupi. Rezultati analize varijanse ukazuju da se prosečne Ct vrednosti između grupa nisu razlikovale statistički značajno kako 0. dana ($F=2,48$; $p=0,070$), tako ni 7. dana ($F=1,88$; $p=0,143$), 14. dana ($F=0,92$; $p=0,439$) i 21. dana ($F=0,64$; $p=0,593$). S obzirom da su za 14. i 21. dan vrednosti F statistike manje od 1, znači da su se podaci u grupama više razlikovali nego između grupa, a i u samim grupama varijabilitet nije bio ispoljen ($cv \leq 30\%$).

Ključne reči: aditivi, stepen infekcije, prasad, B. hyodysenteriae, ishrana

Acknowledgements

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

References

BELLGARD M.I., WANCHANTHUEK P., LA T., RYAN K., MOOLHUIJZEN P., ALBERTYN Z., SHABAN B., MOTRO Y., DUNN D.S., SCHIBECI D., HUNTER A., BARRERO R., PHILLIPS ND., HAMPSON D. (2009): Genome sequence of the pathogenic intestinal spirochete Brachyspira hyodysenteriae reveals adaptations to its lifestyle in the porcine large intestine. PLoS One 4: e4641.

DAVIS A.J., SMITH S.C., MOORE R.J. (2005): The Brachyspira hyodysenteriae ftnA gene: DNA vaccinationand Real-Time PCR quantification of bacteria in a mouse model of disease. Curr Microbiol 50: 285-291.

DELIĆ N., DRAŠKOVIĆ V., STEVANOVIĆ J., SAVIĆ B., LAKIĆ N., BOŠNJAK-NEUMÜLLER
J., STANIMIROVIĆ Z. (2018): The efficacy of two phytogenic feed additives in a control of swine dysentery. Acta Vet-Beograd 68: 178-189.

FELLSTRÖM C., PETTERSSON B., THOMSON J., GUNNARSSON A., PERSSON M., JOHANSSON K.E. (1997): Identification of Serpulina species associated with porcine colitis by biochemical analysis and PCR. J Clin Microbiol 35: 462–467.

HAMPSON D.J. (2012): Brachyspiral colitis. In: Zimmerman, JJ., Karriker, LA., Ramirez, A., Schwartz, KJ., Stevenson, GW. (eds.) Diseases of swine, Tenth Edition. John Wiley & Sons, Inc., pp. 680-696.

HIDALGO Á., CARVAJAL A., LA T., NAHARRO G., RUBIO P., PHILLIPS N.D., HAMPSON D.J. (2010): Multiple-locus variable-number tandem-repeat analysis of the swine dysentery pathogen, Brachyspira hyodysenteriae. J Clin Microbiol 48: 2859–2865.

KENNEDY M.J., ROSNICK D.K., ULRICH R.G., YANCEY R.J. (1988): Association of Treponema hyodysenteriae with porcine intestinal mucosa. J Gen Microbiol 134: 1565–1567.

KENNEDY M.J., ROSEY EL., YANCEY R.J. Y.R. (1997): Characterization of flaA- and flaB- mutants of Serpulina hyodysenteriae: both flagellin subunits, FlaA and FlaB, are necessary for full motility and intestinal colonization. FEMS Microbiol Lett 153: 119-128.

LA T., PHILLIPS N.D., HARLAND B.L., WANCHANTHUEK P., BELLGARD M.I., HAMPSON D.J. (2009): Multilocus sequence typing as a tool for studying the molecular epidemiology and population structure of Brachyspira hyodysenteriae. Vet Microbiol 138: 330–338.

LEE J.I., HAMPSON D.J., COMBS B.G., LYMBERY A.J. (1993): Genetic relationships between isolates of Serpulina (Treponema) hyodysenteriae, and comparison of methods for their subspecific differentiation. Vet Microbiol 34: 35–46.

MILNER J.A., SELLWOOD R. (1994): Chemotactic response to mucin by Serpulina hyodysenteriae and other porcine spirochetes: potential role in intestinal colonization. Infect Immun 62: 4095–4099

NARESH R., HAMPSON D.J. (2010): Attraction of Brachyspira pilosicoli to mucin. Microbiology 156: 191–197.

NRC (2012): Nutrient Requirements of Swine. Eleventh Revised Edition, Nat. Acad. Press, Washington, DC.

RÅSBÄCK T., JOHANSSON K.E., JANSSON D.S., FELLSTRÖM C., ALIKHANI M.Y., LA T., DUNN DS., HAMPSON D.J. (2007): Development of a multilocus sequence typing scheme for intestinal spirochaetes within the genus Brachyspira. Microbiology 153: 4074–4087.

ROSEY E.L., KENNEDY M.J., YANCEY R.J. (1996): Dual flaA1 flaB1 mutant of Serpulina hyodysenteriae expressing periplasmic flagella is severely attenuated in a murine model of swine dysentery. Infect Immun 64: 4154–4162.

SAVIĆ B., RADANOVIĆ O., CVETOJEVIĆ D.J., STEVANČEVIĆ O., STOJANAC N., NEŠIĆ K., KURELJUŠIĆ B., SAVIĆ B. (2017): Multi locus sequence typing of Brachyspira hyodysenteriae isolates from pigs on Serbian farms. Berl Münch Tierärztl 130: 332-336.

STANTON T.B., ROSEY E.L., KENNEDY M.J., JENSEN N.S., BOSWORTH B.T. (1999): Isolation, oxygen sensitivity, and virulence of NADH oxidase mutants of the anaerobic spirochete Brachyspira (Serpulina) hyodysenteriae,etiologic agent of swine dysentery. Appl Environ Microbiol 65: 5028-5034.

STANTON T.B. (2006): The genus Brachyspira. In M Dworkin, S Falkow, E Rosenberg, K-H Schleifer, E Stackebrandt (eds.), The Prokaryotes, Vol. 7. Berlin: Springer, pp. 330–356.

TROTT D.J., OXBERRY S.L., HAMPSON D.J. (1997b): Evidence for Serpulina hyodysenteriae being recombinant, with an epidemic population structure. Microbiology 143: 3357–3365.

WANCHANTHUEK P., BELLGARD M.I., LA T., RYAN K., MOOLHUIJZEN P., CHAPMAN B., BLACK M., SCHIBECI D., HUNTER A., BARRERO R., PHILLIPS N.D. (2010): The complete genome sequence of the pathogenic intestinal spirochete Brachyspira pilosicoli and comparison with other Brachyspira genomes. PLoS ONE 5: e11455.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

ASSESSMENT OF THE EFFECTS OF EDIBLE MICROALGAE IN A CANINE GUT MODEL

Costanza Delsante, Carlo Pinna, Federica Sportelli, Claudio Stefanelli, Carla G. Vecchiato, Giacomo Biagi

Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy
Corresponding author: Giacomo Biagi, giacomo.biagi@unibo.it
Invited paper

Abstract: Gut microbiota plays a fundamental role in maintaining host health and metabolism and is considered a potential target of novel therapeutics. Microalgae represent an interesting source of bioactive compounds such as protein, fatty acids, fibre, and minerals for nutritional supplementation in humans and animals. Nevertheless, there is a lack of information on the effect of microalgae on canine gut microbiota. The aim of the study was to evaluate in a *in vitro* canine gut model the effects of four microalgae *Arthrospira platensis* (AP), *Haematococcus pluvialis* (HP), *Phaeodactylum tricornutum* (PT), *Chlorella vulgaris* (CV), on some faecal microbial populations and metabolites. Following the *in vitro* fermentation, chemical and microbiological analysis displayed significant differences between the control and microalgae groups. In particular, after 6h of incubation, microalgae increased propionate (+36% for CV; $p=0.001$) and butyrate (+24% for CV $p=0,013$), and decreased total BCFA (-47% for both PT and CV; $p=0.006$), isobutyrate (-52% for CV; $p=0.022$) and isovalerate (-43% for AP, CV, PT; $p=0.009$) and *C. hiranonis* (-0.46 log10 copies/75 ng DNA for CV; p=0.052); after 24h microphytes increased propionate (+21% for CV; $p=0.001$) and isovalerate (+10% for CV; $p=0.041$), and decreased the abundance of *Turicibacter* spp. (7.18 vs. 6.69 and 6.56 log10 copies/75 ng DNA for CTRL vs HP and CV, respectively; p=0.018), *C. leptum* (-1.12 log10 copies/75 ng DNA for PT; p=0.008) and *Enterococcus spp.* (-0.37 log10 copies/75 ng DNA for PT; p=0.044). These findings suggest a potential modulatory effect of microalgae on metabolism of canine faecal microbiota.

Key words: dog intestinal microbiota, microalgae, canine nutrition, in vitro fermentation

Introduction

The gastrointestinal tract of mammals harbours numerous bacterial species known to be, along with fungi, protozoa and viruses, one of the largest and most complex ecosystems known. Recently, there has been a growing understanding that the intestinal microbiota plays a crucial role in supporting host health (*Tuddenham and Sears, 2015*). In this vein, modulation of intestinal microbiota should be taken into account as a potential novel therapeutic (*Ercolini and Fogliano, 2018*). Among the bacterial metabolites straight short-chain fatty acids (SCFA) acetate, propionate, and butyrate and branched chain fatty acids (BCFA) isobutyrate and isovalerate (*Mondo et al., 2019*) play a very different but important role in the host body (*Rowland et al., 2018*).

The diet plays a fundamental role in shaping the composition of gut microbiota and its relation with the host (*Ercolini and Fogliano, 2018*). In last decades, scientific research has widely investigated different nutritional strategies aimed to positively influence the microbial ecosystem of human gastrointestinal tract (*Conlon and Bird, 2014*). Among the several dietary components investigated in this context, edible microalgae, also called microphytes, represent an interesting source of bioactive compounds including protein, polyunsaturated fatty acids, polysaccharides, pigments, vitamins, minerals, phenolic compounds, volatile compounds, and sterols, hence offering several possible health benefits (*Camacho et al., 2019*). Microalgae are ancestral living organisms belonging to a phylogenetically diverse group, encompassing a number of different phyla and classes of organisms; in some cases, cyanobacteria are also included (*Kay and Barton, 1991*). For their characteristic, microphytes have been proposed as encouraging sustainable alternatives to conventional animal feed resources and for their possible application as health-promoting ingredients both in human diet (*Guerin et al., 2003; Vaz et al., 2016*) and animal feeds (*de Medeiros et al., 2021*), particularly, in the aquaculture sector (*Charoonnart et al., 2018*). However, successful inclusion of microalgae and microalgae-based products in feed requires a clear understanding of their effects on the intestinal microbiota and bacterial metabolome of the host (*Sagaram et al., 2021*).

Over the last years, studies have identified many positive benefits of microalgae, including immunomodulatory (*Manzo et al., 2017; Satyaraj et al., 2021*), antioxidant (*Guzmán-Gómez et al., 2018*), anti-inflammatory (*Montero-Lobato et al., 2018; Rodríguez-Luna et al., 2018*), and anti-bacterial effects (*Martínez et al., 2019*). In addition, some microalgae are also known to have prebiotic properties (*Patel et al., 2021*), thus modulating the gastrointestinal microbiota. For example, colonic microbiota composition of rats changed after diet supplementation with some edible blue-green algae (cyanobacteria) including

Spirulina (*Rasmussen et al., 2009*). Recently, *Jin et al. (2020)* have demonstrated that supplementation with microalgae, including *Chlorella vulgaris*, increased propionate-producing bacteria in an *in vitro* human gut fermentation model (*Jin et al., 2020*). However, there is a paucity of studies investigating the effects of microphytes on canine gut microbiota and intestinal concentrations of metabolites deriving from the bacterial metabolism such as SCFA, BCFA, ammonia and biogenic amines, which are known to be of crucial relevance in host–microbial interactions (*Mccarville et al., 2020*). Moreover, some of aforementioned metabolites (SCFA in particular) represent important indices of gut health (*Alexander et al., 2019*).

The aim of the present study was to evaluate in an *in vitro* canine gut model the effects of four microalgae *Arthrospira platensis* (AP), *Haematococcus pluvialis* (HP), *Phaeodactylum tricornutum* (PT), and *Chlorella vulgaris* (CV) on some fecal microbial populations and metabolites. We supposed that composition and metabolism of canine faecal microbiota would have been positively influenced by microalgae supplementation.

Materials and Methods

The present study was conducted at the Laboratory of Animal Production of the Department of Veterinary Medical Sciences, University of Bologna, Italy.

Experimental Set Up

In order to simulate the digestion processes that take place in the stomach and small intestine of dogs, the microalgae were preliminarily subjected to *in vitro* digestion, according to the method proposed by *Biagi et al. (2016)*. The undigested fraction was subjected to analysis (Table 1) and subsequently tested as a fermentation substrate.

Table 1. Proximate analysis of the undigested fraction of four microalgae.

	Crude protein%	Crude fat, %	Crude ash, %
Arthrospira platensis	55.7	5.81	4.04
Haematococcus pluvialis	10.2	16.6	1.53
Phaeodactylum tricornutum	18.0	10.7	10.7
Chlorella vulgaris	18.6	3.28	27.0

Five healthy adult dogs (mixed breed; average body weight of 21 kg; age 3.6 years), house hold, were fed the same commercial dry diet for adult dogs (Stuzzy New Zealand & Australia Dry Line with venison, Agras Delic Spa, Italy) for 4 weeks. The diet contained the following ingredients: corn, barley, dehydrated venison, potato protein, purified pork fat, dried beet pulp, sunflower oil, brewer's yeast, dried chicory pulp, FOS, cod liver oil, dicalcium phosphate, potassium chloride, sodium chloride, herbs (dog rose, bearberry, blackcurrant, taraxacum, and thistle), and *Yucca schidigera*. The macronutrient composition of the diet (per kg on dry matter basis) was the following: crude protein (CP) 236 g, ether extract (EE) 125 g, crude ash (ash) 57.1 g, starch 389 g, and crude fibre (CF) 20.8 g.

The same dry food that was fed to the dogs used as faecal donors was subjected to *in vitro* digestion using the two-step procedure proposed by *Biagi et al. (2016)*. After *in vitro* digestion, the undigested fraction was dried at 65°C until a constant dry weight was obtained (18.5 g of undigested residue were obtained from 100 g of food dry matter [DM]) and its chemical composition per kg was the following: CP 173 g, EE 24.3 g, starch 38.7 g, ash 146 g, and CF 99.4 g.

After the 4-week feeding period, a sample of fresh faeces was collected from each dog immediately after excretion; faeces were pooled and suspended at 10 g/L in prereduced Wilkins Chalgren anaerobe broth. The faecal suspension was used to inoculate (100 mL/L) a previously warmed (39°C) and prereduced medium prepared according to *(Sunvold et al., 1995)*. Five 30 mL bottles (each bottle containing 21 mL of faecal culture) were set up per treatment.

Five treatments were carried out: (1) control diet with no addition of experimental substrates and control diet with (2) *Arthrospira platensis* (AP), (3) *Haematococcus pluvialis* (HP), (4) *Phaeodactylum tricornutum* (PT), or (5) *Chlorella vulgaris* (CV). All bottles contained the undigested residue of the commercial dry food for dogs at 10 g/L. The amount of microalgae that was added to the inocula is reported in table 2. Amounts were calculated based on the different *in vitro* digestibility coefficients of microalgae (Table 2). The dose that was used should reflect the amount of microalgae that reach the hindgut when they are included in a commercial extruded food for dogs (with a digestibility of approximately 90%) at a concentration of 40 g/kg.

Table 2. Amount of undigested fraction of the commercial dry food and microalgae that were added to bottles and digestibility coefficients of microalgae subjected to *in vitro* digestion.

Treatment	Commercial dry food, undigested fraction (mg)	Algae, total digestibility (%)	Algae, undigested fraction (mg)
CTRL	210	-	-
AP	210	86.2	11.6
HP	210	7.87	77.4
PT	210	67.5	27.3
CV	210	55.3	37.5

CTRL, control; AP, *Arthrospira platensis;* HP, *Haematococcus pluvialis;* PT, *Phaeodactylum tricornutum*; CV, *Chlorella vulgaris.*

The pH of faecal cultures was adjusted to 6.7; bottles were sealed and incubated for 24 h at 39°C in an anaerobic cabinet (Anaerobic System; Forma Scientific Co., Marietta, OH; under an 85% N2, 10% CO2 and 5% H2 atmosphere). Samples of fermentation fluid were collected from each bottle at 6 and 24 h for the determination of pH, ammonia, biogenic amines, SCFA, and for microbial analysis.

Chemical Analyses

The commercial dry food and its undigested residue were analysed according to the AOAC International standard methods (method 950.46 for water, method 954.01 for CP, method 920.39 for EE, method 920.40 for starch, method 942.05 for ash and method 962.09 for CF). Ammonia was measured using a commercial kit (Urea/BUN—Color; BioSystems S.A., Spain). The SCFA and BCFA were separated on a 2-m glass column (inner diameter, 3 mm) of 10% SP-1000 + 1% H_3PO_4 on 100/120 Chromosorb W AW with nitrogen as the carrier. The chromatograph was a Fisons HRGC MEGA 2 series 8560 with a flame ionization detector. The temperatures of the injector and detector were 200 °C, and the oven temperature was 155 °C. 2-ethylbutyric acid was used as the internal standard. For the determination of biogenic amines, samples were diluted 1:5 with perchloric acid (0.3 M); biogenic amines were later separated by HPLC and quantified through fluorimetry (*Stefanelli et al., 1986*).

Microbial Analysis

At each sampling time, a 1 mL portion of fermentation fluid was collected from each vessel and centrifuged at 4°C for 5 min, at 18,000 X g. The supernatant was removed and immediately frozen at -80°C for further analysis. Bacterial genomic DNA was extracted from remaining pellet using the Stool DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada). Isolated DNA concentration (ng/µL) and purity were measured using a DeNovix DS-11 spectrophotometer (DeNovix Inc., Wilmington, DE, USA). Template DNA was diluted to 50 ng/µL and stored at -20°C until further analysis. Turicibacter, Ruminococcaceae, Blautia, *Escherichia coli*, *Bifidobacterium* spp., *Lactobacillus* spp., *Enterococcus* spp., C*lostridium* cluster XIV, *Clostridium coccoides*, *Clostridium leptum* e *Clostridium hiranonis* were quantified via quantitative polymerase chain reaction (qPCR) using specific primers. The qPCR assay was performed using a CFX96 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). Amplification was performed in duplicate for each bacterial group within each sample, while standard curves were run in triplicate.

Briefly, the PCR reaction contained 7.5 µL 2XSensiFAST No-ROX PCR MasterMix (Meridian Bioscience Inc, Cincinnati, OH, USA), 4.8 µL of nuclease-free water, 0.6 µL of each 10 pmol primer and 1.5 µL of template DNA for a final reaction volume of 15 µL. The amplification cycle was as follows: initial denaturation at 95°C for 2 min, 95°C for 5 s, primer annealing at 56-64°C for 10 s and 72°C for 8 s. The cycle was repeated 40 times. A negative control (without the DNA template) was also run for each primer pair. Standard curves were constructed from eight tenfold dilutions for each target. Cycle threshold values were plotted against standard curves for the quantification of the target bacterial DNA from faecal inoculum. Melting curves were checked after amplification to ensure the single product amplification of a consistent melting temperature.

Statistical Analyses

Kruskal-Wallis One-ANOVA with Dunn's multiple comparisons were performed for data with unequal variances, while normally distributed data were compared using one-way ANOVA with Dunnett's multiple comparison test. Differences between groups were considered significant for $p<0.05$. Each vial represented a single experimental unit. Significance and tendency for statistical tests were set at $p<0.05$ and $0.05<p<0.1$, respectively. Statistical analyses were performed using Statistica 10.0 software (Stat Soft Italia, Padua, Italy).

Results

The chemical parameters evaluated on samples of fermentation fluid collected after 6 and 24 h of incubation are shown in Tables 3 and 4, respectively. After 6 h of incubation, pH was decreased by HP, PT and CV compared to CTRL (6.58, 6.56, 6.63 vs. 6.71 respectively; p=0.005). Conversely, after 24 h of incubation, the pH was not statistically different between CTRL and microphyte groups (p>0.05). Moreover, the concentration of ammonia did not change after 6 and 24 h of incubation. Total concentrations of SCFA were not influenced by treatments after 6 and 24 h. On the contrary total BCFA were decreased in flasks containing PT and CV (-46% for both; p=0.006) at 6 h, however, this effect was no longer present after 24 h. At 6 h, flasks with CV contained higher concentration of propionate (+36%; p=0.001) and butyrate (+24%; p=0.013). Moreover, after 6 h of incubation, isobutyrate was reduced by CV (-52%, p=0.022) and isovalerate was decreased by all treatments, except HP (-43% for AP, CV, PT; p=0.009). At 24 h, propionate was still higher in vessels containing CV (+21%; p = 0.001) while BCFA were not affected by microalgae with the exception of isovalerate concentration that was higher in CV (+10%; p=0.041). In addition, no significant effects were observed in regard to biogenic amines both at 6 and 24 h, as reported in Table 5.

Table 3. pH values, ammonia and short-chain fatty acids concentrations after 6 h of an *in vitro* incubation of canine faecal inoculum supplemented with microalgae.[1]

Item	CTRL	AP	HP	PT	CV	pooled SEM	anova p-value
pH	6.71	6.63	6.58*	6.56*	6.63*	0.03	0.005
Ammonia, mmol/L	30.2	32.2	31.4	31.9	29.6	1.62	0.586
Straight-chain SCFA, mmol/L							
Acetate	8.62	8.66	8.97	8.57	8.85	0.42	0.954
Propionate	4.54	4.92	5.13	5.14	6.19*	0.23	0.001
Butyrate	2.55	2.58	2.62	2.69	3.16*	0.12	0.013
Total SCFA	15.7	15.7	16.7	16.4	18.2	0.78	0.232
BCFA, mmol/L							
Isobutyrate	0.27	0.15	0.15	0.13	0.13*	0.03	0.022
Isovalerate	0.46	0.26*	0.30	0.26*	0.26*	0.03	0.009
Total BCFA	0.73	0.41	0.45	0.39*	0.39*	0.08	0.006

[1] Values are the means of five bottles per treatment.
* Significantly different from CTRL, *p<0.05*
CTRL, control; AP, *Arthrospira platensis;* HP, *Haematococcus pluvialis;* PT, *Phaeodactylum tricornutum*; CV, *Chlorella vulgaris;* SCFA, short chain fatty acid; BCFA, branched chain fatty acid.

Table 4. **pH values, ammonia and short-chain fatty acids concentrations 24 h of an in vitro incubation of canine faecal inoculum with a control diet supplemented with microalgae.**[1]

Item	CTRL	AP	HP	PT	CV	pooled SEM	anova p-value
pH	5.84	5.84	5.81	5.81	5.95	0.01	0.004
Ammonia, mmol/L	39.6	39.9	36.0	35.7	38.0	1.29	0.065
Straight-chain SCFA, mmol/L							
Acetate	16.7	16.9	16.6	16.5	16.5	0.48	0.960
Propionate	9.68	10.5	10.3	10.7	11.7*	0.28	0.001
Butyrate	5.43	5.73	5.31	5.61	5.65	0.14	0.271
Total SCFA	31.81	33.13	32.21	32.81	33.85	0.89	0.536
BCFA, mmol/L							
Isobutyrate	0.60	0.64	0.60	0.62	0.64	0.02	0.289
Isovalerate	0.92	0.95	0.90	0.94	1.01*	0.02	0.041
Total BCFA	1.52	1.59	1.50	1.56	1.65	0.04	0.086

[1] Values are the means of five bottles per treatment.
* Significantly different from CTRL, $p<0.05$
CTRL, control; AP, *Arthrospira platensis;* HP, *Haematococcus pluvialis;* PT, *Phaeodactylum tricornutum*; CV, *Chlorella vulgaris;* SCFA, short chain fatty acid; BCFA, branched chain fatty acid.

Table 5. **Biogenic amines concentrations (nmol/mL) 6 h and 24 h of an in vitro incubation of canine faecal inoculum with a control diet supplemented with microalgae.**[1]

Item	CTRL	AP	HP	PT	CV	pooled SEM	anova p-value
6 h							
Putrescine	177.4	186.6	175.6	179.0	169.2	4.87	0.241
Cadaverine	101.0	124.6	132.4	96.4	87.4	15.1	0.371
Spermidine	24.4	68.8	36.4	23.6	21.6	9.55	0.043
Spermine	3.80	3.70	5.02	1.28	0.98	6.85	0.041
24 h							
Putrescine	166.4	174.0	111.4	107.8	140.4	10.2	0.007
Cadaverine	129.8	154.4	72.4	113.8	97.4	25.6	0.223
Spermidine	22.0	21.8	18.2	24.6	22.6	3.00	0.669
Spermine	1.32	1.16	1.04	2.12	0.58	0.53	0.414

[1] Values are the means of five bottles per treatment.
CTRL, control; AP, *Arthrospira platensis;* HP, *Haematococcus pluvialis;* PT, *Phaeodactylum tricornutum*; CV, *Chlorella vulgari.*

The data relating to the composition of the faecal microbiota evaluated at 6 and 24 h of incubation are presented in Table 6 and 7, respectively. After 6 h, treatments containing CV tended to decrease the abundance of *C. hiranonis* (6.88 vs. 7.34 log10 copies /75 ng DNA; p=0.052). Microphyte treatments decreased the presence of some bacterial population after 24 h. In particular, the abundance of *Turicibacter* spp. was reduced by HP and CV (6.69 and 6.56 vs.7.18 log10 copies /75 ng DNA, respectively; p=0.018). Finally, *C. leptum* (8.26 vs. 9.38 log10 copies /75 ng DNA p=0.008) and *Enterococcus* spp. (6.99 vs. 7.36 log10 copies/75 ng DNA p=0.044) were less abundant in flasks containing PT.

Table 6. Microbial analysis after 6 h of an in vitro incubation of canine faecal inoculum with a control diet supplemented with microalgae.[1]

Target	CTRL	AP	HP	PT	CV	pooled SEM	anova p-value
Bifidobacterium spp.	7.10	7.21	6.91	7.11	6.63	0.23	0.415
Blautia	6.51	6.60	6.42	6.37	6.16	0.19	0.571
Clostridium cluster XIV	8.14	8.21	7.87	8.07	7.97	0.19	0.639
Clostridium coccoides	7.51	7.50	7.26	7.43	7.31	0.25	0.929
Clostridium hiranonis	7.34	7.16	7.20	6.96	6.88*	0.11	0.052
Clostridium leptum	7.42	7.34	7.35	7.20	6.99	0.13	0.183
Escherichia coli	7.01	7.30	7.23	6.93	7.00	0.12	0.346
Enterococcus spp.	7.16	7.27	6.70	7.19	7.32	0.24	0.409
Lactobacillus spp.	5.84	5.77	5.52	5.62	4.87	0.32	0.283
Ruminococcaceae	8.49	8.50	8.32	8.34	8.11	0.19	0.595
Turicibacter spp.	6.30	6.36	6.13	6.10	6.07	0.16	0.631

[1] Values are the means of five bottles per treatment.
The qPCR data was expressed as log10 copies of DNA for each particular bacterial target per 75 ng of isolated total DNA.
* Significantly different from CTRL, $p<0.05$
CTRL, control; AP, *Arthrospira platensis;* HP, *Haematococcus pluvialis;* PT, *Phaeodactylum tricornutum*; CV, *Chlorella vulgaris*

Table 7. Microbial analysis after 24 h of an in vitro incubation of canine faecal inoculum with a control diet supplemented with microalgae.[1]

Target	CTRL	AP	HP	PT	CV	pooled SEM	anova p-value
Bifidobacterium spp.	8.23	7.92	8.11	8.32	7.91	0.11	0.066
Blautia	7.63	7.38	7.60	7.46	7.46	0.09	0.313
Clostridium cluster XIV	8.33	8.07	8.38	8.26	8.31	0.08	0.134
Clostridium coccoides	9.38	8.97	8.88	8.26*	9.09	0.19	0.008
Clostridium hiranonis	8.14	8.06	8.03	8.07	7.95	0.10	0.754
Clostridium leptum	8.41	8.37	8.31	8.15	8.27	0.09	0.310
Escherichia coli	8.45	8.41	8.37	8.16	8.54	0.11	0.206
Enterococcus spp.	7.36	7.19	7.30	6.99*	7.40	0.10	0.044
Lactobacillus spp.	7.29	7.41	7.16	7.19	6.80	0.19	0.256
Ruminococcaceae	9.65	9.47	9.60	9.42	9.48	0.08	0.276
Turicibacter spp.	7.18	6.92	6.69*	6.74	6.56*	0.12	0.018

[1] Values are the means of five bottles per treatment.
The qPCR data was expressed as log10 copies of DNA for each particular bacterial target per 75 ng of isolated total DNA.
* Significantly different from CTRL, $p<0.05$
CTRL, control; AP, *Arthrospira platensis;* HP, *Haematococcus pluvialis;* PT, *Phaeodactylum tricornutum*; CV, *Chlorella vulgaris*

Discussion

The purpose of this investigation was to evaluate the *in vitro* effects of four microalgae on some canine faecal microbial populations and metabolites. It must be emphasized that very few studies have investigated the use of microphytes in dogs and they were mainly focused on anti-inflammatory and immunomodulating activities of microalgae (*Satyaraj et al., 2021*). In this study, supplementation with microalgae partially affected the gut ecology.

Particularly, pH was decreased after 6 h in three of the four microalgae groups (HP, CV, PT). The reduction of intestinal pH could be a desirable effect, as the acidification of the environment has a broad-spectrum inhibitory activity against Gram-positive and Gram-negative bacteria. It is known how the colonic pH is influenced by fermentation processes of bacterial populations, in particular in the proximal colon, where the pH is lower due to the production of SCFA that mainly derive from the fermentation of carbohydrates (*Hamer et al., 2012*). However, in the present investigation, total concentration of SCFA was not affected by treatments.

After 6 h of incubation, the concentration of propionate and butyrate was increased by CV. A previous study conducted in an *in vitro* human gut model demonstrated that supplementation with microphytes, including CV, could affect both intestinal microbiota composition and metabolites. Particularly, *Jin et al. (2020)* investigated the effects of three edible microalgae (*Chlorella vulgaris, Chlorella protothecoides*, and *Schizochytrium* sp.) on gut microbiota showing that microalgae supplementation increased the proportion of propionate in the colonic culture together with the relative abundance of some bacterial populations involved in propionate metabolism (genera *Bacteroides* spp. and *Dialister* spp.). Moreover, total SCFA were significantly increased by *C. vulgaris*. Similar effects were observed in the present study regarding the higher concentration of propionate in CV group, both after 6 and 24 h. Intestinal SCFA are linked with some health-promoting effects, such as anti-inflammatory, anticarcinogenic, and immune-regulatory functions (*O'Keefe, 2016*). Specifically, propionate is metabolized in the liver and plays a role in reducing the concentration of blood sugar and serum cholesterol, while butyrate is an important source of energy for colonocytes (*Guarner and Malagelada, 2003*). In addition, butyrate is known to be effective in preventing colon cancer (*McNabney and Henagan, 2017*).

Nevertheless, in the present trial higher concentration of SCFA did not reflect a change in microbial populations known as SCFA producers. Moreover, after 24 h of fermentation, lower presence of genera *Enterococcus* spp. (PT), *Turicibacter* spp. (CV and HP) and *C. coccoides* subgroup (PT) were detected in three of the groups to which microalgae were added. These last outcomes are in contrast with the results recently obtained by *Wan et al. (2019)* who studied the effect of a bioactive polysaccharide from microalga *Chlorella pyrenoidosa* (CPP) at the dosage of 150 and 300 mg/kg, on gut microbiota of mice fed a high-fat diet. The authors pointed out that the growth of some bacterial genera, including *Turicibacter*, and the concentrations of acetate, propionate, and butyrate were drastically increased in both CPP treatments. *Turicibacter* spp., belonging to the *Firmicutes phylum*, was considered an important producer of SCFA *(Sivaprakasam et al., 2016),* suggesting an important role of *Turicibacter* spp. in promoting gut health.

For what concerns the decrease of *C. coccoides* that was observed in the present study, existing literature appears to report controversial findings regarding the abundance of this bacterial group in host physiology. *C. coccoides*, belongs to the *Firmicutes phylum*, one of the most predominant groups in the human gut, and many species in this class, such as *Eubacterium* spp., *Roseburia* spp., *Subdoligranulum variabile*, and *Faecalibacterium prausnitzii* directly produce butyrate from dietary polysaccharides and other substrates (*Jamar et al., 2018*); moreover, its presence is also correlated with an increased capacity to harvest energy from diet (*Turnbaugh et al., 2006*). Microbial analysis showed that, at 24 h, PT treatment decreased enterococci and *C. leptum* subgroup. The last is known as a butyrate-producing bacterium previously reported to be less abundant in faecal samples of human patients suffering from gastrointestinal disorders like inflammatory bowel disease (*Wang et al., 2014*).

After 6 h of incubation, CV resulted in decreased abundance of *C. hiranonis*. *C. hiranonis* is a bacterial species of interest, as it shows bile acid 7 alpha-dehydroxylating activity, and a decrease in *C. hiranonis* may suggest bile acid dysmetabolism (*AlShawaqfeh et al., 2017*). These findings are apparently in contrast with previously mentioned studies (*Jin et al.,2020; Wan et al., 2019*), in which microphytes seemed to improve intestinal health by promoting the growth of positive bacterial population, such as SCFA-producing bacteria. Certainly, it must be underlined that, in the present study, only few of the main populations of canine microbiota have been evaluated. This fact represents a limitation as we cannot exclude that changes regarding other bacteria could not have been detected.

One of the main reasons for considering microalgae as an interesting source of food is their high protein content (e.g., 55% -70% for *S. platensis* and 42%-55% for *C. vulgaris* on a dry matter basis; *Matos, 2019*). In this study, microalgae were preliminarily subjected to *in vitro* digestion and the undigested fraction was used as the fermentation substrate. Proteins were highly represented in the undigested fraction of AP (55.7%), CV (18.6%), PT (18.0%). Interestingly, the presence of microalgae, despite the increased presence of protein, decreased BCFA after 6 h and did not result in higher concentrations of ammonia and biogenic amines, all metabolites deriving from bacterial proteolysis (*Blachier et al., 2006; Mccarville et al., 2020*). In particular, CV seemed to have the greatest effect on BCFA by decreasing both the concentration of isobutyrate and isovalerate. The biological significance of BCFA and biogenic amines is still poorly understood. The former originate from branched chain amino acids in the colon and it has been hypothesized that BCFA may have a role in the regulation of ionic exchanges in colonic mucosa (*Musch et al., 2001*) and that isobutyrate may act as a potential source of energy for colonocytes after exhaustion of butyrate (*Jaskiewicz et al., 1996*). Similarly, biogenic amines seem to have a beneficial influence on the intestinal mucosa (*Heby, 1981*) but, on the other hand, they could act as precursors in the formation of nitrosamines, known as carcinogens in humans (*Smith and Macfarlane, 1996*). The decrease of BCFA that we observed could indicate a reduction of proteolytic activities operated by some bacterial populations. However, other parameters, including concentration of ammonia and biogenic amines, did not reflect this trend. In this regard, the effects of microalgae supplementation on metabolites deriving from bacterial proteolysis are still poorly investigated, hence it could represent an interesting aspect to be explored.

Conclusion

During the present *in vitro* study microalgae partially affected canine faecal microbiota. Among the four microphytes, CV showed the major effect on microbial metabolites after 6 h of incubation by increasing propionate, butyrate and decreasing BCFA. These outcomes suggest that microalgae, especially CV, could have a potential modulatory effect on the metabolic activities of canine faecal microorganisms. However, CV led to a reduction of *C. hiranonis* at 6 h, while after 24 h HP, PT and CV resulted in a decrease of some beneficial bacterial populations belonging to *Firmicutes*, known to be butyrate-producing bacteria.

The present study regarding the influence of microalgae on the intestinal microbiota of dogs has led to controversial results and should be considered as a preliminary study for future investigations.

References

ALEXANDER C., SWANSON K.S., FAHEY G.C., GARLEB K.A. (2019): Perspective: physiologic importance of short-chain fatty acids from nondigestible carbohydrate fermentation. Advances in Nutrition, 10, 4, 576-589.

ALSHAWAQFEH M., WAJID B., MINAMOTO Y., MARKEL M., LIDBURY J., STEINER J., SERPEDIN E., SUCHODOLSKI J. (2017): A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbiology Ecology 93, 11, 136.

BIAGI G., CIPOLLINI I., GRANDI M., PINNA C., VECCHIATO C.G., ZAGHINI G. (2016): A new in vitro method to evaluate digestibility of commercial diets for dogs. Italian Journal of Animal Science, 15, 4, 617-625.

BLACHIER F., MARIOTTI F., HUNEAU J.F., TOMÉ D. (2006): Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids, 33, 4, 547-562.

CAMACHO F., MACEDO A., MALCATA F. (2019): Potential industrial applications and commercialization of microalgae in the functional food and feed industries: a short review. Marine Drugs, 17, 6, 312.

CHAROONNART P., PURTON S., SAKSMERPROME V. (2018): Applications of microalgal biotechnology for disease control in aquaculture. Biology, 7, 2, 24.

CONLON M.A., BIRD A.R. (2014): The impact of diet and lifestyle on gut microbiota and human health. Nutrients, 7, 1, 17-44.

DE MEDEIROS V.P.B., PIMENTEL T.C., SANT'ANA A.S., MAGNANI M. (2021): Microalgae in the meat processing chain: feed for animal production or source of techno-functional ingredients. Current Opinion in Food Science, 37, 125-

134.
ERCOLINI D., FOGLIANO V. (2018): Food design to feed the human gut microbiota. Journal of Agricultural and Food Chemistry, 66, 15, 3754-3758.
GUARNER F., MALAGELADA J.R. (2003): Gut flora in health and disease. Lancet, 361, 9356, 512-519.
GUERIN M., HUNTLEY M.E., OLAIZOLA M. (2003): Haematococcus astaxanthin: applications for human health and nutrition. Trends in Biotechnology, 21, 5, 210-216.
GUZMÁN-GÓMEZ O., GARCÍA-RODRÍGUEZ R.V., QUEVEDO-CORONA L., PÉREZ-PASTÉN-BORJA R., RIVERO-RAMÍREZ N.L., RÍOS-CASTRO E., PÉREZ-GUTIÉRREZ S., PÉREZ-RAMOS J., CHAMORRO-CEVALLOS G.A. (2018): Amelioration of ethanol-induced gastric ulcers in rats pretreated with phycobiliproteins of *Arthrospira* (*Spirulina*) *maxima*. Nutrients, 10, 6, 763.
HAMER H.M., DE PRETER V., WINDEY K., VERBEKE K. (2012): Functional analysis of colonic bacterial metabolism: relevant to health? American Journal of Physiology - Gastrointestinal and Liver Physiology, 302, 1, G1-G9.
HEBY O. (1981): Role of polyamines in the control of cell proliferation and differentiation. Differentiation, 19, 1-3, 1-20.
JAMAR G., SANTAMARINA A.B., DIAS G.C., MASQUIO D.C.L., DE ROSSO V.V., PISANI L.P. (2018): Relationship between fatty acids intake and clostridium coccoides in obese individuals with metabolic syndrome. Food Research International 113, 86-92.
JASKIEWICZ J., ZHAO Y., HAWES J.W., SHIMOMURA Y., CRABB D.W., HARRIS R.A. (1996): Catabolism of isobutyrate by colonocytes. Archives of Biochemistry and Biophysics, 327, 2, 265-270.
JIN J.B., CHA J.W., SHIN I.-S., JEON J.Y., CHA K.H., PAN C.-H. (2020): Supplementation with *Chlorella vulgaris, Chlorella protothecoides,* and *Schizochytrium* sp. increases propionate-producing bacteria in *in vitro* human gut fermentation. Journal of the Science of Food and Agriculture, 100, 7, 2938-2945.
KAY R.A., BARTON L.L. (1991): Microalgae as food and supplement. Critical Reviews in Food Science and Nutrition 30, 6, 555-573.
MANZO E., CUTIGNANO A., PAGANO D., GALLO C., BARRA G., NUZZO G., SANSONE C., IANORA A., URBANEK K., FENOGLIO, D., FERRERA F., BERNARDI C., PARODI A., PASQUALE G., LEONARDI A., FILACI G., DE PALMA R., FONTANA A. (2017): A new marine-derived sulfoglycolipid triggers dendritic cell activation and immune adjuvant response. Scientific Reports, 7, 1, 1-10.
MARTÍNEZ K.A., LAURITANO C., DRUKA D., ROMANO G., GROHMANN T., JASPARS M., MARTÍN J., DÍAZ C., CAUTAIN B., CRUZ M. DE LA, IANORA A., REYES F. (2019): Amphidinol 22, a new cytotoxic and antifungal

amphidinol from the dinoflagellate amphidinium carterae. Marine Drugs, 17, 7, 385.

MATOS Â.P. (2019): Microalgae as a potential source of proteins. Proteins: sustainable source, processing and applications, 63-96.

MCCARVILLE J.L., CHEN G.Y., CUEVAS V.D., TROHA K., AYRES, J.S. (2020): Microbiota metabolites in health and disease. Annual Review of Immunology, 38, 147-170

MCNABNEY S.M., HENAGAN T .M. (2017): Short chain fatty acids in the colon and peripheral tissues: a focus on butyrate, colon cancer, obesity and insulin resistance. Nutrients, 9,12, 1348.

MONDO E., MARLIANI G., ACCORSI P.A., COCCHI M., LEONE A. DI. (2019): Role of gut microbiota in dog and cat's health and diseases, 9, 253-258.

MONTERO-LOBATO Z., VÁZQUEZ M., NAVARRO F., FUENTES J.L., BERMEJO E., GARBAYO I., VÍLCHEZ C., CUARESMA M. (2018): Chemically-induced production of anti-inflammatory molecules in microalgae. Marine Drugs, 16, 12, 478.

MUSCH M.W., BOOKSTEIN C., XIE Y., SELLIN J.H., CHANG E.B. (2001): SCFA Increase intestinal na absorption by induction of NHE3 in rat colon and human intestinal C2/Bbe cells. American Journal of Physiology - Gastrointestinal and Liver Physiology, 280, 4, 43-4.

O'KEEFE S.J.D. (2016): Diet, microorganisms and their metabolites, and colon cancer. Nature Reviews Gastroenterology & Hepatology, 13, 12, 691-706.

PATEL A.K., SINGHANIA R.R., AWASTHI M.K., VARJANI S., BHATIA S.K., TSAI M.-L., HSIEH S.-L., CHEN C.-W., DONG C.-D. (2021): Emerging prospects of macro- and microalgae as prebiotic. Microbial Cell Factories, 1, 1-16.

RASMUSSEN H.E., MARTÍNEZ I., LEE J.Y., WALTER J. (2009): Alteration of the gastrointestinal microbiota of mice by edible blue-green algae. Journal of Applied Microbiology, 107, 4, 1108-1018.

RODRÍGUEZ-LUNA A., ÁVILA-ROMÁN J., GONZÁLEZ-RODRÍGUEZ M.L., CÓZAR M.J., RABASCO A.M., MOTILVA V., TALERO E. (2018): Fucoxanthin-containing cream prevents epidermal hyperplasia and UVB-induced skin erythema in mice. Marine Drugs, 16, 10, 378.

ROWLAND I., GIBSON G., HEINKEN A., SCOTT K., SWANN J., THIELE I., TUOHY K. (2018): Gut microbiota functions: metabolism of nutrients and other food components. European Journal of Nutrition, 57, 1-24.

SAGARAM U.S., GAIKWAD M.S., NANDRU R., DASGUPTA S. (2021): Microalgae as feed ingredients: recent developments on their role in immunomodulation and gut microbiota of aquaculture species microalgae as feed ingredients. FEMS Microbiology Letters, 368, 11.

SATYARAJ E., REYNOLDS A., ENGLER R., LABUDA J., SUN P. (2021):

Supplementation of diets with *Spirulina* influences immune and gut function in dogs. Frontiers in Nutrition, 8: 667072.

SIVAPRAKASAM S., PRASAD P.D., SINGH N. (2016): Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. Pharmacology & Therapeutics, 164, 144-151.

SMITH E.A., MACFARLANE G.T. (1996): Studies on amine production in the human colon: enumeration of amine forming bacteria and physiological effects of carbohydrate and pH. Anaerobe, 2, 5, 285-297.

STEFANELLI C., CARATI D., ROSSONI C. (1986): Separation of N1- and N8-acetylspermidine isomers by reversed-phase column liquid chromatography after derivatization with dansyl chloride. Journal of Chromatography B: Biomedical Sciences and Applications, 375, 49-55.

SUNVOLD G.D., HUSSEIN H.S., FAHEY G.C., MERCHEN N.R., REINHART G.A. (1995): *In vitro* fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. Journal of Animal Science, 73,12, 3639-3648.

TUDDENHAM S., SEARS C.L. (2015): The intestinal microbiome and health. Current Opinion in Infectious Diseases, 28, 5, 464.

TURNBAUGH P.J., LEY R.E., MAHOWALD M.A., MAGRINI V., MARDIS E.R., GORDON J.I. (2006): An obesity-associated gut microbiome with increased capacity for energy harvest. Nature, 444, 7122, 1027-1031.

VAZ B. DA S., MOREIRA J.B., MORAIS M.G. DE, COSTA J.A.V. (2016): Microalgae as a new source of bioactive compounds in food supplements. Current Opinion in Food Science, 7, 73-77.

WAN X., AI C., CHEN Y., GAO X., ZHONG R., LIU B., CHEN, X. ZHAO C. (2019): Physicochemical characterization of a polysaccharide from green microalga *Chlorella pyrenoidosa* and its hypolipidemic activity via gut microbiota regulation in rats. Journal of Agricultural and Food Chemistry, 68, 5, 1186-1197.

WANG W., CHEN L., ZHOU R., WANG X., SONG L., HUANG S., WANG G., XIA B. (2014): Increased proportions of Bifidobacterium and the Lactobacillus group and loss of butyrate-producing bacteria in inflammatory bowel disease. Journal of Clinical Microbiology, 52, 2, 398-406.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

THE EFFECTS OF PRE-SLAUGHTER RESTING PERIODS ON CARCASS AND MEAT QUALITY DETERMINED BY DIGITAL IMAGE ANALYSIS

Yalcin Bozkurt[1], Serkan Ozkaya[1], Sukran Kuleasan[2]

[1]Isparta University of Applied Sciences, Faculty of Agriculture, Department of Animal Science, 32260, Isparta, Turkey
[2]Mehmet Akif Ersoy University, Faculty of Engineering, Department of Food Science, 15030, Burdur, Turkey
Corresponding author: Yalcin Bozkurt, yalcinbozkurt@isparta.edu.tr
Invited paper

Abstract: In this study, the effects of different pre-slaughter resting periods on the carcass and meat quality of slaughtered beef cattle by Digital Image Analysis (DIA) and traditional methods (colorimeter) were determined. There were 4 resting periods, for those slaughtered without resting (RP0); 3 hours resting (RP3), 6 hours (RP6) and 12 hours (RP12) and formed as four different groups of animals and 375 samples in total from 10 animal carcasses from each group were collected. The measurements values of pH for the meat in each group, L*, a* and b* values and the RGB values obtained by the image analysis were subjected to ANOVA analysis by using GLM (General Linear Model) procedure and Tukey test was applied for pairwise mean comparisons. The samples of 2.5 cm thick *Longissimus dorsi muscle* area (LMA) between the 12th and 13th ribs were taken and pH measurements were obtained from the lumbar region. LMAs were measured using a planimeter and colour values with a colorimeter (L*, a* and b*) were determined. Digital images of the LMA samples taken from the carcass were subjected to image analysis. Both LMA and the colour distribution of red, green and blue (RGB) values and their density were determined by DIA. A-10 cm reference card was placed next to each object to eliminate the effects of shooting distance between the object and a digital camera. There were no statistically significant differences in LMAs of any group of animals obtained by of either method used in this study. All RGB colour values of the meat of the animals slaughtered without resting period (RP0) were significantly higher than those obtained in other resting periods. Similarly, L*, a* and b* values of the meat of the animals slaughtered without resting period (RP0) were significantly higher (P=0.01) than those in other resting periods. It was also found that there were no statistical differences in the colour values of the meats of the animals subjected to resting time 6 and 12 hours in both methods. Therefore, these results suggested that it would be appropriate for the animals to be rested for at least 6 hours before slaughter. It can also be concluded that DIA may open up new dimensions to determine some other meat quality parameters (texture, visual appearance, marbling, lean to fat ratio, lipid/protein oxidation levels etc.).

Keywords: carcass, beef cattle, digital image analysis, meat quality

Introductıon

Rapidly developing computer technology has enabled new dimensions in the agriculture sector as well as speeding up the flow of agricultural information and facilitating the rapid transfer of information. The priority aim in the development in the agriculture sector in the developing countries is to increase productivity as well as to use the existing production potential effectively.

Digital image analysis can be used to obtain accurate descriptive values (*Sapirstein, 1995*), to be quick and objective (*Lefebvre et al., 1993*; *Gerrard et al., 1996; Liu et al., 1997; Ni et al., 1997*), to save people from tedious and time consuming operations (*Gunasekaran, 2001*), the ability to store data in such a way that objects are often able to analyse without corrupting the structures (*Trenkle and Limas, 1999*), is stable, effective and low cost (*Lu et al., 2000*), automating many processes requiring high technological skills (*Tarbell and Reid, 1991*).

Traditional methods are currently used in determining carcass and meat quality, and besides this method, advanced technology such as Digital Image Analysis, which can be used easily in practice, has been developed and the studies on the development of new systems that will increase productivity by determining values such as carcass fat thickness, *Longissimus dorsi* muscle area and marbling have accelerated in meat quality research areas (*Tylutki et al., 1994*).

In a study on the use of Digital Image Analysis, it was stated that significant relationships were obtained between the percentage of marbling and R, G, B (red, green, blue) colour values in the evaluation of meat quality characteristics of some cattle breeds (*Dasiewicz et al., 2003*). It was also emphasized that RGB and L*a*b* values can be measured on a given area of jpeg images (*OSullivan et al., 2003*).

It was reported by *Gregory (2008)* that animals taken to slaughterhouses become dizzy due to the accumulation of carbon dioxide due to fatigue during transportation and oxidative deterioration caused by *Escherichia coli* bacteria that occurs after slaughter. Therefore, animals are required to have a resting period before slaughter.

Traditional carcass and meat quality determination methods require knowing the pH value, meat colour and texture of meat as well as carcass yield. In recent years, computer-aided software programs have been developed and these programs have begun to be used in animal production. As a result of the studies, the Digital Image Analysis method has been widely used both in the private sector and in scientific studies (*Bozkurt et al., 2009, 2017*). Therefore, in this study, the determination of the effects of resting periods on meat quality was assessed by the colour densities of the meat acquired by Digital Image Analysis.

Materials and Methods

This study was conducted in the Lake District, Isparta and Burdur provinces of Turkey in the private sector and municipal slaughterhouses. The slaughtered Holstein, Brown Swiss and their crosses were recorded for the study purposes. Regardless of transportation distance of the slaughtered male animals was studied. Additionally, all the animals fasted in all resting periods before the slaughter. Animals were weighed before slaughtering by an electronic Tru-Test weighing scale (Haptner-Germany), the dressing percentage of the same animal carcasses was determined after slaughter.

Traditional method: Eye muscle area (*Longissimus dorsi*) samples split into 2.5 cm thick were taken from 10 carcasses each group, between the 12th and 13th ribs and pH measurements, were made at lumbar regions of the post-mortem in the first 45 minutes and 24th hours after slaughter by Crison pH meter. Eye muscle area was then determined by grid (Figure 1) and planimeter using and colour values with minolta colourimeter (L*, a* and b* values).

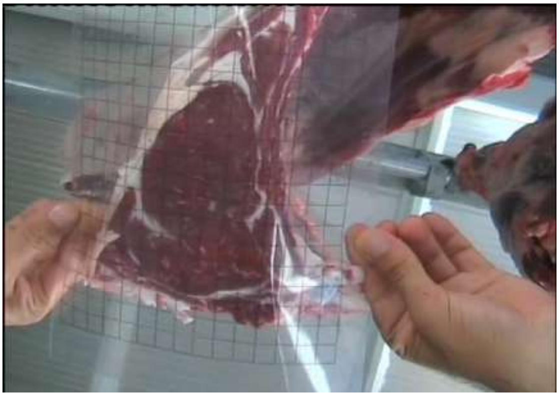


Figure 1. The eye muscles area measured by acetate grid

Digital image analysis method: A 10 cm reference card (Figure 2) was put next to each side of the carcass to eliminate the effects of the captured photo shooting distance. The sizes of parameters in images were transformed from the pixel unit to the metric units (cm), (spatial calibration). After slaughtering carcasses and *Longissimus dorsi* images were taken by using a digital camera then these images were subjected to the DIA method using Image-Pro Plus 6.0 and IQ-studio software programs (Figure 3). For the eye muscle area, the distribution of the colours red, green and blue (RGB) and the density was determined.

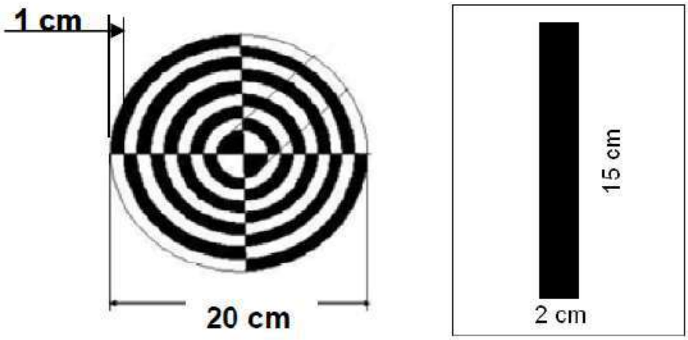


Figure 2. Some reference cards

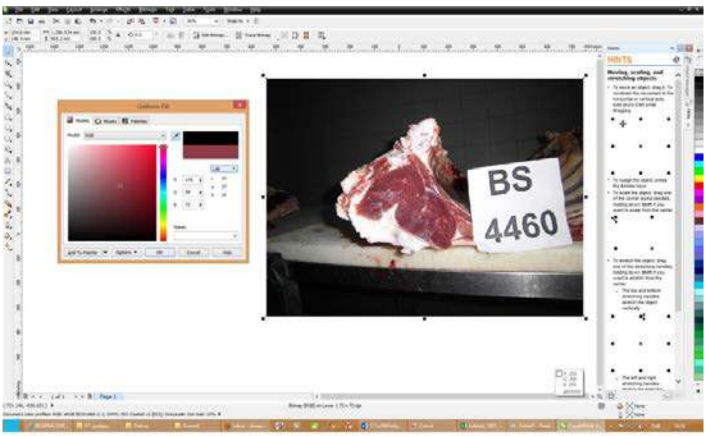


Figure 3. Digital methods of determining the colour intensity

Statistical Analysis

The observations obtained in the experiment were analysed by one-way analysis of variance technique (MİNİTAB V. 16). The values determined by DIA and the values determined by the metric measurements were associated with each other by correlation. pH measurements of each group, and differences between the colour values (L*, a* and b*) and the RGB values obtained by the image analysis were subjected to one-way ANOVA. Statistical analysis differences between mean values of characteristics are determined by the difference with Tukey test.

Results and Discussion

In this study, in total 375 cattle were used regardless of breed differences and were exposed to 4 different resting periods and 162 animals without resting (RP0), 66 animals 3-hour resting (RP3), 45 animals 6-hour resting (RP6) and 102 animals 12-hour resting (RP12). There were no statistically significant differences in the eye muscle areas of any group of animals obtained by the methods used in this study. Therefore, the results related to eye muscle area measurements were not presented.

The average colour values in meat samples according to different resting times are presented in Table 1.

Table 1. The average colour values in the meat samples according to resting time by DIA

	RED(R)			GREEN(G)			BLUE(B)		
	N	Mean*	S.E	N	Mean*	S.E	N	Mean*	S.E
RP0	162	157.5^{a}	2.8	162	88^{a}	2.2	162	90.9^{a}	2.1
RP3	66	76.0^{b}	2.9	66	44.5^{b}	1.5	66	46.7^{b}	1.8
RP6	45	71.9^{b}	3.9	45	46.4^{b}	1.9	45	47.3^{b}	2.4
RP12	102	64.3^{b}	2.4	102	43.4^{b}	1.2	102	44.7^{b}	1.5

N: The number of animals in each group, S.E.: Standard error, RP0: No resting, RP3: 3-hour resting, RP6: 6-hour resting, RP12: 12-hour resting.
*The means with the same superscripts within the same columns are not statistically significant ($P > 0.05$).

The red, green and blue colour values of the meat samples of the animals that were not resting (RP0) were found to be statistically significant ($P < 0.05$) and higher than the colour values of the meat samples obtained from other resting time animals (Table 1). In the evaluation of meat quality characteristics of some cattle breeds, with the help of the numerical image analysis method, there are important relationships between marbling percentage and R, G, B (red, green, blue) colour values. It is stated that it can be used as a method (*Dasiewicz et al., 2003*).

Table 2. The average colour intensity in meat samples according to their resting time

	L*			a*			b*		
	N	Mean	S.E	N	Mean	S.E	N	Mean	S.E
RP0	162	45.7^{a}	0.9	162	28.7^{a}	0.4	162	10.9^{a}	0.3
RP3	66	22.2^{b}	0.8	66	13.9^{b}	1.0	66	4.4^{b}	0.4
RP6	45	21.8^{b}	1.1	45	11.4^{bc}	1.2	45	3.4^{bc}	0.3
RP12	102	20.1^{b}	0.7	102	8.9^{c}	0.7	102	2.2^{c}	0.2

L* Brightness, a*: Red, b*: Yellow.

The means with the same superscripts within the same columns are not statistically significant (P >0.05).

The most colour intensity of the colour values L* brightness was detected in meat samples of animals slaughtered without resting with 45.7. In no-resting groups (RP0), the mean values of all L* colour intensities were statistically different (P <0.05) and greater than those values at other resting periods. The results are in agreement with other studies (*Nanni et al., 2002*) where the meat from the animals with a higher *ante mortem* stress before the slaughter process had a lower pH, a pale colour with high L* values.

In the case the red hue (a*), colour values the RP0 group were different (P <0.05) than the groups subjected to other resting periods. It was observed that this value was higher in the meat samples of the animals slaughtered in the same colour without resting in the yellow colour (b*), and these differences were found to be statistically significant (P<0.05). Similarly, yellow colour (b*) values the RP0 group were more than others, and these differences were statistically significant (P <0.05). However, there were no statistically significant differences in all colour values between RP3 and RP6; and also between RP6 and RP12. Similarly, Sima et al., (2015) reported that directly slaughtered animals had higher meat colour values (L*, a* and b*) than those animals rested at least for 3 hours.

As it was suggested by *Viljoen et al. (2002)* that exposing animals to chronic or long time stress such as long hours of transportation, food and water deprivation and overcrowding of animals in the lairage can cause Dark, Firm and Dry (DFD) carcasses. Pale Soft Exudative (PSE) and DFD meats are unattractive and more likely to face discrimination by consumers. PSE meat looks pale and lean, has soft texture and low water holding capacity and poor functional attributes. DFD meat looks dark, has variations in tenderness, poor functional attributes and is prone to spoilage. Moreover, the results found in this study were in line with those reported by *Marenčić et al. (2012)* that bulls rested in lairage had significantly higher pH24 and significantly lower L*, b* and a* value compared with unrested bulls on the contrary the rested heifers had significantly lower pH24 and significantly higher L* and a* value, compared with unrested heifers.

Conclusion

The results obtained from this study showed that both L*, a*, b* and RGB values were high in the animals slaughtered without resting, and as an expression the dark-cutting carcass, it was determined that the optimum resting time should be at least 6 hours. In addition, the results of this study can be advised to apply in slaughterhouses and will provide new dimensions for determining the shelf life of meat and processed meat products marketed in markets and on shelves.

Studies are showing that the values used in the determination of meat colour, as in many areas of DIA. To carcass and meat quality evaluation, the colour values to be determined by DIA are increasing research trends in this field. Digital image analysis methods used in this study, contributed to determining the effects of the pre-slaughtering periods on the carcass and meat quality of the animals and also determining the optimum resting period to decrease dark cutting cases.

This study has shown that it will provide more reliable and accurate results in studies to be conducted in more controlled conditions. Furthermore, care must be taken to ensure that the resolution of the cameras used for image acquisition is good and that the ambient light has the effect of eliminating the effects on the image. It is considered that the image quality measurement techniques can be useful to consider breed differences in the quality estimations to be performed by the digital image method.

Uticaj perioda odmora pre klanja na kvalitet trupova i mesa utvrđeni analizom digitalne slike

Yalcin Bozkurt, Serkan Ozkaya, Sukran Kuleasan

Rezime

U ovom istraživanju utvrđeni su efekti različitih perioda odmora pred klanje na kvalitet trupova i mesa goveda pomoću digitalne analize slike (DIA) i tradicionalnih metoda (kolorimetar). Istraživanje je obuhvatilo 4 perioda odmora, grla zaklana bez odmora (RP0); 3 sata (RP3), 6 sati (RP6) i 12 sati odmora (RP12) i formirane su četiri različite grupe životinja i ukupno 375 uzoraka od 10 trupova životinja iz svake grupe. Merenja pH vrednosti mesa u svakoj grupi, L*, a* i b* i RGB vrednosti dobijene analizom slike podvrgnute su ANOVA analizi primenom GLM (General Linear Model) procedure, a Tukey test je primenjen za poređenja srednjih vrednosti. Uzeti su uzorci mišića *Longissmus dorsi* debljine 2,5 cm (LMA) između 12. i 13. rebra i dobijena su merenja pH iz lumbalne regije. LMA su mereni pomoću planimetra i određene su vrednosti boje sa kolorimetrom (L*, a* i b*). Digitalne slike LMA uzoraka uzetih sa trupa podvrgnute su analizi slike. DIA je odredila i LMA i distribuciju boja crvenih, zelenih i plavih (RGB) vrednosti i njihovu gustinu. Referentna kartica A-10 cm postavljena je pored svakog objekta kako bi se eliminisali efekti udaljenosti snimanja između objekta i digitalnog fotoaparata. Nije bilo statistički značajnih razlika u LMA između grupa životinja dobijenih metodama korišćenih u ovoj studiji. Sve vrednosti boje RGB mesa životinja zaklanih bez perioda odmora (RP0) bile su značajno veće od onih dobijenih u drugim periodima mirovanja. Slično tome, vrednosti L*, a* i b* mesa životinja zaklanih bez perioda odmora (RP0) bile su značajno veće (P=0,01) od onih u drugim periodima mirovanja. Takođe je utvrđeno da nema statističkih razlika u vrednostima boje mesa životinja koje su odmarale 6 i 12 sati primenom obe metode. Stoga su ovi rezultati sugerisali da bi bilo poželjno da se životinje odmore najmanje 6 sati pre klanja. Takođe se može zaključiti da DIA metoda može da otvori nove dimenzije u smislu određivanja nekih drugih parametara kvaliteta mesa.

Ključne reči: trup, govedina, digitalna analiza slike, kvalitet mesa

Acknowledgments

This study is presented as a part of the research project 2020-BTAP1-0073 supported by Scientific Research Unit (BTAP) of Isparta University of Applied Sciences.

References

BOZKURT Y., ÖZKAYA S., KILIÇ B. (2009): Comparison of two different methods to predict meat quality and prediction possibility using digital image analyses, Journal of Kafkas University Veterinary Faculty., 15, 485-489.

BOZKURT Y., MIKAIL N., ULUŞAR U.D., AKTAŞ H., DOGAN C. (2017): Prediction of Bodyweight of Holstein and Brown-Swiss Male Cattle by Using Digital Images, Scientific Papers. Series D. Animal Science, Vol. LX, 196-201.

DASIEWICZ K., SLOWİNSKİ M., SAKOWSKİ T., OPRZADEK J., WİSNİSCH A., DYMNİCKİ E., SLONİEWSKİ K. (2003): The Attempt of Video İmage Analysis Use For Estimation of Meat Quality of Beef Breeds Bulls. http://www.ejpau.media.pl/volume6/issue2/food/art-12.html.

GERRARD D. E., GAO X., TAN J. (1996): Beef Marbling and Colour Score Determination by Image Processing. Journal of Food Science, 61(1), 145–148.

GREGORY N. G. (2008): Animal welfare at markets and during transport and slaughter, Meat Science, 80, 2–11.

GUNASEKARAN S. (2001): Non-destructive Food Evaluation Techniques to Analyze Properties and Quality, Food Science and Technology Series. Marcel Dekker, New York. p105.

LEFEBVRE M., GİL S., BRUENT D., NATONEK E., BAUR C., GUGERİL P., PUN T. (1993): Computer Vision and Agricultural Robotics for Diease Control: The Potato Operation, Computers and Electronics in Agriculture, 8, 102.
LIU W., TAO Y., SİEBENMORGEN T. J., CHEN H. (1997): Digital Image Analysis Method for Rapid Measurement of Rice Degree of Milling. In: 1997 ASAE Annual International Meeting Technical papers, Paper No. 973028, ASAE, 2950 Niles Road, St. Joseph, MI 49085–9659, USA.
LU J., TAN J., SHATADAL P., GERRARD D. E. (2000): Evaluation of pork Color by Using Computer Vision. Meat Science, 56, 56-60.
MARENČİĆ D., IVANKOVİĆ A., PİNTİĆ V., UGARKOVİĆ N. K., JAKOPOVİĆ T. (2012): Effect Of Pre-Slaughter Rest Perıod On Some Physicochemical Properties Of Simmental Beef. Stočarstvo 66:2012 (4) 253-262.
NANNI COSTA L., LO FIEGO D., DALL'OLIO S., DAVOLI R., RUSSO V. (2002): Combined effects of pre-slaughter treatments and lairage time on carcass and meat quality in pigs of different halothane genotype. Meat Science. 61(1):41-7.
NI B., PAULSEN M. R., LİAO K., REİD J. F. (1997): Desing of an Automated Corn Kernel Inspection System for Machine Vision. Transactions. Misset World Poultry, 13(8), 19–20.
OSULLİVAN M., BYRNE D., MARTENS H., GİDSKEHAUG L., ANDERSEN H., MARTENS M. (2003): Evaluation of pork colour: Prediction of visual sensory quality of meat from instrumental and computer vision methods of color analysis. Meat Science, 65(2), 909-18.
SAPIRSTEIN H.D. (1995): Variety identification by digital image analysis. In: C.W. Wrigley (Ed.), Identification of Food Grain Varieties. AACC, USA, 91–130.
SIMA C., HERES L., KLONT R., MIRESAN V. (2015): The Effect of Pre-Slaughter Resting Time on the Quality of Pork Meat. Bulletin UASVM Animal Science and Biotechnologies, pp.72.
TARBELL K.A., REİD J.F. (1991): A Computer Vision System for Characterising Corn Growth and Development. Transactions of the ASAE, 34(5), 2245–2249.
TRENKLE A., LİAMS C. (1999): Use of A Digital Camera to Collect Carcass Data from Experimental Cattle, A. S. Leaflet R1650, 1999 Research Report_Iowa State University.
TYLUTKİ T. P., FOX D. G., ANRİQUE R. G. (1994): Predicting net energy and protein requirements for growth of implanted and nonimplanted heifers and steers and nonimplanted bulls varying in body size. Journal of Animal Science, 72, 1806-1813.
VİLJOEN H.F., DE KOCK H.L., WEBBB E.C. (2002): Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. Me

TECHNO-FUNCTIONAL PROPERTIES OF THREE DIETARY FIBERS USED IN THE MEAT PROCESSING INDUSTRY

Nikola Stanišić[1], Milo Mujović[2], Slaviša Stajić[3], Maja Petričević[4], Čedomir Radović[4], Marija Gogić[4], Aleksandar Stanojković[4]

[1]AVEBE U.A., Prins Hendrikplein 20, 9641 GK Veendam, The Netherlands
[2]Meat industry Gombit d.o.o., Kralja Petra I 194c, Inđija, Republic of Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Republic of Serbia
[4]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade, Republic of Serbia
Corresponding author: Nikola Stanišić, nikola0135@yahoo.com
Invited paper

Abstract: Fibers are naturally occurring compounds present in a variety of vegetables, fruits and cereals. They are used as additives in the food processing industry for not only their nutritional value, but for their versatility as a functional ingredient. This study was carried out to investigate the techno-functional characteristics of three dietary fibers namely, potato, wheat and oat, and their effect on the yield and texture of meat burgers. The findings revealed interesting functional properties for potato fiber. This fiber displayed significantly higher water (9,5 g/g) and oil (5,9 g/g) holding capacity compared to wheat and oat fibers ($p<0,05$), probably due to higher starch content and a bigger porosity of the fiber structure. Better emulsion stability of potato fiber, after cooking and frying, suggests their possible usage in comminuted meat products to enhance texture and improve cooking yield. The application of potato and oat fibers significantly improved the firmness (N) of meat burgers after frying. Overall, the findings demonstrate the potential functional and economic utility of potato fiber, as a promising source of dietary fiber.

Key words: potato fiber, wheat fiber, oat fiber, techno-functional properties, meat burgers

Introduction

Traditional sources of fibers in the diet are cereals, such as wheat, oats and barley, however nowadays nutritional fibers are obtained from all sorts of grains, vegetables and fruits *(Sze et al., 2017)*. Vegetable-based fibers are mixtures of β-glucans, amylopectin and celluloses *(Warner et al., 2001; Brewer, 2012)*. Dietary fibers have many nutraceutical benefits, that range from a digestive aid to their ability to improve colonic health and to prevent cancer (the latter is more relevant to cereal and potato fibers, as they have a larger insoluble fraction) and the lack of fibers in the diet is often associated with gastrointestinal diseases, colon cancer, increased risks of cardiovascular and metabolic diseases, including obesity and diabetes *(Schneeman, 1999; Jimenez-Colmenero et al., 2001; Fuller et al., 2016)*.

In the modern food industry, fibers are of interest to food processors for not only their nutritional value, but for their versatility as a functional ingredient *(Leão, et al., 2013; Yangilar, 2013; McGill et al., 2015)*. Fibers offer many desired functions, such as improving texture, appearance, moisture control and shelf life in a wide range of products like beverages, meat and dairy products, pasta, cereals and baked goods *(Mansour and Khalil, 1997; Abdul-Hamid and Luan, 2000; Paraskevopoulou et al., 2005; Montesinos-Herrero et al., 2006)*.

Dietary fibers isolated from various plants have diverse functional properties namely solubility, viscosity, gel forming ability, water-binding and oil adsorption capacity, which affect final product quality and characteristics. These functional properties of fibers depend on the plant source, their structure and chemical composition *(Chau and Huang, 2003)*. Much of the functionality of the various fibers comes from their ability to absorb and in some cases to bind water at two to ten times their weight. Water can serve as an economical and noncaloric addition to many products, and in its bound form may increase product shelf life. Additionally, in formulating comminuted or emulsified products, the addition of fibers can enhance the emulsion stability by retaining the fat/oil present in the formula *(Tungland and Meyer, 2002)*.

The most important technological effects of fibers in the meat processing industry include: moisture and fat/oil retaining capacity, improving the stability of emulsions, substitution or reduction of fat content, increasing the yield, improving the texture and retaining the shape of the product after heat treatment, increasing storage stability and shelf-life *(Grigelmo- Miguel et al., 1999; Kim and Paik, 2012; Zinina et al., 2019)*. Cereal based fibers (e.g. oat and wheat) and potato fibers are widely used in the meat processing industry. With a bland taste and a light colour, these fibers have good water retention and emulsification capacity, which make them very suitable for a wide range of meat products.

This study was carried out to investigate the techno-functional properties of potato, wheat and oat fibers in an effort to improve the understanding of the difference that these fibers have on functional properties important to meat processors. Effects on water and oil holding capacity, emulsification and texture properties were studied. Finally, the functionality of the three dietary fibers was compared in a meat burger application.

Material and Methods

Potato fibers (product name: Paselli FP) for this study were obtained from AVEBE U.A. (The Netherlands). Wheat fibers (product name: Vitacel WF200) were obtained from Rettenmaier & Sohne (Germany) and oat fibers (product name: Canadian Harvest) from Barentz International B.V. (The Netherlands). Vegetable oil used in this trial was sunflower oil (Reddy, The Netherlands), with a composition of 58% monounsaturated, 35% polyunsaturated and 7% saturated fat (according to labelled product information). Pork back-fat was obtained from the local butcher.
The basic composition of each fiber (according to product specification on the package) is given in Table 1.

Table 1. Fiber properties[1]

Composition (%)	Potato fiber	Wheat fiber	Oat fiber
Dietary fiber	70 - 75	97	94
Starch	< 25	0.8	0.4
Protein	5	0.4	-

[1] Supplier product information

For the measurement of the water holding capacity of fibers, a simple filtration method was used. A filter paper was placed into a plastic funnel and weighed (F1). 2 g of the fiber was weighed into 100 ml tap water. After stirring for 2 min, the sample was poured into the funnel and filtered into a cylinder until there was no further drip loss. The funnel, containing the filter paper and wet filtrate was then weighed (F2). All samples were measured in triplicate. The water holding capacity (WHC) was calculated as: WHC (g water/g fiber) = (F2 –F1)/2.

To compare the oil holding capacity (OHC) of three different fiber products a centrifuge method was used. 5 g of fiber and 45 g of sunflower oil were mixed in a centrifuge tube. The samples were stirred and left to rest for 5 min. They were then put into a centrifuge (Centaur 1, Beun de Ronde b.v.). After 30 min centrifugation at a speed of 2000 rpm, the supernatant was poured out and the remaining in the tube weighed (S). All samples were measured in triplicate. The amount of bounded oil was calculated as: OHC (g oil/g fiber) = S/5.

In order to compare the emulsion stability of fibers, emulsions were made under a 1:7:7 ratio (1 part fiber to 7 parts pork back fat to 7 parts water). Using a Stephan mixer (UM5, The Netherlands), the fat is first very roughly chopped under vacuum with approximately one-eighth of the water at a medium speed (1500 rpm) for 1 min. The fiber was then added and gradually the remaining water is added and further mixed at high speed (3000 rpm) for 2 min. The emulsion was poured into the 200 ml cans and pasteurized in a water bath at 75°C for 40 min, until reaching 72°C in the center of the can. The cans are then cooled with tap water before being refrigerated overnight. The cooked emulsion stability of fibers was assessed after opening the cans and described sensorially using a 5-point grading system (from 5 = no fat loss, to 1 = much fat loss). After that, each emulsion was fried in sunflower oil at ~180°C for 1 min and a similar sensorial test was conducted to describe emulsion stability after frying, using a scale from 5 = stable structure, to 1 = loss of structure. All samples were scored in triplicate.

The effect of adding different fiber on the burger yield and texture was investigated. Burgers were prepared according to the recipe given in Table 2. All the ingredients were obtained from the local store and are widely available on the market. The ingredients were mixed together and burgers of approx. 90 g were formed and pan-fried. Three different groups of burgers were made, depending on the fiber source.

Table 2. Burger formulation

Ingredient	%
Beef (15% fat)	60.5
Pork (20% fat)	15.2
Water	17.8
Salt (NaCl)	1.2
Spices and flavors	4.0
Polyphosphate (STPP)	0.3
Fiber[1]	1.0
TOTAL	100.0

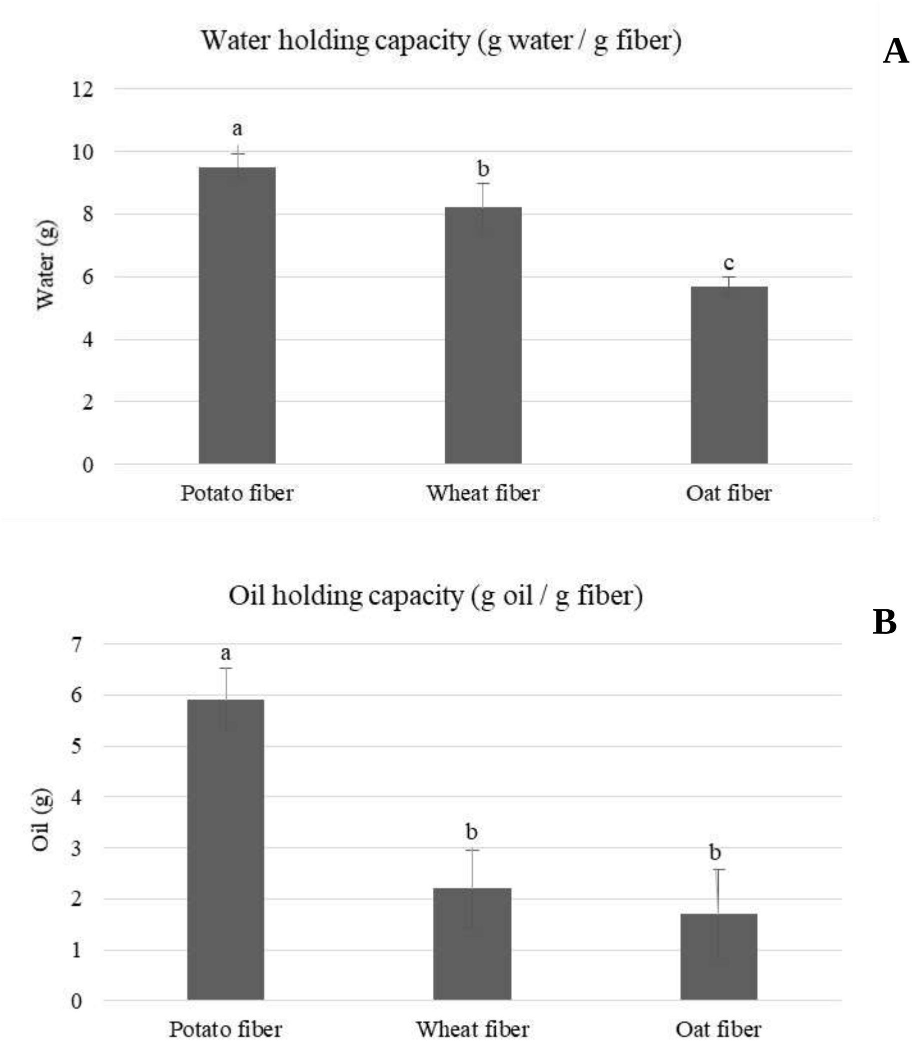
[1] Three groups were made: potato, wheat, oat

Process loss was calculated by measuring the difference in weight before and after frying the burgers, and expressed as a percentage of the initial weight. Texture measurements were performed on fried burgers using a Shimadzu EZ-SX texture analyzer (Shimadzu Corp, Japan) equipped with a 5 kg load cell and a cylindrical probe with a diameter of 36 mm. The firmness was measured as the maximum force (N) required to compress a burger sample by 50% at a speed of 1 mm/s. Nine replicate samples were tested from each group.

The results were analyzed statistically using one-way analysis of variance with the ANOVA procedure from SPSS 20.0 software (IBM SPSS Statistics, Version 20, IBM Corp, USA). Statistically significant differences between samples were defined as $p<0.05$. All the data in are expressed as means ± standard deviation.

Results and Discussion

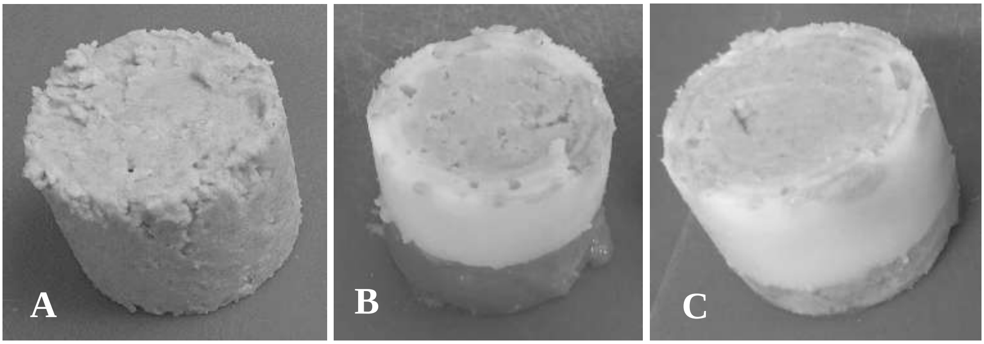
Significant differences ($p<0.05$) were observed between the WHC and OHC of the three fibers and these results are represented in Graph 1.



Graph 1. Water (A) and oil (B) holding capacity of three different fibers (different letters (a-c) denote a significant difference between means at p<0.05)

Potato fiber had a significantly higher water and oil holding capacity compared to wheat and oat fibers ($p<0.05$). Higher water holding capacity can be correlated with a higher starch content of potato fibers (Table 1). On the other hand, oil binding is in part related to fiber chemical composition, but is more largely a function of the porosity of the fiber structure *(Biswas et al., 2011)*. The composition differences between the three fibers could be mainly attributed to their different origins and to the different extraction procedures *(Ktari et al., 2014)*. Wheat fibers had a better water holding capacity compared to oat fibers ($p<0.05$). There was no significant difference in oil holding capacity between wheat and oat fibers. Somewhat lower water and oil holding capacity of potato fibers (6 g/g and 2g/g, respectively) was reported by *Ktari et al. (2014)*, although the authors investigated the potato fibers from a different producer (Vitacel KF500), which can explain the differences with current research.

The superior fat emulsification capacity of potato fibers is presented in Picture 1 and the sensory scores for cooked and fried emulsions are given in Table 3. Potato fibers had significantly higher scores for both attributes tested, compared to wheat and oat fibers ($p<0.05$). There were no significant differences between wheat and oat fibers in emulsion characteristics (Picture 1, Table 3). Oat fibers are often used in the production of emulsion-type products such as sausages and pâtés, as they reportedly enhance the flavor and texture *(Chang and Carpenter, 1997; García et al., 2002; Desmond and Troy, 2003; Serdaroglu, 2006; Talukder and Sharma, 2010)*. Based on the better emulsion stability of potato fibers presented in this trial, we can conclude that they can be used in higher extended emulsion-type products, compared to oat and wheat fibers.



Picture 1. Potato (A), wheat (B) and oat (C) fiber emulsions (1:7:7, fiber:fat:water) after cooking

Table 3. Cooked and fried emulsion ratings (mean ± standard deviation)[1]

Fiber	Cooked emulsion[2]	Fried emulsion[3]
Potato	4.9 ± 0.2^{a}	4.7 ± 0.3^{a}
Wheat	3.2 ± 0.0^{b}	2.2 ± 0.1^{b}
Oat	2.0 ± 0.1^{b}	1.4 ± 0.1^{b}

[1] Different letters (a-b) within the column denote a significant difference between means at $p<0.05$
[2] Scale from 5 = no fat loss, to 1 = much fat loss
[3] Scale from 5 = stable structure, to 1 = structure loss

Considerable variations in literature data can be found on different fibers effects on the cooking yield and texture of various meat products. The presence of different fibers was previously reported to induce effects ranging from no effect on cooking yield in low-fat beef burgers *(Desmond et al., 1998)* to significant improvements in cooking yield in low-fat bologna *(Claus and Hunt, 1991)*. *Thebaudin et al. (1997)* reported that the addition of fiber seems to favor water binding and fat absorption of meat products, but the effect was depended on the fiber source. It has previously been shown that adding oat fiber to low-fat sausages can improve the cooking yield *(Hughes et al., 1997; Aleson-Carbonell et al., 2005)*. A decrease in frying loss as a result of adding oat or barley fiber to low-fat meat patties has also previously been shown *(Kumar and Sharma, 2004; Pinero et al., 2008)*. *Besbes et al. (2008)* reported that the use of wheat dietary fibers increased cooking yield, decreased the shrinkage during frying and minimized the production costs without degradation of sensory properties of beef patties. *Desmond et al. (1998)* reported that adding oat fiber had limited effects on the yield and water holding capacity of low-fat beef burgers.

In the present trial, the process losses of the burgers made with the addition of potato fibers were significantly lower compared to wheat and oat fibers (Table 4). This can be correlated to a higher water and oil binding capacity of potato fibers (Graph 1).

Table 4. Process loss and firmness of fried burgers (mean ± standard deviation)[1]

Burger group	Process loss (%)	Firmness (N)
Potato	4.93 ± 0.74^{b}	12.71 ± 2.10^{a}
Wheat	8.72 ± 1.15^{a}	7.12 ± 1.08^{b}
Oat	10.69 ± 1.61^{a}	11.08 ± 1.54^{a}

[1] Different letters (a-b) within the column denote a significant difference between means at $p<0.05$

The difference in burger firmness (N) when the different fibers were added is presented in Table 4. When adding potato and oat fibers, the firmness of the burgers was significantly higher compared to the wheat fiber group ($p<0.05$), with no significant difference between these two. Interestingly, the higher starch content of potato compared to oat and wheat fibers (Table 1) didn't had a significant effect on lowering the firmness of the final product. Similar findings were also reported by *Petersson et al. (2014).*

Conclusion

The values recorded for the water and oil binding capacity of the three dietary fibers could be related to their origins and their processing procedures that could have significantly affected their compositions, physical structures, porosities, and particle sizes. The ability of fibers to assist in the stabilization of fat and water during the production process provides enhanced tolerances that are very important in the modern meat processing industry. The high water and oil holding capacity of the potato fibers suggest that they could be used as a functional ingredient in meat formulations to modify texture and viscosity, increase yield and improve the texture of the final product. The increased insoluble fiber content also offers a nutritional benefit for the consumer.

Tehno-funkcionalne karakteristike tri dijetalna vlakna korišćena kao aditivi u industriji mesa

Nikola Stanišić, Milo Mujović, Slaviša Stajić, Maja Petričević, Čedomir Radović, Marija Gogić, Aleksandar Stanojković

Rezime

Vlakna su prirodno prisutna u raznim vrstama povrća, voća i žitarica. Koriste se kao aditivi u prehrambenoj industriji ne samo zbog svoje nutritivne vrednosti, već i zbog svojih raznovrsnih funkcionalnih svojstava. Ovo istraživanje je sprovedeno kako bi se istražile tehno-funkcionalne karakteristike tri dijetalna vlakna, preciznije krompira, pšenice i zobi, i njihov uticaj na prinos i teksturu hamburgera. Dobijeni podaci ukazuju na zanimljiva funkcionalna svojstva vlakana krompira. Ovo vlakno je pokazalo značajno veći kapacitet vezivanja vode (9,5 g/g) i ulja (5,9 g/g) u poređenju sa vlaknima pšenice i zobi ($p < 0{,}05$), verovatno zbog većeg sadržaja skroba i veća poroznosti u strukturi samih vlakana. Bolja stabilnost emulzije vlakana krompira, nakon kuvanja i prženja, sugeriše njihovu moguću upotrebu u emulgovanim mesnim proizvodima radi poboljšanja teksture i poboljšanja prinosa tokom termičke obrade. U poređenju sa pšeničnim vlaknima, primena vlakana krompira i zobi poboljšala je čvrstinu (N) hamburgera nakon prženja. Rezultati

istraživanja pokazuju potencijalnu funkcionalnu upotrebljivost vlakana krompira u industriji mesa, kao obećavajućeg izvora dijetetskih vlakana.

Ključne reči: vlakna krompira, pšenična vlakna, vlakna zobi, tehno-funkcionalna svojstva, hamburger

References

ABDUL-HAMID A., LUAN Y.S. (2000): Functional properties of dietary fiber prepared from defatted rice bran. Food Chemistry, 68, 15-19.
ALESON-CARBONELL L., FERNANDEZ-LOPEZ J., PEREZ-ALVAREZ J.A., KURI V. (2005): Functional and sensory effects of fibre-rich ingredients on breakfast fresh sausages manufacture. Food Science and Technology International, 11, 2, 89-97.
BESBES S., ATTIA H., DEROANNE C., MAKNI S., BLECKER C. (2008): Partial replacement of meat by pea fiber: Effect on the chemical composition, cooking characteristics and sensory properties of beef burgers. Journal of Food Quality, 31, 480-489.
BISWAS K., KUMAR V., BHOSLE S., SAHOO J., CHATLI M.K. (2011): Dietary fibers as functional ingredients in meat products and their role in human health. International Journal of Livestock Production, 2, 4, 45-54.
Brewer M.S. (2012): Reducing the fat content in ground beef without sacrificing quality: A review. Meat Science, 91, 385-395.
CHANG H.C., CARPENTER J.A. (1997): Optimizing quality of frankfurters containing oat bran and added water. Journal of Food Science, 62, 194-197.
CHAU C.F., HUANG Y.L. (2003): Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of Citrus sinensis L. Cv. Liucheng. Journal of Agricultural and Food Chemistry, 51, 2615-2618.
CLAUS J.R., HUNT M.C. (1991): Low-fat, high-added water bologna formulated with texture-modifying ingredients. Journal of Food Science, 56, 643-647.
DESMOND E.M., TROY D.J. (2003): Sensory and physical characteristics of pork sausages manufactured with dietary fibres. Irish Journal of Agriculture and Food Research, 42, 1, 161.
DESMOND E.M., TROY D.J., BUCKLEY D.J. (1998): Comparative studies of nonmeat adjuncts used in the manufacture of low-fat beef burgers. Journal of Muscle Foods, 9, 3, 221-241.
FULLER S., BECK E., SALMAN H., TAPSELL L. (2016): New horizons for the study of dietary fiber and health: A Review. Plant Foods for Human Nutrition**,** 71, 1-12.

GARCIA L., DOMINGUEZ R., GALVEZ D., CASAS C., SELGAS D. (2002): Utilization of cereal and fruit fibres in low fat dry fermented sausages. Meat Science, 60, 227-232.

GRIGELMO-MIGUEL N., ABADIAS-SEROS M., MARTIN-BELLOSO O. (1999): Characterization of low fat high-dietary fiber frankfurters. Meat Science, 52, 247-256.

HUGHES E., COFRADES S., TROY D.J. (1997): Effects of fat level, oat fiber and carrageenan on frankfurters formulated with 5, 12 and 30% fat. Meat Science, 45, 3, 273-2810.

JIMENEZ-COLMENERO F., CARBALLO J., COFRADES S. (2001): Healthier meat and meat products: their role as functional foods. Meat Science, 59, 5-13.

KIM H.J., PAIK H.D. (2012): Functionality and application of dietary fiber in meat products. Korean Journal for Food Science of Animal Resources, 32, 6, 695-705.

KTARI N., SMAOUI S., TRABELSI I., NASRI M., SALAH R.B. (2014): Chemical composition, techno-functional and sensory properties and effects of three dietary fibers on the quality characteristics of Tunisian beef sausage. Meat Science, 96, 521-525.

KUMAR M., SHARMA B.D. (2004): Quality and storage stability of low-fat pork patties containing barley flour as-fat substitute. Journal of Food Science and Technology-Mysore, 41, 5, 496-502.

LEÃO D.P., MELO J.C.S., FRANCA A.S., OLIVEIRA L.S. (2013): Comparative evaluation of agricultural residues in the production of dietary fibers. Proceedings of 2nd International Conference on Nutrition and Food Sciences, Singapore: IACSIT Press, IPCBEE, 53, 7, 34-38.

MANSOUR E.H., KHALIL A.H. (1997): Characteristics of low fat beef burgers as influenced by various types of wheat fibers. Food Research International, 30, 199-205.

MCGILL C.R., FULGONI V.L.III., DEVAREDDY L. (2015): Ten-year trends in fiber and whole grain intakes and food sources for the United States population: National Health and Nutrition Examination Survey 2001-2010. Nutrients, 7, 2, 1119-1130.

MONTESINOS-HERRERO C., COTTELL D.C., O'RIORDAN E.D., O'SULLIVAN M. (2006): Partial replacement of fat by functional fiber in imitation cheese: effects on rheology and microstructure. International Dairy Journal, 16, 910-919.

PARASKEVOPOULOU A., BOSKOU D., KIOSSEOGLOU V. (2005): Stabilization of olive oil–lemon juice emulsion with polysaccharide. Food Chemistry, 90, 627-634.

PETERSSON K., GODARD O., ELIASSON A.C., TORNBERG E. (2014): The effects of cereal additives in low-fat sausages and meatballs. Part 2: Rye bran, oat bran and barley fibre. Meat Science, 96, 503-508.
PINERO M.P., PARRA K., HUERTA-LEIDENZ N., DE MORENO L.A., FERRER M., ARAUJO S. (2008): Effect of oat's soluble fibre (beta-glucan) as a fat replacer on physical, chemical, microbiological and sensory properties of low-fat beef patties. Meat Science, 80, 3, 675-680.
SCHNEEMAN B.O. (1999): Fiber, inulin and oligofructose: Similarities and differences. Journal of Nutrition, 129, 1424S-1427S.
SERDAROGLU M. (2006): The characteristics of beef patties containing different levels of fat and oat flour. Journal of Food Science Technology, 41, 147-153.
SZE N., SATHYASURYA R., WAN N. (2017): Incorporation of dietary fibre-rich oyster mushroom (Pleurotussajor-caju) powder improves postprandial glycaemic response by interfering with starch granule structure and starch digestibility of biscuit. Food Chemistry, 227, 358-368.
TALUKDER S., SHARMA D. (2010): Development of dietary fiber rich chicken meat patties using wheat and oat bran. Food Science and Technology, 47, 2, 224-229.
THEBAUDIN J.Y., LEFEBVRE A.C., HARRINGTON M., BOURGEOIS C.M. (1997): Dietary fibers: nutritional and technological interest. Trends in Food Science and Technology, 8, 41-48.
TUNGLAND B.C., MEYER D. (2002): Non-digestible oligo- and polysaccharides (Dietary Fiber): Their physiology and role in human health and food. Comprehensive Review in Food Science and Food Safety, 3, 90-109.
WARNER K., ESKINS K., FANTA G.F., NELSEN T.C., ROCKE J.W. (2001): Use of starch– lipid composites in low fat ground beef products. Food Technology, 55, 2, 36-41.
YANGILAR F. (2013): The application of dietary fibre in food industry: Structural features, effects on health and definition, obtaining and analysis of dietary fibre: A Review. Journal of Food and Nutrition Research, 1, 3, 13-23.
ZININA O., MERENKOVA S., TAZEDDINOVA D., REBEZOV M., STUART M., OKUSKHANOVA E., YESSIMBEKOV ZH., BARYSHNIKOVA N. (2019): Enrichment of meat products with dietary fibers: a review. Agronomy Research, 17, 4, 1808-1822.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

TECHNOLOGICAL AND SENSORY PROPERTIES OF SERBIAN TRADITIONAL MINCED MEAT PRODUCT ĆEVAPI WITH IMPROVED NUTRITIONAL PROPERTIES

Slaviša Stajić[1], Ana Kalušević[2,3]

[1]University of Belgrade, Faculty of Agriculture, Department of Animal Source Food Technology, Nemanjina 6, Belgrade 11080, Serbia
[2]University of Belgrade, Faculty of Agriculture, Department of Food Technology and Biochemistry, Nemanjina 6, Belgrade, Serbia
[3]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia
Corresponding Author: Slaviša Stajić, stajic@agrif.bg.ac.rs
Invited paper

Abstract: Ćevapi and pljeskavica are Serbian traditional minced meat products. Fatty tissue and salt are very important ingredients in the formulation of these products due to their significant influence on sensory properties. However, high intake of salt and fat increases the risk of cardiovascular disease. The aim of this paper was to examine the technological and sensory properties of ćevapi prepared with reduced sodium content and with a partial substitution of backfat with encapsulated pumpkin seed oil (EPSO). Two groups of ćevapi were prepared: first with NaCl (Na treatments), and second the salt mixture containing 30% of KCl (K treatments). Within both groups, three treatments were prepared with different amounts (0%, 15% and 30%) of backfat replaced with EPSO. Cooking loss (CL), length reduction (dL), instrumental texture and sensory properties were examined. Salt mixture and backfat replacement with EPSO did not have a significant influence on CL while opposite was observed in dL. All K treatments had significantly higher dL values compared to Na0, while fat replacement with EPSO led to higher length reduction only within Na treatments. Hardness and chewiness were significantly influenced by salt mixture and backfat replacement – lower values were measured in K treatments and in treatments with EPSO. Regarding sensory evaluation, all treatments were graded with similar and relatively high grades in all observed properties: appearance, colour, odour, taste, and overall acceptability. Healthier products could be produced by using a salt mixture with lower sodium content and encapsulated pumpkinseed oil without affecting production yield and sensory quality.

Key words: minced meat product, ćevapi, sodium reduction, pumpkinseed oil, sensory quality

Introduction

Minced meat products (such as burgers and patties), which are intended for heat treatment (e.g. grill, barbecue) before consumption are worldwide popular food nowadays. The burger and the patty are probably the most popular products within this group, and a hallmark of the fast-food industry.

As for Serbia, according to Serbian Regulation 50/2019, these types of meat products are classified under semi-finished meat products, namely, meat products intended for heat treatment (e.g. grill, barbecue) before consumption. Within this category, the most popular are pljeskavica (pronounced [pʎɛ̂skaʋitsa]) – a patty type minced meat product made of different meats, and ćevapi (pronounced [tɕeʋǎːpi]), a minced meat product made of different meats and formed into cylindrical shapes usually about 2 cm in diameter and 6–8 cm in length, and mostly served in batches of five and ten. Both pljeskavica and ćevapi are served either on a plate as a meat dish or as a fast-food variant – placed into a special type of bread (called lepinja and somun) with chopped fresh onions and different salads. These minced meat products are also very popular in all Balkan countries, with several local variants which are often considered local and national dishes. Several annual manifestations are organized in the region to showcase these meat products. Among them, probably the most popular is the Leskovac Grill Festival that has been held since 1965 in the town of Leskovac with several thousand visitors each year (up to 600,000 in 2013).

Burgers, patties and in general this type of meat products are highly appreciated because of their attractive sensory characteristics, as well as because they are a source of proteins of high biological value and other nutrients (energy, minerals). Fatty tissue and salt are very important ingredients in the formulation of minced meat products (and meat products in general) due to their significant influence on sensory properties.

Salt affects taste, shelf-life and safety, and especially texture properties by promoting protein solubilisation and extraction, improving water-holding capacity (WHC), therefore increasing processing yield and juiciness (*Petit et al., 2019*). Sodium cation (Na+) mainly contributes to the salty taste, while Cl- promotes protein solubilisation and increases WHC (*Petit et al., 2019*). In Europe 16.3% of the salt dietary intake comes from meat products, while in USA the figure is 21.0% (*Desmond, 2006; Petit et al., 2019*). High sodium intake increases the risk of cardiovascular disease and kidney diseases (*Yotsuyanagi et al., 2016*). Therefore, over the past several decades, recommendations and strategies of sodium reductions were introduced. A simple reduction of the salt level had limitations because of the significance on Na+ and Cl- ions on technological and sensory properties of meat products. Further reduction of sodium content can be achieved by replacing the NaCl with other chloride salts (*Ruusunen and Puolanne*, *2005*). Several research studies show that replacing 30% of NaCl with KCl should not have an adverse influence on technological and sensory properties of meat products.

In general, fat contributes to the colour, flavour and texture of meat products. Meat products may contain up to 50% (or even more) of total fat, like some traditional dry-fermented sausages (*Salgado et al.*, *2005*), while patty-type products usually contain up to 30% (*Heck et al.*, *2017*). However, high intakes of meat lipids, due to the high content of saturated fatty acids (SFA), are associated with the increase in the risk of cardiovascular disease (*Gogus and Smith*, *2010*). Improvement of the fatty acid profile of meat products can be achieved by partial animal fat substitution with oils high in polyunsaturated and monounsaturated fatty acid contests such as olive, fish, flaxseed, grape seed, canola, etc. (*Stajić et al.*, *2020a; Stajić et al.*, *2020b*). The use of pumpkin seed oil is rare, though this type of oil is rich in oleic and linoleic acids, and other bioactive compounds (*Montesano et al., 2018*). The inclusion of oils into the meat product formulation is limited due to its physico-chemical characteristics, therefore in order to obtain stable products, oils were added as an emulsion system, gelled emulsion system or encapsulated (*Stajić et al.*, *2020a*).

There are numerous research studies about the improvement of the fatty acid profile of patty-type meat products. However, as far as the authors are aware, no research was conducted on traditional Serbian minced meat products such are pljeskavica and ćevapi. Hence, the aim of this study was to examine the technological and sensory properties of traditional Serbian minced meat product ćevapi, prepared with a reduced sodium content and with a partial substitution of backfat with encapsulated pumpkin seed oil.

Materials and Methods

Preparation of ćevapi

Two groups were prepared, each containing three different treatments. The first group was prepared with the addition of NaCl (Na treatments), while the second with the addition of the NaCl+KCl mixture containing 70% NaCl and 30% KCl (K treatments). Within both groups, three treatments were prepared with different amounts (0%, 15% and 30%) of backfat replaced with encapsulated pumpkin seed oil (EPSO): 0, 15 and 30 treatments (Table 1). All treatments were prepared in the same manner and, except salt and fat type, with the same amount of other ingredients (Table 1).

Beef (shoulder muscles) and backfat were bought at a local store and kept in the refrigerator for 24 h. Before usage, the visible fat and connective tissue were trimmed off the meat, and backfat and meat were cut into small pieces. Then, meat and backfat were weighed, mixed by hand with other ingredients (except EPSO and onion), ground through an 8 mm plate (KENWOOD PRO 1600, ZEMLJA), covered with foil, and kept refrigerated for 24 h. Afterwards, onion and encapsulated pumpkin seed oil (previously weighted) were added to all treatments, mixed by hand and ground again through a 4.5 mm plate. The mixtures were formed into cylindrical shapes about 2 cm in diameter and 6–8 cm in length. After shaping, ćevapi were baked on an electric grill (Tefal OptiGrill™ Plus) at 230–235°C until an internal temperature of 80°C was reached, cooled at room temperature, covered with foil, and kept refrigerated for 24 h before analysis. The experiment was conducted in two replicates.

Table 1. Formulation of experimental batches

	Salt 1			Salt 2		
	Fat 1	Fat 2	Fat 3	Fat 1	Fat 2	Fat 3
	Na0	Na15	Na30	K0	K15	K30
beef meat	67	67	67	67	67	67
backfat	20	17	14	20	17	14
water	5.5	5.5	5.5	5.5	5.5	5.5
EPSO	/	3	6	/	3	6
Ingredients						
onion	5	5	5	5	5	5
salt*	1.5	1.5	1.5	1.5	1.5	1.5
dextrose	0.5	0.5	0.5	0.5	0.5	0.5
Na-bicarbonate	0.5	0.5	0.5	0.5	0.5	0.5

*Na treatments with NaCl; K treatments with mixture: 70%NaCl+30%KCl;
0 – treatments with all backfat; 15 – treatments with replaced of 15% backfat with EPSO; 30 – treatments with replaced of 30% backfat with EPSO; EPSO – encapsulated pumpkinseed oil

The encapsulation of PSO in the calcium alginate matrix was realized as was describe by *Stajić et al.* (*2014*) with some modifications. Firstly, a shell solution was prepared by dissolving sodium alginate (Sigma-Aldrich, St. Louis, MO, USA) in distilled water (0.02 g/mL), followed by the preparation on alfinate/oli emulsion (20%, w/w) using Ultra-Turrax T25 (T25 digital ULTRA-TURRAX ®, IKA, Germany) at a speed of 10,000 rpm for 5 min. The prepared alginate/oil emulsion was extruded through blunt stainless still needle (22 gauge) using a syringe pump under a constant flow rate of 50 mL/h. Electrostatic potential (5.0 kV) was formed by a high voltage DC unit (Model 30R, Bertan Associates, Inc., New York). Calcium chloride (0.02 g/mL, Analytika, Czech Republic) was the collecting solution, while the distance between the needle tip and the collecting solution was 2.5 cm. After extrusion, the beads were left in the collecting solution for 30 min and after the gelling period, microbeads were rinsed with distilled water.

Methods

Cooking loss (CL) was determined by measuring 10 individual ćevapi before heat treatment and after cooling, and this represents the mass difference (as %) between these measurements.

Length reduction (dL) represents the difference in length (as %) before heat treatment and after cooling. The measurements of length were carried out with a digital nonius.

Texture profile analysis was performed using the universal texture analyzer (TA.XT Plus; Stable Micro System, Ltd., Godalming, UK) in the same manner as described by *Stajić et al.* (*2018a*). Six ćevapi from each treatment were held for equilibration to room temperature, samples 10 mm in height and 12 mm in radius were taken from the center of each individual ćevapi and compressed twice to 50% of their original height, with a compression aluminum platen of 25mm (P/25) and a 50 kg load cell. Pretest speed was 60 mm/min, test speed was 60 mm/min, and posttest speed was 300 mm/min. Hardness, adhesiveness, springiness, cohesiveness, and chewiness were evaluated and obtained using the available computer software.

Sensory analysis was performed by 25 untrained students from the Department of Animal Source Food Technology, Faculty of Agriculture, University of Belgrade. The assessors evaluated the appearance, colour, odour, taste, and overall acceptability using a nine-point hedonic scale (1 – extremely unacceptable, 5 – neither like nor dislike, 9 – extremely acceptable). Prior to evaluation, samples were heated in a microwave (for 20s at 650W, about 50 °C in the centre of the sample), coded with a randomly selected three-digit number and served to the assessors in broad daylight, randomly. Assessors were instructed to cleanse their palates between samples with water.

The results were subjected to two-way ANOVA to evaluate the effect of the salt mixture, fat replacement and its interaction. Statistical analyses were performed using software Statistica 12.5 (StatSoft, Inc., Tulsa, OK, USA) and the results were presented as mean±standard deviation (SD). Differences between means were determined using Tukey’s HSD test at the significance level $p < 0.05$.

Results and Discussion

Technological properties

Salt mixture and backfat replacement with EPSO did not have a significant influence on CL (Figure 1). Na0 treatment, which can be considered as the control treatment (100% NaCl and backfat only), differed significantly only relative to K0 and Na15 treatments which had lower CL values. By replacing 50% of backfat with encapsulated chia and flaxseed oil, *Heck et al.* (*2017*) obtained lower CL, while (*Carvalho et al., 2020*) found significantly lower CL values when beef fat was completely replaced with tiger nut oil emulsion.

Regarding length reduction (Figure 2), salt mixture and backfat replacement had a significant influence. All K treatments had significantly higher dL values compared to Na0, while fat replacement with EPSO led to higher length reduction only within Na treatments. Contrary to this, *Heck et al.* (*2017*) did not find any differences in diameter reduction in burgers with encapsulated chia and flaxseed oil, which could be attributed to the different shape of burgers compared to ćevapi, despite belonging to the same type of meat products.

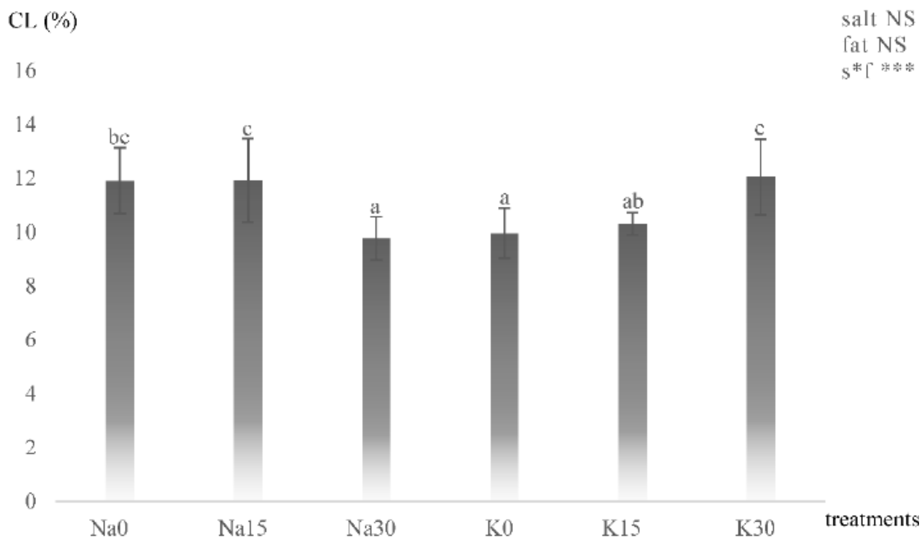


Figure 1. Cooking loss (CL) results of examined treatments; different letters indicate significant differences (p<0.05)

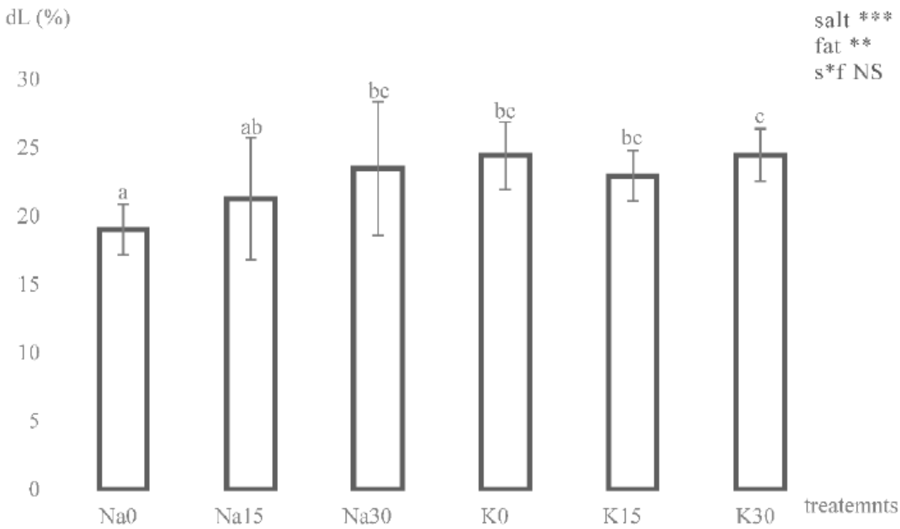


Figure 2. Length reduction (dL) of examined treatments; different letters indicate significant differences (p<0.05)

Regarding TPA results (Table 2), salt mixture had no significant influence on adhesiveness, springiness and cohesiveness, while backfat replacement with EPSO had no significant influence on adhesiveness and springiness. Hardness and chewiness were significantly influenced by salt mixture and backfat replacement with EPSO (and their interaction as well). All treatments with salt mixture containing KCl had lower hardness and chewiness values compared to the corresponding Na treatments. However, significantly only compared to Na0 treatment which can be considered as control. Backfat replacement with EPSO led to lower hardness and chewiness values compared to the treatments with all backfat. However, an effect of the fat reduction level was not observed. The results indicate that the products with the salt mixture containing KCl and with EPSO were softer and easy to chew, which should be examined by sensory evaluation to determine whether this could be perceived negatively. *Heck et al.* (*2017*) did not find a significant influence of backfat replacement in the amount of 50% with encapsulated chia and flaxseed oil on hardness, springiness and chewiness. Similar to this, *Carvalho et al.* (*2020*) did not find a significant influence on hardness, cohesiveness, gumminess and chewiness even when beef fat was completely replaced with tiger nut oil emulsion in beef burgers. On the other hand, (*Martins et al., 2019*) reported lower hardness and chewiness values when replacing 75% of backfat with oleogel containing flaxseed oil.

Sensory analysis

The results of the sensory analysis (Table 2) indicated that the use of salt mixture with 30% of KCl and backfat replacement with EPSO in the amount of up to 30% had no significant influence on the observed sensory properties. Although the results of the length reduction indicated that treatments containing salt mixture (NaCl+KCl) and EPSO had higher deformation level compared to Na0 treatment (which can be considered as control treatment – 100% NaCl and backfat only), the assessors did not perceive it negatively, and gave similar and relatively high grades regarding appearance (7.48–7.68) to all treatments. Similar to this, all treatments were graded with similar and relatively high grades regarding colour and odour. On the other hand, Na15 received the lowest grade in terms of taste, which was more than 1 grade lower than Na0, although not significantly.

Literature data indicates that the replacement of 25–40% of NaCl with KCl in salt mixtures will not impair flavour in meat products (*Desmond, 2006*), which is confirmed by the results of this study. Regarding the use of oils as fat substitutes in meat products, research studies indicate that it depends on oil type, oil amount and oil pre-treatment (*Stajić et al., 2018b; Stajić et al., 2020b; Stajić and Živković, 2021*). In that sense, (*Heck et al., 2017*) found no significant differences (compared to control) in any of the observed sensory properties of burgers when 50% of backfat was replaced with encapsulated flaxseed oil, as was the case in this study, while burgers with the same level of encapsulated chia oil had significantly lower grades, compared to control, in all observed sensory properties (except texture). Moreover, *Carvalho et al.* (*2020*) reported that the complete replacemenet of beef fat with tiger nut oil emulsion in beef burgers did not significantly alter the sensory quality of burgers.

Table 2. Results of texture profile analysis (TPA) and sensory evaluation

	Salt 1			Salt 2					
	Fat 1	Fat 2	Fat 3	Fat 1	Fat 2	Fat 3	Significance		
	Na0	Na15	Na30	K0	K15	K30	salt	fat	s*f
TPA									
Hardness (N)	14.08±075^{c}	9.87±1.34^{ab}	11.53±1.31^{b}	11.44±1.18^{b}	9.79±0.50^{ab}	9.31±0.57^{a}	***	***	**
Adhesiveness (N*s)	-0.78±0.81	-0.18±0.17	-0.72±0.80	-0.46±0.46	-0.28±0.39	-0.78±0.80	NS	NS	NS
Springiness	0.87±0.01	0.88±0.03	0.86±0.04	0.86±0.01	0.89±0.04	0.86±0.04	NS	NS	NS
Cohesiveness	0.72±0.03^{b}	0.66±0.10^{ab}	0.67±0.042^{ab}	0.72±0.04^{b}	0.72±0.04^{b}	0.57±0.08^{a}	NS	***	*
Chewiness (N)	8.87±0.69^{c}	5.70±0.84^{ab}	6.60±1.16^{b}	7.10±0.78^{b}	6.23±0.48^{b}	4.57±0.98^{a}	***	***	**
Sensory evaluation									
Appearance	7.68±1.03	7.48±0.92	7.48±1.16	7.48±0.92	7.56±1.16	7.60±1.00	NS	NS	NS
Colour	7.76±0.83	7.12±1.51	7.60±1.22	7.32±1.31	7.72±1.21	7.72±1.14	NS	NS	NS
Odour	7.40±1.04	7.28±1.24	7.32±1.31	7.44±1.08	7.64±1.25	7.92±1.12	NS	NS	NS
Taste	7.80±0.87	6.76±1.98	7.36±1.50	7.40±1.38	7.24±1.81	7.52±1.36	NS	NS	NS
Overall acceptance	7.84±0.55	7.20±1.38	7.56±1.33	7.52±0.96	7.52±1.42	7.72±1.21	NS	NS	NS

Na treatments – with NaCl; K treatments – with mixture: 70%NaCl+30%KCl; 0 – treatments with all backfat; 15 – treatments with replaced of 15% backfat with EPSO; 30 – treatments with replaced of 30% backfat with EPSO; EPSO – encapsulated pumpkinseed oil;
NS – not significant; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$
$^{a–c}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p<0.05$).

Conclusions

Ćevapi, a traditional Serbian minced meat product, are very popular and gladly consumed either as a meat dish or as a fast-food variant, which is why it could be

The results of this study indicate that the use of salt mixture with 30% of KCl and a simultaneous replacement of back fat with encapsulated pumpkinseed oil did not affect cooking loss. On the other hand, length reduction, instrumental hardness and chewiness were affected by these two factors, indicating more deformed products and products which could be softer and easier to chew compared to control. However, the results of the sensory evaluation showed no significant differences between all evaluated products.

All in all, healthier products could be produced by using a salt mixture with lower sodium content and encapsulated pumpkinseed oil without affecting production yield and sensory quality. Further studies could focus on the use of other oils (and their mixture) and formulation improvement of other similar products, such as pljeskavica.

Tehnološka i senzorska svojstva ćevapa sa poboljšanim nutrtivnim svjstvima

Slaviša Stajić, Ana Kalušević

Rezime

Ćevapi i pljeskavice su tradicionalni poluproizvodi od mesa u Srbiji. Masno tkivo i so su veoma bitni sastojci u pripremi ovih proizvoda zbog značajnog uticaja na njihova senzorska svojstva. Međutim, prekomerni unos soli i masti povećava rizik od pojave kardiovaskularnih bolesti. Cilj ovog rada jeste da ispita tehnološka i senzorska svojstva ćevapa sa smanjenim sadržajem natrijuma i delimično zamenjenim masnim tkivom inkapsuliranim tikvinim uljem (ITU). Napravljeno je dve grupe ćevapa: prva sa NaCl (Na ćevapi) i druga sa smešom soli koja sadrži 30% KCl (K ćevapi). U okviru svake grupe napravljeno je po tri varijante sa različitim udelom zamenjenog masnog tkiva ITU – 0%, 15% i 30%. Ispitivana su: kalo toplotne obrade (KTO), promena dužine nakon toplotne obrade (dL), instrumentalno određena tekstura i senzorska svojstva napravljenih ćevapa. So i zamena masnog tkiva ITU nisu imali značajan uticaj na KTO, ali je značajan uticaj ovih faktora utvrđen na dL – sve K varijante su imale veće vrednosti dL u odnosu na Na0 ćevape, dok je veći udeo zamene masnog tkiva ITU uticao na veće vrednosti dL samo kod ćevapa sa NaCl. Značajan uticaj posmatranih faktora uočen je na instrumentalno određenu tvrdoću i žvakljivost – manje vrednosti su izmerene kod K varijanti i kod varijanti sa ITU. Sve ispitivane varijante ćevapa su ocenjene sličnim i relativno visokim ocenama u pogledu svih ispitivanih senzorskih svojstava. Upotrebom soli sa smanjenim sadržajem natrijuma i zamenom masnog tkiva inkapsuliranim tikvinim uljem mogu se dobiti proizvoda sa poboljšanim nutritivnim svojstvima a bez značajnih promena tehnoloških i senzorskih svojstava.
Ključne reči: poluproizvodi od mesa, ćevapi, smanjenje sadržaja natrijuma, tikvino ulje, senzorski kvalitet

Acknowledgments

The research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, ref. number: 451-03-9/2021-14/200116. The authors thank Mrs. Marija Stajić, MA, for English proofreading.

References

CARVALHO BARROS J., MUNEKATA P. E. S., DE CARVALHO F. A. L., PATEIRO M., BARBA F. J., DOMÍNGUEZ R., TRINDADE M. A., LORENZO J. M. (2020): Use of tiger nut (*Cyperus esculentus* L.) oil emulsion as animal fat replacement in beef burgers. Foods, 9 (1), 44.

DESMOND E. (2006): Reducing salt: A challenge for the meat industry. Meat Science, 74(1), 188–196.

GOGUS U., SMITH C. (2010): n-3 Omega fatty acids: a review of current knowledge. International Journal of Food Science and Technology, 45(3), 417-436.

HECK R. T., VENDRUSCOLO R. G., ETCHEPARE M. A., CICHOSKI A. J., MENEZES C. R., BARIN J. S., LORENZO J. M., WAGNER R., CAMPAGNOL P. C. B. (2017): Is it possible to produce a low-fat burger with a healthy n−6/n−3 PUFA ratio without affecting the technological and sensory properties? Meat Science, 130, 16–25.

MARTINS A. J., LORENZO J. M., FRANCO D., VICENTE A. A., CUNHA R. L., PASTRANA L. M., QUIÑONES J., CERQUEIRA M. A. (2019): Omega-3 and polyunsaturated fatty acids-enriched hamburgers using sterol-based oleogels. European Journal of Lipid Science and Technology, 121(11), 1900111.

MONTESANO D., ROCCHETTI G., PUTNIK P., LUCINI L. (2018): Bioactive profile of pumpkin: an overview on terpenoids and their health-promoting properties. Current Opinion in Food Science, 22, 81–87.

PETIT G., JURY V., DE LAMBALLERIE M., DURANTON F., POTTIER L., MARTIN, J. L. (2019): Salt intake from processed meat products: benefits, risks and evolving practices. Comprehensive Reviews in Food Science and Food Safety, 18(5), 1453–1473.

RUUSUNEN M., PUOLANNE E. (2005): Reducing sodium intake from meat products. Meat Science, 70(3), 531–541.

SALGADO A., FONTAN M. C. G., FRANCO I., LOPEZ M., CARBALLO J. (2005): Biochemical changes during the ripening of Chorizo de cebolla, a Spanish traditional sausage. Effect of the system of manufacture (homemade or industrial). Food Chemistry, 92(3), 413–424.

SERBIAN REGULATIONS 50/2019 (2019): Regulation on the quality of minced meat, meat preparations and meat products. Official Gazette of the Republic of Serbia, 50/2019.

STAJIĆ S., ŽIVKOVIĆ D. (2021): Hemijski sastav i senzorna svojstva frankfurtera sa biljnim uljima. Proceedings of the XXVI Savetovanje o biotehnologiji, Martch 12–13, Čačak, Srbija, 467–472.

STAJIĆ S., KALUŠEVIĆ A., TOMASEVICI., RABRENOVIĆ B., BOŽIĆ A., RADOVIĆ P., NEDOVIĆ V. ŽIVKOVIĆ D. (2020a): Technological properties of model system beef emulsions with encapsulated pumpkin seed oil and shell powder. Polish Journal of Food and Nutrition Sciences, 70(2), 159–168.

STAJIĆ S., TOMASEVIC I., STANIŠIĆ N., TOMOVIĆ V., LILIĆ S., VRANIĆ D., BARBA F., LORENZO J., ŽIVKOVIĆ D. (2020b): Quality of dry-fermented sausages with backfat replacement – Fermented sausages with high content of flaxseed oil and pre-treated with soy protein isolate and alginate. Allemand, 100(7), 74–81.

STAJIĆ S., STANIŠIĆ N., TOMASEVIC I., DJEKIC I., IVANOVIĆ N., ŽIVKOVIĆ D. (2018a): Use of linseed oil in improving the quality of chicken frankfurters. Journal of Food Processing and Preservation, 42(2), e13529.

STAJIĆ S., STANIŠIĆ N., LEVIĆ S., TOMOVIĆ V., LILIĆ S., VRANIĆ D., JOKANOVIĆ M., ŽIVKOVIĆ D. (2018b): Physico-chemical characteristics and sensory quality of dry fermented sausages with flaxseed oil preparations. Polish Journal of Food and Nutrition Sciences, 68(4), 367–375.

STAJIĆ S., ŽIVKOVIĆ D., TOMOVIĆ V., NEDOVIĆ V., PERUNOVIĆ M., KOVJANIĆ N., LEVIĆ S., STANIŠIĆ N. (2014): The utilisation of grapeseed oil in improving the quality of dry fermented sausages. International Journal of Food Science & Technology, 49(11), 2356–2363.

YOTSUYANAGI S. E., CONTRERAS-CASTILLO C. J., HAGUIWARA M. M. H., CIPOLLI K. M. V.A .B., LEMOS A. L. S. C., MORGANO M. A., YAMADA E. A. (2016): Technological, sensory and microbiological impacts of sodium reduction in frankfurters. Meat Science, 115, 50–59.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

EXAMINATION OF CERTAIN BEEF QUALITY TRAITS UNDER THE INFLUENCE OF FLAXSEED DIET

Maja Petričević, Tamara Stamenić, Dragan Nikšić, Ljiljana Samolovac, Veselin Petričević, Marija Gogić, Violeta Mandić

Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia.
Corresponding author: Maja Petričević, majanovakovic@live.com
Invited paper

Abstract: The experiment was set up with the objective of examining the effect of adding flax seeds to the cattle diet on the quality of meat, in the final phase of fattening. Thirty Simmental young bulls of uniform initial body weight were selected for the experiment and divided into 2 groups (CON (control) and LS (experimental)). The control group did not consume flaxseed as a dietary supplement. Flaxseed was added to the experimental group in the amount of 3.75% (300 g per day) of the concentrated part of the meal in the final 90 days of fattening. The research included the examination of certain traits of beef meat quality. The results of the study show that the addition of flaxseed in the diet did not have a statistically significant effect on pH, water binding capacity (WBC), weight loss during cooking and meat tenderness. The chemical composition of the selected muscles, as well as the content of total pigments did not change significantly in the experimental animals compared to the control group. It was found that the addition of flax seeds to the diet during the final phase of fattening had an effect on the colour change of the meat reflected in the brightness L* of the muscles of *M. longissimus dorsi* and *M. semitendinosus.*

Key words: meat quality, meat colour, water binding capacity, flax seed

Introduction

Meat consumption is related to standard of living, eating habits and livestock production. Compared to other products, meat is characterized by high production costs and high product prices. Higher demand for meat in the diet is associated with changes in food consumption that favours increased protein intake deriving from animal products. Beef consumption is the third most common type of meat consumption with 15.1 kg per capita, after consumption of poultry and pork, 6.3, 11.8 and, respectively (*OECD, 2021*). The demand for beef grows proportionally with the increase of the population, as well as with the increase in household income. Beef consumption is estimated to be 8% higher in developed countries and 21% higher in developing countries by 2027 (*Mwangi et al., 2019*). Beef is rich in macro- and micro-nutrients - 100 g of beef provides more than 25% of the recommended dietary intake of protein, vitamin B6, vitamin B12, zinc and selenium and more than 10% of phosphorus, iron and riboflavin (*Troy et al., 2016*), and also has certain antioxidant properties (*Asp et al., 2012*).

Meat quality is defined by various technological and physical-chemical properties, such as pH value, water binding capacity, colour intensity, firmness and uniformity of meat structure and reflects the potential of this food for different processing operations. Postmortem changes take place in muscle, connective and adipose tissue and significantly affect the quality of meat (tenderness, structure, ability to bind water, sensory properties and digestibility). In the first stage of meat ripening, the pH value of the meat decreases (*Mancini and Hunt, 2005;Dalmau et al., 2009).* The final pH value is reached in 18 to 40 hours after slaughter. Immediately after slaughter, the pH value of meat ranges from 7.2 to 7.3, and with the transition of glycogen to lactic acid, the pH gradually decreases to 5.6 to 5.7. The pH value affects various meat quality parameters, including WBC. As the pH drops to values close to 5.2, WBC decreases and the amount of released meat juice increases. A decrease in pH leads to stabilization in muscle colour (*Aalhus et al., 2001*).

Muscle tissue contains about 75% water. The characteristic of meat to retain its own water, as well as water added under certain conditions, to a greater or lesser extent, and when an external force, such as pressure or heating, is applied, is referred to as water binding capacity (WBC) (*Rede and Petrović, 1997*). WBC is an important characteristic of meat quality. Loss of water from fresh meat is one of the most common quality problems of this food stuff, which results in numerous economic losses - from weight loss before sales and lower sales of meat, to reduced nutritional value of meat due to loss of valuable water-soluble proteins and vitamins (*Dalmau et al., 2009*).

Sensory quality of meat (colour, tenderness) has a great influence on the attractiveness of these products and consumer satisfaction with them (*Dransfield et al., 2003).* In order to be able to assess the quality of meat, a good knowledge of these characteristics is required. Meat quality is affected by the characteristics of the animal's muscles and the post-mortem biochemical reaction (*Ouali, 1990; Dransfield et al., 2003*). Colour intensity depends on: species, age and diet; the method of rearing and the function that the muscle has in a living organism (*Coleman et al., 2016*), as well as on the gender (*Muir et al., 2000*). Fresh beef is usually dark red in colour. The extremely dark (purple) red colour of beef is an undesirable sign and is most often a consequence of the action of premortal factors, among which stress is of great importance. The more desirable meat colour can be achieved by resting the animal before slaughter and better preparation for slaughter.

Materials and Methods

The research was performed in the experimental cattle farm and experimental slaughterhouse of the Institute of Animal Husbandry in Zemun (Serbia). Male animals of the domestic Simmental breed were used in the experiment. 30 Simmental cattle of uniform body weight were selected for the experiment. At the age of 390 days, two groups of 15 cattle were formed: control group (CON) in which the cattle did not consume heat-treated flaxseed and experimental group (LS) in which part of the concentrate was replaced by heat-treated flaxseed, so that each animal consumed 300 g of flax seeds per day. The final pre-slaughter weight was about 570 kg. Slaughter and primary processing were performed in the experimental slaughterhouse of the Institute of Animal Husbandry.

The pH of meat (*M. longissimus dorsi*) was determined in a muscle sample 45 minutes and 24 hours post mortem, with a pH meter with a combined prick electrode Hanna HI 83141 (Hanna Instruments, USA). The pH meter was pre-calibrated using standard buffer solutions, pH 4.0 and 7.0.

The water-binding capacity (WBC) of meat (*M. longissimus dorsi*, *M. triceps brachii* and *M. semimebranosus*) was determined by two methods: according to *Grau and Hamm (1953)* where the WBC value is expressed in cm^2 of wetted area and according to *Rede and Rahelić (1969),* where the WBC value is expressed in ml of released fluid.

Weight loss during cooking of meat (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus)* was determined based on the difference in weight of a piece of meat size: 3 x 4 x 1.5 cm before and after cooking in a closed glass jar at 100°C for 10 minutes in distilled water (meat to water ratio 1: 2). It is expressed as a percentage in relation to the weight of the sample before cooking (*Official Gazette of the SFRY, No. 2/85, 12/85 and 24/86*).

The meat tenderness (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus*), expressed by cutting force (kg), was determined after cooking at 100°C for 10 minutes and cutting the meat into pieces of 0.5 x 1 x 2 cm in the direction of extending muscle fibres using the consistometer by *Volodkevich (1938).*

The water content (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus*) was determined by drying the sample to constant weight at 102°C ± 2°C *(SRPS ISO 1442, 1998*) and expressed as a percentage of the weight. The fat content (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus*) was determined by the Soxhlet extraction method with petroleum ether as solvent (*SRPS ISO 1444, 1998)* on a Soxtherm multistat apparatus (Gerhardt, Germany). The fat content is expressed as a percentage of the weight. The protein content (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus*) was determined by the Kjeldahl method (*SRPS ISO 937, 1992*) on the Kjeltec system 1026 (Foss Tecator, Denmark) and expressed as a percentage of the weight. The ash content (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus*) was determined by burning the sample to a constant weight at 550°C ± 25 ° C (*SRPS ISO 936, 1999*) and is expressed as a percentage of the weight.

The content of total pigments (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus*) was determined by the Hornsey method (Bunning and Hamm, 1970) and expressed in mg/kg (ppm).

Instrumental measurements of meat colour (*M. longissimus dorsi, M. triceps brachii* and *M. semimebranosus)* were performed on fresh meat samples (24 hours post mortem). Meat samples were cut and left for 30 minutes to stabilize the colour (the samples were in contact with air during that time). The test was performed with the device Chroma Meter CR-400 (Minolta, Japan), which was previously calibrated in relation to the standard white surface (illumination D65, observer angle 20° and at aperture size 8 mm). The colour values are presented in the system CIE L* a* b* (*CIE, 1976*) where the measure L* indicates the lightness of the flesh, a* the relative share of red and b* the relative share of yellow. Three readings were performed on each meat sample and their mean value was used for statistical data processing. The hue angle (H° - “real red”) was calculated as: arctangent arctangent (b*/a*) * 180/3,142. The chroma value (C* - “colour intensity”) was calculated as $(a^{*2} + b^{*2})^{0,5}$.

The obtained data were processed by analysis of variance in one-way ANOVA program SPSS Statistics 20, and all results are displayed as the mean value ± standard deviation. The statistical significance of the difference between mean values was determined by t-test.

Results and Discussion

The effect of the addition of flax seeds to the cattle diet on individual meat quality traits is shown in Table 1. The use of flax seeds in the final stage of cattle fattening did not statistically significantly affect the pH value (pH_{45} and pH_{24}) in the analysed muscle *M. longissimus dorsi*.

Hernàndez-Calva et al. (2011) reports values of pH_{45} 6.89 and pH_{24} 5.68 in cattle that consumed flaxseed as a dietary supplement. *Uchockis et al. (2014)* did not find significant effects of feeding flaxseed meal to cattle on pH_{24} value (5.92), as did *Suksombat et al., (2016)* who did not determine the effect of flaxseed oil addition on the pH of *M. longissimus dorsi*. *Ragni et al. (2014)* believe that the consumption of flax seeds by cattle does not cause differences in the values of pH_{45} (6.08) and pH_{24} (5.48). Similar values for pH_{24} (5.54) are given by *Petričević et al. (2015)* for Simmental cattle. A mean pH of 5.50 reflects a low pre-slaughter stress level and thus ensures good meat quality (*De Smet et al., 2004*).

Table 1. The effect of the addition of flax seeds in the cattle diet on certain meat quality traits

	K	O-1	p
	M. longissimus dorsi		
pH_{45min}	6.50 ± 0.10	6.64 ± 0.41	ns (0.403)
pH_{24h}	5.57 ± 0.30	5.63 ± 0.12	ns (0.732)
WBC (cm^2)	11.04±0.92	10.92±0.53	ns (0.735)
WBC (ml)	8.06±0.15	8.03±0.12	ns (0.778)
WL cooking (%)	41.83 ± 1.52	41.66 ± 0.91	ns (0.633)
Tenderness (kg)	10.61 ± 3.08	10.28 ± 1.14	ns (0.722)
	M. triceps brachii		
WBC (cm^2)	11.98±0.80	12.33±0.51	ns (0.075)
WBC (ml)	8.25±0.17	8.43±0.21	ns (0.052)
cooking (%)	44.20 ± 2.27	45.20 ± 2.14	ns (0.699)
Tenderness (kg)	8.00 ± 2.19	7.95 ± 0.53	ns (0.917)
	M. semitendinosus		
WBC (cm^2)	11.20±0.38	11.68±0.03	ns (0.270)
WBC (ml)	8.12±0.10	8.23±0.06	ns (0.070)
WL cooking (%)	44.25 ± 5.27	42.63 ± 0.31	ns (0.822)
Tenderness (kg)	8.57 ± 0.63	8.18 ± 0.26	ns (0.124)

[1] WBC-Water binding capacity; [2] WL – weight loss

The cattle diet with the addition of flaxseed did not statistically significantly affect WBC, WL cooking and meat tenderness in all three muscles (*M. longissimus dorsi, M. triseps brachii* and *M. semitendinosus*) (Table 1). The water WBC in *M. longissimus dorsi* was approximately the same between the groups, while in case of *M. triseps brachii* and *M. semitendinosus* it was more favourable in the control group. WBC changes under the influence of post mortem factors (*Aalhus et al., 2001*). Higher final temperatures after slaughter can cause a decrease in protein solubility which accompanies reductions in water binding capacity (*Hernàndez-Calva et al., 2011*).

The WL cooking values in the selected muscles were approximately the same between the groups. *Uchockis et al. (2014)* reports no significant differences for the value of meat WL cooking, although cattle from the group that consumed flaxseed meal had a higher value of WL cooking (44.46%) compared to the control group (40.84%). Similar results are reported by *Hernàndez-Calva et al. (2011)* and *Corazzin et al. (2012)* who state that the WL of cooking is higher in cattle that consumed flaxseed in the diet than those that did not, but that the established difference is not significant. *Piasentier et al. (2009)* state the value of WL cooking of 33.50%.

The meat was more tender in the samples of the experimental group in all three muscles. The established differences for meat tenderness between the examined groups were not statistically significant. *Suksombat et al. (2016)* in their study find no statistically significant differences in tenderness for *M. longissimus dorsi* and *M. semitendinosus*, but state that meat tenderness was better in cattle that consumed flaxseed as a dietary supplement, which is confirmed by the results of our study.

The results of the chemical composition of meat are shown in Table 2. The chemical composition of the selected muscles did not change significantly under the influence of the cattle diet with the addition of flaxseed. It should be noted that the treatment had a slight effect on increasing the content of intramuscular fat and protein. The differences in water and protein content were not statistically significant, but the cattle of the experimental group had lower water content and higher protein content in all three muscles.

Table 2. The effect of the addition of flax seeds in the cattle diet on the chemical composition of meat

%	**K**	**O-1**	**p**
	M. longissimus dorsi		
Water	74.97 ± 0.73	74.66 ± 1.53	ns (0.769)
Fat	1.56 ± 0.52	1.55 ± 1.08	ns (0.957)
Ash	1.14 ± 0.04	1.11 ± 0.04	ns (0.335)
Protein	22.31 ± 0.27	22.66 ± 0.63	ns (0.091)
	M. triceps brachii		
Water	76.48 ± 0.58	75.60 ± 0.95	ns (0.154)
Fat	1.42 ± 0.35	1.43 ± 0.65	ns (0.997)
Ash	1.04 ± 0.06	1.19 ± 0.10	ns (0.081)
Protein	21.01 ± 0.68	21.73 ± 1.06	ns (0.202)
	M. semitendinosus		
Water	75.16 ± 0.60	74.91 ± 0.53	ns (0.778)
Fat	1.12 ± 0.46	1.15 ± 0.57	ns (0.996)
Ash	1.17 ± 0.03	1.16 ± 0.04	ns (0.656)
Protein	22.52 ± 0.25	22.75 ± 0.32	ns (0.202)

Uchockis et al. (2014) state that the use of flaxseed cake in the diet of cattle did not have a statistically significant effect on the chemical composition of *M. longissimus dorsi,* where the water content was 76.37%, fat 1.25%, ash 1.05% and protein 21.17%. *Maurić et al. (2016)* report that the water content in *M. longissimus dorsi* ranges from 74.78–76.22%, the intramuscular fat content from 1.92–4.49%, the ash content from 1.08–1.17% and protein content of 19.66–21.25% in Simmental cattle weighing 526–588 kg. In most studies, the chemical composition of meat did not change under the influence of a flaxseed diet. Similar results are reported by *Juárez et al. (2012). Bures et al. (2006)* report a lower water and fat content and a higher protein content in *M. longissimus dorsi* for cattle weighing 550 kg compared to 630 kg cattle. In contrast, *Corazzin et al. (2012)* show a higher water content in *M. longissimus dorsi* in the control group than in the group of cattle fed with the addition of flaxseed.

The effect of the addition of flax seeds to the diet of cattle on the content of total pigments and meat colour is shown in Table 3. The addition of flax seeds to the cattle diet affected the production of lighter coloured meat. No significant effect of flax seed consumption on the content of total pigments in the analysed muscles was found. Higher content of total pigments in *M. longissimus dorsi, M. triceps brachii* and *M. semitendinosus* was determined in the control group.

Table 3. The effect of the addition of flax seeds in the cattle diet on the content of total pigments and meat colour

	K	**O-1**	**p**
	M. longissimus dorsi		
TP (mg/kg) [1]	138.58 ±35.53	136.00 ± 31.87	ns (0.929)
L*	34.99± 2.71	36.15 ± 2.55	* (0.087)
a*	18.91 ± 2.67	18.52 ± 2.42	ns (0.535)
b*	7.16 ± 1.48	7.17 ± 1.50	ns (0.898)
H^0 [2]	20.57 ± 2.07	20.97 ± 1.99	ns (0.661)
C* [3]	20.23 ± 2.97	20.04 ± 3.02	ns (0.630)
	M. triceps brachii		
TP (mg/kg) [1]	213.69 ±30.18	179.97 ± 30.18	ns (0.320)
L*	39.30 ± 2.25	40.99 ± 2.99	ns (0.054)
a*	23.75 ± 2.31	23.69 ± 2.44	ns (0.984)
b*	9.91 ± 1.87	9.90 ± 2.03	ns (0.313)
H^0 [2]	22.61 ± 3.50	22.32 ± 2.44	ns (0.113)
C* [3]	25.78 ± 2.53	25.73 ± 3.00	ns (0.954)
	M. semitendinosus		
TP (mg/kg) [1]	128.01 ±33.32	82.96 ± 17.78	ns (0.104)
L*	38.82± 3.94	43.05 ± 3.66	** (0.002)
a*	20.49 ± 4.57	17.28± 2.89	* (0.042)
b*	8.66 ± 2.86	8.55 ± 1.42	ns (0.844)
H^0 [2]	22.47 ± 3.87	26.53 ± 4.69	* (0.014)
C* [3]	22.29 ± 5.19	19.34 ± 2.86	ns (0.109)

[1] UP – Total pigments; [2] H^o (Hue) = [arctangent (b*/a*) × 180/3,142]; [3] C* (Chroma) = $[(a^{*2} + b^{*2})^{0,5}]$.

The use of flax seeds in the cattle diet affected a significant change in colour, namely the light L* value in *M. longissimus dorsi* and *M. semitendinosus*. Cattle in the experimental group had a statistically significantly ($p<0.05$) higher L* value in *M. longissimus dorsi* than cattle from the control group. In *M. triceps brachii*, the L* value did not differ between the examined groups, but the higher L* value was recorded in the experimental group. The experimental group had significantly ($p<0.01$) higher L* values in *M. semitendinosus*. The proportion of red (a*) was statistically significantly ($p<0.05$) different only in *M. Semitendinosus* and was higher in the control group. The use of flax seeds in the diet of cattle in the final phase of fattening caused a statistically significant ($p<0.05$) change in the Hue (H^0) value of *M. semitendinosus*.

Hernàndez-Calva et al. (2011) report no statistically significant differences for Chroma and Hue values which were higher in cattle fed silage and flax seed compared to cattle that consumed hay instead of silage. According to the authors *Merera et al. (2010)* differences in Chroma and Hue values may be related to the presence of adipose and connective tissue on the surface of meat. In their study, *Corazzin et al. (2012)* report no statistically significant differences for the meat colour of *M. longissimus dorsi*. According to them, the L* values of *M. longissimus dorsi* are higher in the control group, while the proportions of red (a*) and yellow (b*) are lower compared to the group fed flaxseed. These results are consistent with the results of *Mach et al. (2006). Drouillard et al. (2004)* report better meat colour results when using vitamin E with flaxseed.

Conclusion

The addition of flaxseed in the diet did not have a statistically significant effect on pH, water binding capacity (WBC), weight loss during cooking and meat tenderness. Also, the chemical composition of selected muscles and the content of total pigments did not change significantly under the influence of flaxseed. It was found that the addition of flax seed to the diet during the final phase of fattening had only an effect on the colour change of the meat reflected in the light L* in the muscles of *M. longissimus dorsi* and *M. semitendinosus Semitendinosus*. The share of red (a*) colour was higher in the control group, while the change in Hue (H^0) value was higher in the experimental group. The share of red (a*) colour and the Hue value (H^0) were statistically significant ($p<0.05$) distinguished in M. Semitendinosus.

Ispitivanje pojedinih karakteristike kvaliteta mesa junadi pod uticajem ishrane sa semenom lana

Maja Petričević, Tamara Stamenić, Dragan Nikšić, Ljiljana Samolovac, Veselin Petričević, Marija Gogić, Violeta Mandić

Rezime

Eksperiment je postavljen sa ciljem da se ispita efekat dodavanja semena lana u ishranu junadi na kvalitet mesa, u završnoj fazi tova. Za ogled je odabrano 30 muških junadi simentalske rase ujednačenih početnih telesnih masa, koja su podeljena u 2 grupe (CON (kontrolna) i LS (ogledna)). Kontrolna grupa nije konsumirala seme lana kao dodatak ishrani. Junadima ogledne grupe je dodavano seme lana u količini od 3,75% (300 g dnevno) koncentrovanog dela obroka u poslednjih 90 dana tova. Istraživanje je obuhvatilo ispitivanje pojedinih karakteristika kvaliteta mesa junadi. Rezultati istraživanja su pokazali da dodatak semena lana u ishrani nije imao statistički značajan uticaj na pH vrednost, sposobnost vezivanja vode, gubitak mase tokom kuvanja i pečenja i mekoću mesa. Hemijski sastav odabranih mišića, kao i sadržaj ukupnih pigmenata nisu se značajno menjali kod oglednih grla u poređenju sa kontrolnom grupom. Utvrđeno je da dodatak semena lana u ishranu tokom završne faze tova imao uticaj na promenu boje mesa koja se ogledala u svetloći L* u mišićima *M. longissimus dorsi* i *M. semitendinosus*.

Key words: kvalitet mesa, boja mesa, sposobnost vezivanja vode, lan

Acknowledgements

Research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

Reference

AALHUS J. L., JANS A. M., TONG A. K. W., JONES S. D. M., ROBERTSON, W. M. (2001): The influence of chilling rate and fat cover on beef quality. Canadian Journal of Animal Science, 81, 321–330.

ASP M. L., RICHARDSON J.R., COLLENE A. L., DROLL K. R., BELURY M. A. (2012): Dietary protein and beef consumption predict for markers of muscle mass and nutrition status in older adults. Journal of Nutrition, Health & Aging, 16, 784–790.

BUNNING K., HAMM R. (1970): Über die Haminbestimmung in fleisch mittels der methode von Horsney. Fleischwirtschaft, 50, 1541–1545.

BUREŠ D., BARTOŇ L., ZAHRÁDKOVÁ R., TESLÍK V., KREJČOVÁ M. (2006): Chemical composition, sensory characteristics, and fatty acid profile of muscle from Aberdeen Angus, Charolais, Simmental, and Hereford bulls. Czech Journal of Animal Science, 51, 279–284.

CIE (1976): Commission Internationale de l'Eclairage. Colorimetry, 2nd ed., Vienna.

CORAZZIN M., BOVOLENTA S., SEPULCRI A., PIASENTIER E. (2012): Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. Meat Science, 90, 99–105.

COLEMAN L. W., HICKSON R. E., SCHREURS N. M., MARTIN N. P., KENYON P. R., LOPEZ-VILLALOBOS N., MORRIS S. T. (2016): Carcass characteristics and meat quality of Hereford sired steers born to beef-cross-dairy and Angus breeding cows. Meat Science, 121, 403–408.

DALMAU A., VELARDE A., GISPERT M. (2009): Standardisation of the measure „meat quality" to assess the welfare of pigs at slaughter, in Forkman B., Keeling L., Assesment of Animal Welfare Measures for Sows, Piglets and Fattening Pigs, Welfare Quality Reports, 10.

DE SMET S., RAES K., DEMEYER D. (2004): Meat fatty acid composition as affected by fatnes and genetic factors. Animal Research, 53, 81–98.

DROUILLARD J. S., SEYFERT M. A., GOOD E. J., LOE E. R., DEPENBUSCH B., DAUBERT R. (2004): Flaxseed for finishing beef cattle: Effects on animal performance, carcass quality, and meat composition. Proceedings, 60th Flax Institute, 108–117.

DRANSFIELD E., MARTIN J. F., BAUCHART D., ABOUELKARAM S., LEPETIT J., CULIOLI J., JURIE C., PICARD B. (2003): Meat quality and composition of three muscles from French cull cows and young bulls. Animal Science, 76, 387–399.

GRAU R., HAMM R., BAUMANN A. (1953): Über das wasserbindungsvermögen des toten Säugetiermuskels. I. Mitteilung. Der Einfluß des pH Wertes auf die Wasserbindung von zerkleinertem Rindermuskel. Biochem. Z., 325, 1–11.

HERNÀNDEZ-CALVA L.M., HE M., JUÀREZ M., AALHUS J. L., DUGAN M. E. R., MCALLISTER T. A. (2011): Effect of flaxseed and forage type on carcass and meat quality of finishing cull cows. Canadian Journal of Animal Science, 91, 613–622.

JUÁREZ M., DUGAN, M. E. R., ALDAI N., BASARAB J. A., BARON V. S., MCALLISTER T. A., AALHUS J. L. (2012): Beef quality attributes as affected by increasing the intramuscular levels ofvitamin E and omega-3 fatty acids. Meat Science, 90 (3), 764–769.

MACH N., DEVANT M., BACH A., DÍAZ I., FONT M., OLIVER M. A., GARCÍA J. A. (2006): Increasing the amount of omega-3 fatty acid of meat from intensively fed young Holstein bulls through nutrition. Journal of Animal Science, 84, 3039–3048.

MAURIĆ M., STARČEVIĆ K., ŠTOKOVIĆ I., MAŠEK T. (2016): The influence of sires selection index on the intramuscular fat fatty-acid profile of their progeny. Veterinarski Arhiv, 86 (6), 753–766.

MERERA C., ABEBE G., SEBSIBE A., GOETSCH, A.L. (2010): Effects and interactions of origin of sheep in Ethiopia (Highland vs Lowland areas), feeding, and lengths of rest and feeding on harvest measures. Journal of Applied Animal Research, 37, 33–42.

MANCINI R. A., HUNT M. C. (2005): Current research in meat color. Meat Science, 71, 100–121.

MUIR P. D., WALLACE G. J., DOBBIE P. M., BOWN, M. D. (2000): A comparison of animal performance and carcass and meat quality characteristics in Hereford, Hereford×Friesian, and Friesian steers grazed together at pasture. New Zealand Journal of Agricultural Research, 43, 193–205.

MWANGI F.W., CHARMLEY E., GARDINER C. P., MALAU-ADULI B. S., KINOBE R. T., MALAU-ADULI A. E. O. (2019): Diet and Genetics Influence Beef Cattle Performance and Meat Quality Characteristics. Foods, 8(12), 648.

PETRIČEVIĆ M., ALEKSIĆ S., PETROVIĆ M. M., PANTELIĆ V., OSTOJIĆ-ANDRIĆ D., STANIŠIĆ N., NIKŠIĆ D. (2015): Comparative study of fattening and slaughter traits of male simmental breed and crosses with charolais breed. Biotechnology in Animal Husbandry, 31 (2), 265–272.

OECD (2021): Meat Consumption (indicator) https://data.oecd.org/agroutput/meat-consumption.htm

Official Gazette of the SFRY, No. 2/85, 12/85 and 24/86)/SL. LIST SFRJ, BR. 2/85, 12/85 i 24/86: Pravilnik o kvalitetu zaklanih svinja i kategorizaciji svinjskog mesa.

OUALI A. (1990): Meat tenderization: Possible causes and mechanisms. A review. Journal of Muscle Foods, 1, 129–165.
PIASENTIER E., BOVOLENTA S., MOIOLI B., ORRÙ L., VALUSSO R., CORAZZIN M. (2009): Fatty acid composition and sensory properties of Italian Simmental beef as affected by genes frequency of Montbéliarde origin. Meat Science, 83, 543–550.
RAGNI M., TOTEDA F., TUFARELLI V., LAUDADIO V., FACCIOLONGO A., DIPALO F., VICENTI A. (2014): Feeding of Extruded Flaxseed (Linum usitatissimum L.) and Pasture in Podolica Young Bulls: Effects on Growth Traits, Meat Quality and Fatty Acid Composition. Pakistan Journal of Zoology, 46 (4), 1101–1109.
REDE R., RAHELIĆ S. (1969): Priručnik za laboratorijske vežbe. Tehnologija mesa, Novi Sad.
REDE R. R., PETROVIĆ LJ. S. (1997): Tehnolologija mesa i nauka o mesu. Tehnološki fakultet Novi Sad.
SUKSOMBAT W., MEEPROM C., MIRATTANAPHRAI R. (2016): Performance, Carcass Quality and Fatty Acid Profile of Crossbred Wagyu Beef Steers Receiving Palm and/or Linseed Oil. Asian Australasa Journal Animal Science, 29, 1432-1442.
TROY D. J., TIWARI B. K., JOO S. (2016): Health implications of beef intramuscular fat consumption. Korean Journal Food Science Animal Resources, 36, 577–582.
UCHOCKIS V., ŠVIRMICKAS G. J., BALTRUKONIENĖ G. (2014): The effects of linseed cake supplementation on the growth and quality of carcass and meat of fattening bulls. Veterinary medicine and zootechnics., 66 (88), 73–77.
VOLODKEVICH N. N. (1938): Apparatus for measurement of chewing resistance or tenderness of foodstuffs. Food Research., 3, 221–225.
(1985): Pravilnik o kvalitetu mesa stoke za klanje, peradi i divljači, Sl. list SFRJ, br. 34/74, 26/75, 13/78 - dr. pravilnik, 1/81 - dr. pravilnik i 2/85 - dr. pravilnik). https://www.scribd.com/doc/183009807/Meso-i-Proizvodi-Od-Mesa
SRPS ISO 1442 (1998): Određivanje sadržaja vode.
SRPS ISO 1444 (1998): Određivanje sadržaja slobodne masti.
SRPS ISO 936 (1999): Određivanje sadržaja pepela.
SRPS ISO 937 (1992): Određivanje sadržaja belančevina

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

BIOFORTIFICATION AS A WAY OF NUTRIENT DENSE FEED PRODUCTION

Vesna Dragičević[1], Milena Simić[1], Milan Brankov[1], Milena Šenk[1], Vesna Krnjaja[2], Violeta Mandić[2], Branka Kresović[1]

[1]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185 Zemun Polje, Republic of Serbia
[2]Institute for Animal Husbandry Belgrade-Zemun, Autoput 16, 11080 Zemun, Serbia
Corresponding author: Vesna Dragičević, vdragicevic@mrizp.rs
Invited paper

Abstract: Malnutrition is a global problem, hitting both, people and animals. Due to the many factors, such as climate change, soil depletion, anthropogenic impact, including irresponsible soil management, high fertilization rates with macro-nutrients, crops lack in essential nutrients, particularly minerals, Mg, Fe, Zn, etc., as well as important vitamins, like carotenoids, vitamins from B group. Also, some arable soils have naturally low fertility. All of that negatively affect production of domestic animal, including animal health and quality of livestock products. To mitigate malnutrition, a bio-fortification strategy was developed. It is based on increase in the concentration of essential nutrients in food and feed, and also to promote further bio-availability from digestive organs. Bio-fortification implies various practices. Two main types of bio-fortification were developed: genetic bio-fortification, and agronomic bio-fortification. The first one uses standard breeding techniques, marker assistant selection, transgenic approaches, genome editing, etc., to develop highly-efficient genotypes, that are able to absorb and accumulate higher concentrations of essential nutrients in biomass and grains. Agronomic bio-fortification uses different practices, such is special fertilizers, enriched with essential minerals, foliar fertilizers, bio-fertilizers, growth hormones and enhancers, and some lesser known sustainable practices, like inter-cropping, cover cropping, in order to increase crop yields, as well as the concentration of essential nutrients. The bio-fortification approach, based on the development and commercialisation of highly efficient genotypes, as well as agricultural practices that enable and support better absorption and accumulation of essential nutrients option that is safer for both, people and domestic animals. Mutual increase in concentration of essential minerals, vitamins, and other promoting substances is of a particular importance for bio-fortification programs, increasing efficiency and success of applied practices, thus positively reflecting on animal health and wellbeing.

Key words: breeding, cropping practices, mineral nutrients, antinutrients, promoters.

Introduction

Humans and animals require variety of mineral elements, but in relatively small amounts, 1-2500 mg day^{-1}. They present important body constituents. For example, Ca is an important part of bones, nerves, and muscle structures; P is a part of ATP and nucleic acids molecules, as well as bones and is important for maintaining of pH balance; K, Na, and Cl maintain osmotic balance at the cellular and inter-cellular level; Fe is an important part of haemoglobin and cytochrome molecules involved in cell respiration; Mg, Fe, Zn, Cu, Mn, Se, and Mo are important co-factors of various enzymes, and they are also involved in numerous biochemical pathways; Se and Zn are important for the proper immune system function; I is an important part of thyroid hormones; vitamin A is important for optimal vision, function of the immune and reproductive system; vitamins from B group are important for metabolism, cell reproduction, blood, skin, and health of many other organs in human and animal bodies, etc. Excessive intake, as well as deficiency of essential minerals and vitamins disrupts homeostasis of mineral balance, metabolism, and hormone balance in humans and animals, causing as a consequence various diseases in humans and animals.

An inadequacy in the intake of minerals and vitamins is referred as malnutrition. The malnutrition is a worldwide problem, affecting not just people, but also animals. Nowadays, when sufficient food/feed amount is mainly enabled, malnutrition is predominantly connected to the lack of particular components, such as protein, minerals, and vitamins, thereby affecting human and animal health and welfare. *Welch (2008*) and *Keding et al. (2013)* pointed that the goal of the farming systems is the maximization of the production, with minimal costs, rather than maximization of nutrient output to produce food/feed for nutrient dense and balanced diet. The industrialization of an agricultural sector, particularly a crop production is based on a yield increase, including breeding of high yield genotypes, applying of agricultural practices that support high yields of biomass and grain, based on high inputs of agro-chemicals, such as pesticides and mineral fertilizers (mainly N and NPK fertilizers). All mentioned contributed to the sufficient production of food and feed, reflected through increased amounts of carbohydrates, i.e. calories. The "Green revolution" paradigm changed face of the agriculture, with multiply higher crop and livestock production, CO_2 emission, as well as an increased share of processed foods in nutrition (*Gomez et al., 2013; Zeng et al., 2014*). *Fardet and Rock (2020)* indicated that the livestock production occupy globally 26% of areas aimed for rangelands and 33% of the agricultural areas aimed for fodder production, whereas the intensive livestock and the production of other animal species use less land, higher inputs, and high concentration of animals, thus contributing to nutrient pollution, increased greenhouse gas emission (GHGE), habitat destruction and diversity reduction. It was accentuated that among 6300 domestic animal breeds, about 1350 are close to extinction or already disappeared over against breeds that have high growth rate and altered body structure and composition (*Hocquette, 2010; Hayes et al., 2013*).

As a consequence of all mentioned, an environmental depletion and pollution rose, withaltered soil pH (to low or high values), mineral imbalance, erosion, increased greenhouse gasses emission, etc. Even more, climate change, recognized through meteorological extremes additionally worse the situation. In such conditions, agricultural plants are unable to provide basic elements for growth and development, whereas deficit of essential elements and vitamins, particularly Fe, Mg, Zn, Se, I, carotenoids and other vitamins in food and feed is present worldwide (*Welch, 2005; Clemens, 2014*). One of the most important aspects of food security, irrespective that food and/or feed are considered is to provide optimal amounts/concentration of the essential elements in edible parts of plants. Insufficient concentrations of essential elements and vitamins could be reduced by an introduction of a food diversity (for humans), as well as supplementation and/or increased concentration of highly available nutrients in food and feed, by application of various bio-fortification practices. In regard to supplementation, advantage of bio-fortification is reflecting through improved concentration of mineral and other nutrients, with reduced possibility of overdose or toxicity, due to the fact that nutrients in plant tissues are mainly in highly available forms, what in combination to the other important plant constituents, such as carotenoids, vitamin C, some amino acids, soluble fibre, etc. additionally enhance their bio-availability during digestion.

It is also important to underline that concentration of mineral nutrients in animal tissues does not follow increased concentration of mineral elements in biomass and/or grains used for feed, since bio-availability depends on feed composition, where so called anti-nutrients, such as phytic acid, certain fiber (cellulose, hemicelluloses, lignin, suberin etc.), lignans and other polyphenolics, hemagglutinins, goitrogens, heavy metals restrain bio-availability and absorption of mineral nutrients. Though, so called promoters: organic acids (e.g. ascorbate, fumarate, malate, citrate), hemoglobin, certain amino acids (methionine, cysteine, histidine, lysine), long-chain fatty acids, fats and lipids, Se, Fe, Zn, β-carotene, inulin and other non-digestible carbohydrates increase the absorption of nutrients, diminishing negative effects of anti-nutrients (*Welch and Graham, 2005*). Anti-nutrients and promoters are normal plant metabolites, thus variation in their concentrations could seriously affect bioavailability of mineral nutrients for humans and non-ruminants. Nevertheless, ruminants, due to the presence of specific microorganisms in their guts are able to digest most of the mentioned anti-nutrients, having better conversion of mineral elements from feed into their bodies. Some strategies, like addition of enzyme preparations into feed (either they are active prior or after feed consumption) could also contribute to increased nutrient bio-availability (*Pariza and Cook, 2010*).

As an answer to a globally rising problem of the malnutrition in people and animals, bio-fortification, as a sustainable practice was developed. It combines various techniques and practices, aimed to increase concentration of important nutrients, as well as their further bio-availability, in regard that absorption and accumulation of mineral nutrients in plants, particularly in grains is under the control of various physiological processes. Several important strategies as a parts of bio-fortification programs were developed. Some of them use diverse genetic resources and breeding of genotypes with improved absorption and accumulation of mineral elements, as well as factors that contribute their further bio-availability from digestive system. Other strategies combine various cropping practices, particularly fertilizers with desirable composition, also the bioactive stimulants that enhance absorption and accumulation of mineral elements. Many practices, commonly used in sustainable and organic agriculture, which improve soil fertility, diversity and quality, at the same time contribute to the better absorption and accumulation of mineral nutrients, thus enhancing the nutritional quality of feed crops.

Abiotic and Biotic Factors Affecting Utilization of Mineral Nutrients for Feed Production

An agricultural production is highly dependable on the environment, particularly variations in meteorological conditions. Extreme fluctuations of meteorological conditions are able not just to reduce, but also to destroy crop yields. Besides that, climate change affects socio-economic aspects of agricultural production, livestock production, transport, demography, altering production and food security (*Tirado et al., 2010*). Climate change is closely tied to the increased atmospheric concentration of CO_2 and other GHGE-s, thus increasing C portion in plant tissues, which is not in parallel, followed by the increased absorption from the soil and accumulation of mineral elements (*Loladze, 2002*), thus reducing minerals:C ratio. Increased CO_2 reduces transpiration intensity, accelerate plant growth, affect flowering and grain filling and reduce absorption and accumulation of Ca and Si, contributing to the decreased resistance to pathogens attack, as well as lower concentrations of protein and lipids in grains (*Fernando et al., 2012*). Fabaceous and gramineous are C3 plants, which are mainly used for forage production, accumulate lesser Zn and Fe concentrations, lesser water usage and increased accumulation of carbohydrates in green parts, when they were grown in atmosphere with high CO_2 concentration, while C4 plants were mainly unaffected

(*Myers et al., 2014*). According to *Bornman et al. (2019*) changes in climate caused by ozone depletion and thus increased exposure to UV radiation is connected to increases and reductions in the growth, survival and reproduction of plants and animals. What is more, the fluctuations in meteorological conditions, like drought, could also affect nutrient uptake, distribution, and accumulation in plants, thus altering its chemical composition and nutritional quality (*Hart et al., 1998; Rouphael et al., 2012*).

The primary source of mineral nutrients for plants is the soil. Beside the amount of mineral nutrients in the soil, its characteristics and condition are mainly responsible for availability, absorption, and accumulation of mineral nutrients in plants. Mineral nutrients are present in the form of free ions, salts, adsorbed by various minerals and organic components, parts of soil solution, as well as parts of soil living and decomposing organisms (microbiota, worms and other living systems) (*White and Broadley, 2009*). An optimal mineral nutrition, provided by fertilizer inputs enables normal plant growth and development, particularly when they are grown on poorer soils with low fertility. Accordingly, connection between the Zn deficiency in humans and animals and low Zn concentration in soil is well known (*Cakmak and Kutman, 2018; Dhaliwal et al., 2020*). Chemical reactions in a soil, such as redox reactions, could affect an availability of mineral nutrients. Besides, Fe- and Mn-oxides, organic matter, including humic substances, various microorganisms, and products of their decompositions presents active sorbents of various mineral elements, influencing their availability to plant roots (*Violante et al., 2010; Lin et al., 2019; Hacquardet al., 2015*). Physical characteristic, such as high soil hardness, low penetration or high drainage degree, high or low pH, high salinity, toxicity of some ions and heavy metals (like Na, Cl, Al, Fe, Mn, Cd, Pb etc.) affect not just availability of important mineral elements, but also could induce abiotic stress to plants grown on these soils. Mineral elements with similar characteristics, including association to group in Periodic System of Elements, atom mass, charge ant other, could compete each to other during absorption by roots. Thus, Ca and Cd could compete with Zn, contributing additionally to oxidative stress increase (*Rose et al., 2013; Slamet-Loedin et al., 2015*). It is important to underline that deficiency symptoms will be observable on older leaves, if plants are grown on soils with poor availability of particular mineral elements that have better mobility, and *vice versa*, (*Soetan et al., 2010*).

The important factor that contributes to imbalance in mineral nutrients in a soil, through degradation, erosion, desertification, acidification, increasing salinization, high irrigation rates, and inadequate fertilization is an anthropogenic factor. From this viewpoint, high P fertilization rates conducts to the low Zn availability. Some, commonly used amelioration practices, such as increased fertilization rates with macro-elements (N, P, K, S, Ca, and Mg), including practices that affect soil fertility and pH balance, incorporation of Ca–carbonate and –oxide, gypsum, high amount of organic fertilizers could negatively affect the availability of some mineral elements, particularly, micro-elements. When plants were grown on soils where long-term mineral fertilization is present, stoichiometric balance between N and P in plant leaves that is closely tied to the yield losses is actually consequence of unbalanced N and P inputs through fertilization (*McKenzie and Williams, 2015*). On the other hand, the positive influence of the anthropogenic factor is reflected through inputs of organic matter, bio-fertilizers (containing mycorhizzal fungi, N-fixing bacteria, microorganisms that accelerate crop residues decomposition) which could positively affect, absorption of mineral elements (particularly of P, Fe, Mn, Zn, and Cu), thus contributing to the enhanced stress tolerance, photosynthesis and increased crop yield (*El–Sirafy et al., 2006; Bhardwaj et al., 2014; Lehmann and Rillig, 2015*).

Other than soil characteristics, there are genotypes that have high efficiency in nutrient elements absorption from soil, even from soils with low level or deficient in a particular nutrient(s), such as soils with poor fertility, acid, saline or similar soils. According to *Eckhard et al. (2012)*, high efficient genotypes are able to achieve high yields on soils deficient in one or several mineral nutrients, due to the genes that could synthetize transporters with high affinity to particular mineral elements, even in conditions of deficiency or poor availability. For instance, some rice genotypes, with high efficiency for Zn absorption have genes that encode Zn absorption and translocation from older to physiologically more active, younger leaves (*Impa et al., 2013; Tiong et al., 2015*), even more these plants easier translocate metabolites from older leaves to the grains.

Breeding and Genetic Engineering as an Integral Part of the Bio-fortification

Modern genotypes must be able to produce high yields of biomass and grains, and to be able to grow in conditions with low inputs of water, fertilizers, and other agro-chemicals. What is more important, their nutritional status has to meet requirements of animals for all necessary elements. Nevertheless, nutritionally quality and yield potential are reversely correlated. Thus, new wheat genotypes with high yield potential are also low in Zn, Fe, and Se in regard to older genotypes (*Garvin et al., 2006*). The necessity for crops that are able to have high yields, with improved efficiency to use resources is an important objective of modern agricultural production (*Raboy, 2013*).

Highly-efficient genotypes could achieve by different methods, such as standard breeding techniques, marker assistant selection (MAS), transgenic approaches, genome editing, etc. Environmental impacts are important obstacle for breeding. Genotype × environment interaction direct Fe and Zn accumulation in maize grain two-fold more than each factor individually (*Oikeh et al., 2004a*). Accordingly, conventional breeding is a long-term strategy, due to the variable agro-ecological factors, primary soiland climate, while MAS is better solution, when low heritability traits are considered, due to the fact that genotypes without desirable traits were excluded from further tests at the early stages. For MAS application, high variability of desirable traits (efficiency of mineral nutrient absorption) must be present, and an important part of this method is results proofing in a field conditions. Both mentioned strategies are important for selection and development of genotypes which possess high variability in efficiency to utilize and accumulate mineral elements, as well as factors that restrain or promote bio-availability of absorbed minerals. From this viewpoint, maize, as one of the most important crops used for feed production, either biomass, or grain are used, has high variability in concentration of carotenoids, phenolics, phytic acid, Fe, Mn, Zn, and other minerals (*Dragičević et al., 2013; Gupta et al., 2015*), what is particularly referred to the genotypes with relative low phytic acid concentration in grain (*Mladenović Drinić et al., 2009; Dragičević et al., 2010; Dragičević et al., 2013; Dragičević et al., 2016*). This was supported by results present in Table 1, done on 78 maize lines, where Mn and Zn concentration in the grain correlated significantly on the phytate concentration, from which Zn correlated negatively, thus indicating its better accumulation in grain of genotypes lover in phytic acid, as well potentially enhanced further bio-availability. Based on conventional breeding techniques, it was established that heterotic background presents an important source of highly-efficient genotypes, when concentration of mineral nutrients and promoters was considered. Hence, based on results obtained on 51 maize lines (Figure 1), it was established that in grain of genotypes from *Lancaster* heterotic group relative low concentration of phytic acid was present, together with enhanced Zn concentration while in lines from Independent source variations in Mg, Fe, and Mn concentration were independent on phytic acid status, indicating that they could serve as an advantageous source for increased Mg, Fe, and Mn concentration and improved bio-availability (*Dragičević et al., 2016*). In parallel, combination of conventional breeding and MAS could be successfully used for mapping and selection of maize lines with desirable traits, such as genotypes high in mineral elements and low phytic acid (*Šimić et al., 2012*).

Table 1. Correlation between examined traits: phytic (P_{phy}), inorganic (P_i) and total (P_{tot}) phosphorus, and β-carotene, Fe, Mn and Zn in grain of 78 maize lines (*Dragičević et al. 2013*).

	P_{phy}	P_i	P_{tot}	β-carotene	Fe	Mn
P_i	0.05					
P_{tot}	0.43*	0.31*				
β-carotene	0.12	-0.11	-0.01			
Fe	0.01	0.23	0.15	0.06		
Mn	0.36*	0.17	0.30*	0.21	0.20	
Zn	-0.25*	0.17	0.06	0.05	0.34*	-0.04

*The significant values at the level of significance of 0.05.

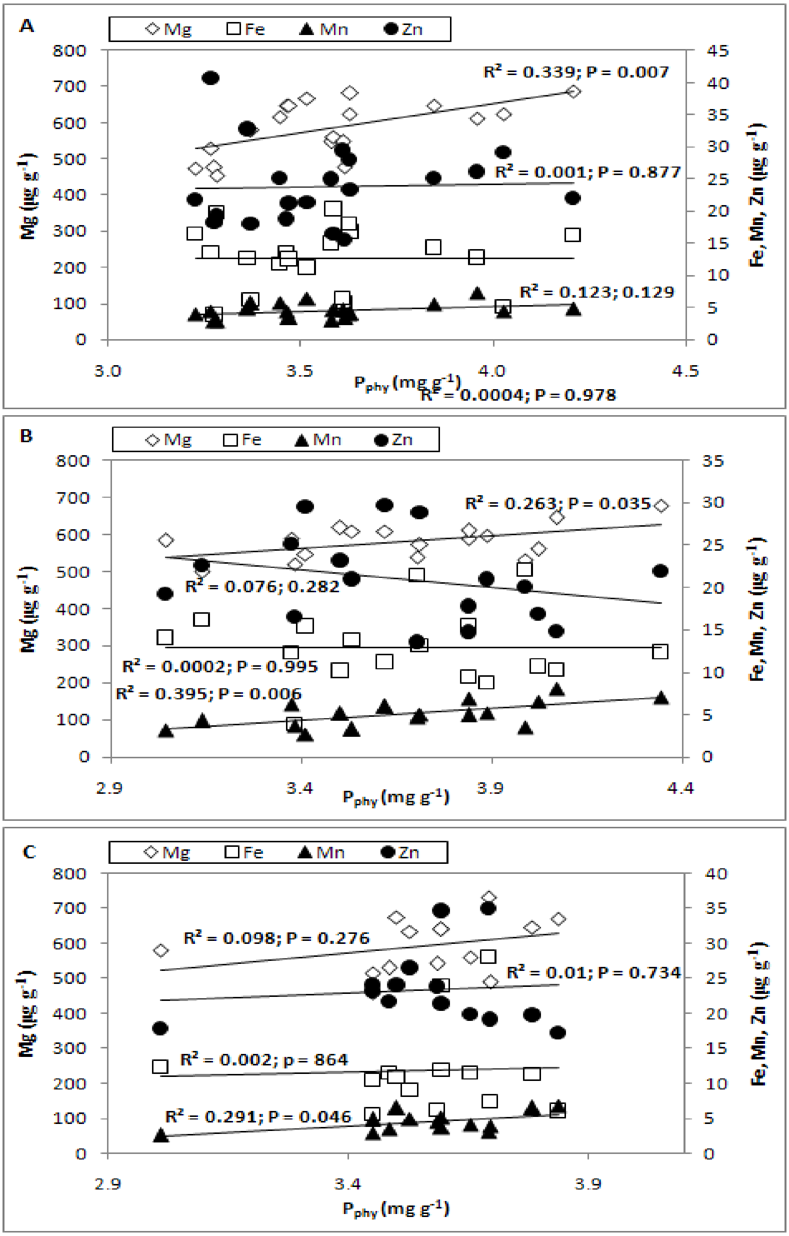


Figure 1. Interdependence between phytic P (P_{phy}) and mineral elements in grain of maize lines belong to (A) *BSSS* heterotic group, (B) *Lancaster* heterotic group, and (C) Independent source (*Dragicevic et al., 2016*).

The application of transgenic techniques depends, not only on an identification and introduction of genes responsible for the improved absorption and accumulation of mineral nutrients into crop biomass and/or grain, but also on acceptance of transformed genotypes by the market, including consumers (for human nutrition) as well as farmers engaged to the livestock production. Criterion for the commercialisation of bio-fortified genotypes lies in their ability to achieve same or even higher yield, reached concentration of nutrients must be stable in various environments, including positive effects on human or animal health, by the definition (*Welch and Graham, 2005*). Bio-availability and absorption into animal organisms must be tested in practice, prior to releasing of modified genotypes to the market.

There are many examples of bio-fortified transgenic crops, where strategic role play chelators that enhance mobility and accumulation of mineral elements, including nicotinamine, of which three genes that encode nicotinaminesynthetase and genes that encode regulatory proteins involved into Fe accumulation were successfully used for rice transformation with six-fold higher Fe concentration (*Clemens, 2014*). Even more, the combination of genes for nicotinamine synthetase and ferritin increased 1.5-fold Zn concentration. From the viewpoint of increased Fe accumulation, soybean ferritin genes in combination with genes that encode mugineic acid synthesis resulted in four-fold higher Fe concentrations in the grain of polished rice that was grown in conditions of the Fe deficiency (*Masuda et al., 2013*). One of the important strategies, when transgenic approach is considered, is isolation of genes from hyper-accumulators involved into metabolism and accumulation of particular mineral elements, such as ZIP-transporters from *Thlaspi caerulescens* and *Arabidopsis* genome involved into Zn transport, as well as genes from *Astragalus* species, known as a Se accumulators (*Guerinot and Salt, 2001*). Transformations of crops in bio-fortification programs are not based only on improvements in accumulation of mineral elements, but also on the increased accumulation of promoters, such as carotenoids, vitamin C, and folate. There are many genes involved in synthesis of various carotenoids that have been used in improvements of genome of different plant species (*Suwarno et al., 2015*). Introduction of some of these genes resulted in maize grain with 169-folds higher β-carotene concentration + 6-folds higher vitamin C level + 2-folds higher folate concentration (*Naqvi et al., 2009*). Due to the fact that P is mainly present in the plant tissues in the form of phytic acid, its utilization efficiency in the livestock production is below 40%. *Kebreab et al. (2012)* summarized that usage of low phytate transgenic plants and transgenic animals increased P availability by 14% and 52–99%, respectively, whereas combination of phase feeding and enzymes that decompose phytic acid increased P availability from 42 to 95%, having the greatest importance in practice.

Irrespective that breeding and genetic method are promising bio-fortification tools, their combination with other techniques could give better results in enrichment of forage crops with mineral elements and vitamins, particularly when it was taken into consideration that even highly efficient genotypes are unable to absorb elements that lack in soil.

The Agronomic Bio-fortification – Cropping Practices for Enhanced Feed Quality

The most important factor that supports high availability, confident and stable absorption, and accumulation of mineral elements in crops is the soil fertility. The application of various fertilizers could contribute to the maintaining, increase, or depletion of the soil fertility. The usage of various supplements as feed additives with intention to enable optimal and balanced livestock nutrition have potential risks, reflected through overdosing and increased potential toxicity, as well as increased excretion, what is environmentally unacceptable (*Anderson et al., 2012; Titcomb and Tanumihardjo, 2019*). The same authors pointed that the introduction of bio-fortified crops as feed is safer for animals and environmentally rational, since nutrients are mostly in the highly available forms, what with promoters from feed enable enhanced absorption and usage on the cellular level.

When the bio-fortification was considered in general, it is important to underline that the agronomic bio-fortification and genetic bio-fortification should be jointly used, to provide high efficiency in nutrient usage and delivery to the human and animal organisms. For instance, Zn enriched NPK fertilizers could present complementary amendment of breeding programs (*Cakmak, 2008*). It is well known that macro-elements from fertilizers affect availability and further absorption of micro-elements, such as N, which positively affect the absorption of micro-elements by rice plants (*Hao et al., 2007*). Also, an optimization of Zn-enriched urea for the agricultural production was developed as a part of the bio-fortification (*Shivay and Prasad, 2012; Cakmak and Kutman, 2018*). Some of the bio-fortification techniques include utilization of plants originating from locations under fitoremediation programs, such as soils with extreme high Se or Zn content, as a green manure, as well as part of feed mixtures (*Wu et al., 2015; Schiavonand Pilon-Smits, 2017; Wang et al., 2021*).

One of the most efficient agronomic bio-fortification practices is an application of foliar fertilizers, since unfavourable soil conditions that could affect availability and absorption of mineral nutrients were avoided and metabolizing of mineral nutrients by plants was assured. Therefore, the method for increase in Zn, Se, and Fe concentration in rice by the foliar fertilization was developed (*Fang et al., 2008*). Many other agro-chemicals, such as bio-stimulants, plant, and algae extracts, phytohormone preparations, herbicides, etc. could be successfully used to alleviate status of mineral elements and factors that positive or negative affect their further bio-avilability, such asphytate, phenolics carotenoids, glutathione, and some other vitamins in the plant tissue (*Dragičević et al., 2015a; Dragičević et al., 2016b; Mesarović et al., 2019; Đurović et al., 2019; Brankov et al., 2020*). It is important to underline that efficiency of foliar fertilizers is highly dependable on agro-meteorological factors, particularly when crops were grown in dry farming conditions (*Dragičević et al., 2016b*), where low precipitation level and particularly drought could severely decrease grain yield, but in parallel, it could positively affect accumulation of essential mineral elements in barley grain (Figure 2), what additionally, in combination with reduced concentration of phytic acid, could be positively reflected on further bio-availability during digestion.

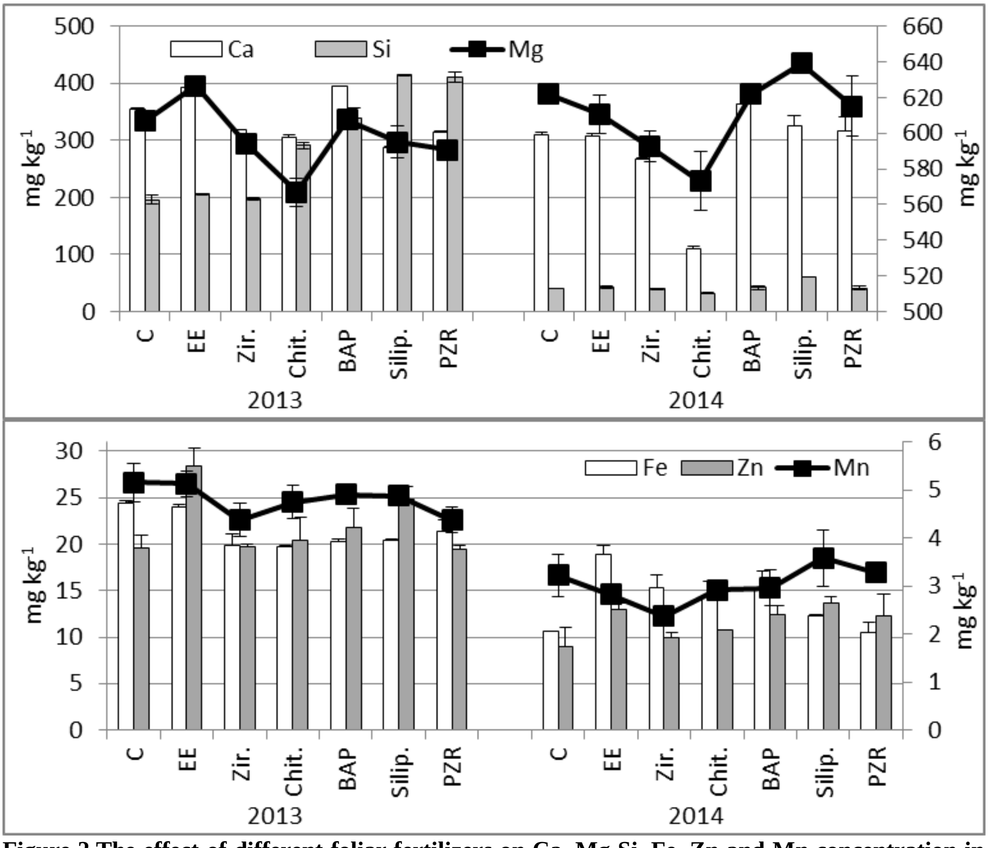


Figure 2.The effect of different foliar fertilizers on Ca, Mg Si, Fe, Zn and Mn concentration in barley grain (C – control, EE – Epin Extra, Zir. – Zircon, Chit. – Chitosan, BAP –

Benzyladenine, Silip. – Siliplant, PZR – Propikonazole); Mean ± SD (standard deviation) (*Dragicevic et al., 2016b*).

Some other practices, included in sustainable agricultural systems could play an important role in the availability and absorption of mineral elements into crop biomass and grains, due to the fact that sustainable systems basically tend to maintain or even increase soil fertility, increase biodiversity, including crop yields and their quality. From this standpoint bio-fertilizers are very important: N fixing bacteria, symbiotic bacteria, fungi, and other microorganisms have pivotal role in metal homeostasis in soils (*González-Guerrero et al., 2014*; *Lehmann and Rillig, 2015*), what could present a great potential for bio-fortification programs. Bio-fertilizers are able to stimulate crop growth, productivity, stress tolerance mechanisms, synthesis of antioxidants and photosynthetic pigments, to enhance of absorption and accumulation of P, Fe, Mn, Zn and Cu (*El–Sirafy et al., 2006; Bhardwaj et al., 2014*). Bio-fertilizers are also able to increase a protein level, parallel with reduction of phytic acid concentration in soybean grains (*Zarei et al., 2012; Hussain et al., 2020*). It is interesting that microbiota that are present in plant roots and animal gut, and are highly responsible for acquisition of mineral elements and some vitamins, evolved in different directions, possible due to the differences in environments, such as oxygen levels, temperature, pH, and organic carbon availability, but there are some similar taxa - overlapping, present in both kingdoms (*Hacquardet al., 2015*), emphasizing importance of further research in microbiota role for plants and animals/humans.

Furthermore, growing of inter- and cover- crops presents unavoidable part of sustainable agricultural systems. By combining of different crops on the same field at the same time, support of different crop species in acquisition of mineral elements was enabled. Phytosiderophores, excreted by maize root help other crops that were grown in combination, like peanut or soybean to absorb and accumulate greater amounts of Mg, Fe, and Zn in grains (*Xiong et al., 2013; Dragicevic et al., 2015b*). In parallel, crop combining could positive reflect on increased synthesis and accumulation of promoters, such as β-carotene (*Dragicevic et al., 2015b*). In experiment with soybean and proso millet, grown in different intercrop combinations it was shown that combinations, particularly with two rows of soybean and four rows of millet (SS-MMMM combination, Figure 3) was responsible for greater Fe accumulation in biomass of both crops, while Zn was greater in soybean biomass (one row of soybean and one row of millet, S-M combination), what was not the case for proso millet, with lower accumulation ability present in all combinations. This refers of potential competitivity between crops, what is an important part, when intercropping design is establishing.

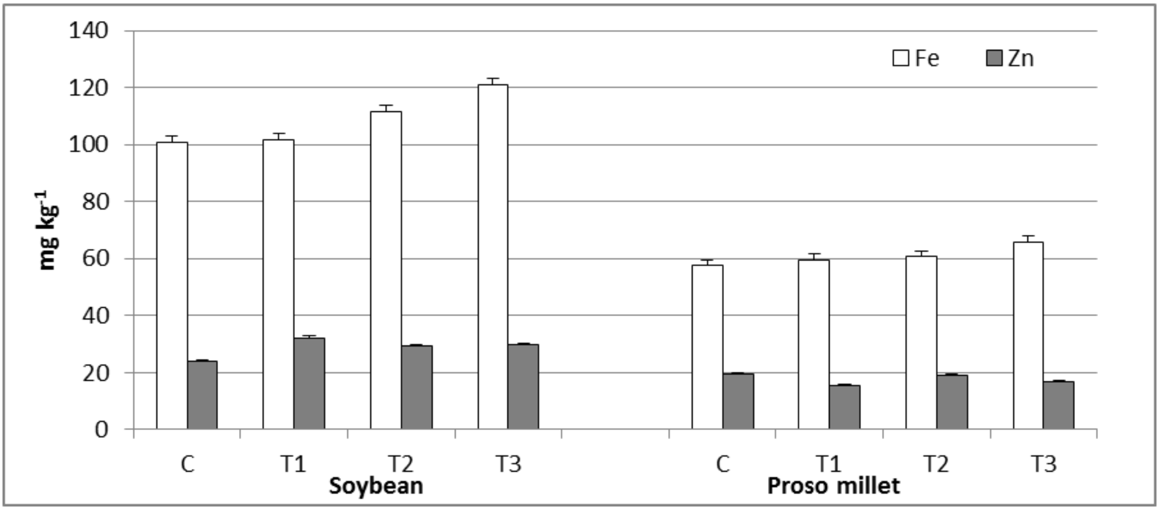


Figure 3.The effect of different soybean and proso millet intercrop combinations on P, S, Fe and Zn accumulation in biomass (C - sole crop, control; T1 - S-M combination, T2 - SS-MM combination and T3 - SS-MMMM combination); S - soybean, M - proso millet: Results present mean ± SD (standard deviation)

Cover crops, that are commonly used as sustainable practice for protection from erosion, leaching of mineral nutrients into soil depth, weed suppression, could be also successfully used for soil enrichment with N (particularly leguminous covers), as well as improved absorption and accumulation of mineral elements and other bioactive compounds, which could serve as promoters (*Baligar et al. 2006; Janošević et al., 2017; Dragicevic et al., 2021*)

Conclusion

Past and still on-going changes and fluctuations in the world, including climate, soil devastation, biodiversity reduction, etc., affected severely agricultural sector, including livestock. From this standpoint, malnutrition is recurring problem, which does not affect human health, but also health and wellbeing of domestic animals. When essential elements, including minerals and vitamins lack from animal diets, i.e. feed, they suffer from various diseases, production is reduced and further livestock products have low quality, and are also unable to meet human requirements for vital nutrients, that could be provided only by animal products. This problem is especially important for domestic animals, since their diets are specific, in regard to humans. Supplementation is not always the best solution, due to the poor bio-availability or even toxicity, since overdosing of ingested amount of some nutrients is hard to control.

Thus, it is important to increase concentration of essential nutrients in crops used for feed, in which nutrients are in highly available forms, in combination with lot of other important nutrients, which could support or suppress bio-availability from animal intestines. The bio-fortification approach, based on development and commercialisation of highly efficient genotypes, as well as agricultural practices that enable and support better absorption and accumulation of essential nutrients is safer option. Mutual increase in concentration of essential minerals, vitamins, and other promoters is of the particular importance for bio-fortification programs, increasing efficiency and success of applied practices, thus positively reflecting on animal health and wellbeing.

Biofortifikacija, kao način proizvodnje nutritivno bogate hrane za domaće životinje

Vesna Dragičević, Milena Simić, Milan Brankov, Milena Šenk, Vesna Krnjaja, Violeta Mandić, BrankaKresović

Rezime

Neishranjenost predstavlja globalni problem, pogađajući i ljude i domaće životinje, paralelno. Zahvaljujući broujnim faktorima, kao što su promena klime, ispošćavanje zemljišta, uticaj antropogenog faktora preko neodgovornog upravljanja zemljištem, visokih unosa mineralnih đubriva baziranih na makro-elementima, u usevima se javlja se nedostatak esencijalnih hraniva, kao što su Mg, Fe, Zn, itd., kao i važnih vitamina, kao što su karotenoidi i vitamini iz B grupe. Takođe, neke obradive površine imaju prirodno nisku plodnost. Sve navedeno se negativno odražava na proizvodnju domaćih životinja, uključujući zdravlje životinja, kao i kvalitet životinjskih proizvoda. Kao odgovor u borbi protiv neishranjenosti, razvijena je strategija bio-fortifikacije, koja se bazira na povećanju koncentracije esencijalnih hraniva u hrani i hranivima, kao i većoj pristupačnosti iz organa za varenje. Bio-fortifikacija koristi različite mere. Dva osnovna tipa bio-fortifikacije su razvijena: genetička i agronomska bio-fortifikacija. Prva koristi stndardne tehnike selekcije, marker asastiranu selekciju, transgene pristupe, editovanje genoma, i dr. u dobijanju vioko-efikasnih genotipova koji su sposobni da apsorbuju i akumuliraju esencijana hraniva u većim koncentracijama u biomasi i zrnu. Agrnonomska bio-fortifikacija koristi različite tehnike, kao što je primena specijalnih đubriva obogaćenih sa esencijalnim mineralima, folijarnih đubriva, hormona i poboljšivača rasta, kao i nekih manje poznatih mera koje se koriste u održivoj poljoprivredi, kao što su kombinovani i pokrovni usevi, koji su prvenstveno namenjeni povećanju prinosa, kao i koncentracije esencijalnih hraniva. Strategija bio-fortifikacije, bazirana na razvijanju i komercijalizaciji visoko efikasnih genotipova, kao i agronomskih tehnika, koje omogućavaju i podržavaju bolje usvajanje i akumulaciju esencijanih hraniva je bezbednija opcija i za ljude i za domaće životinje. Paralelno povećanje koncentracije esentijalnih minerala, vitamina i drugih supstanci koje pomažu usvajanje je od posebnog značaja za programe bio-fortifikacije, povećavajući efikasnost i uspešnost primenjenih mera, i odražavajući se pozitivnona zdravlje i blagostanje domaćih životinja.

Ključne reči: uzgoj, prakse u ratarstvu, mineralni nutrijenti, antinutrijenti, promoteri

Acknowledgment

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-68/2020-14/200040.

References

ANDERSON C.W.N., ROBINSON B.H., WEST D.M., CLUCAS L., PORTMANN D. (2012): Zinc-enriched and zinc-biofortified feed as a possible animal remedy in pastoral agriculture: Animal health and environmental benefits, Journal of Geochemical Exploration, 121, 30-35. https://doi.org/10.1016/j.gexplo.2012.01.009.

BALIGAR V.C., FAGERIA N.K., PAIVA A.Q., SILVEIRA A., POMELLA A.W.V., MACHADO R.C.R. (2006): Light intensity effects on growth and micronutrient uptake by tropical legume cover crops. Journal of Plant Nutrition, 29, 1959-1974.

BHARDWAJ D., ANSARI M.W., SAHOO R.K., TUTEJA N. (2014): Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories, 13, 66.

BORNMAN J.F., BARNES P.W., MATTHEW ROBSON T., S.A. ROBINSON JANSEN M.A.K., BALLARÉ C.L., FLINT S.D. (2019): Linkages between stratospheric ozone, UV radiation and climate change and their implications for terrestrial ecosystems. Photochemical & Photobiological Sciences, 18, 681-716. https://doi.org/10.1039/C8PP90061B

BRANKOV M., SIMIC M., MESAROVIC J., KRESOVIC B., DRAGICEVIC V. (2020): Integrated effects of herbicides and foliar fertilizer on corn inbred line. Chilean Journal of Agricultural Research, 80, 1, 50-60. doi:10.4067/S0718-58392020000100050

CAKMAK I. (2008): Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant and Soil, 302, 1-17.

CAKMAK I., KUTMAN U.B. (2018): Agronomic biofortification of cereals with zinc: a review. European Journal of Soil Science, 69, 1, Special Issue: Including Landmark Papers No. 7, 172-180. https://doi.org/10.1111/ejss.12437

CLEMENS S. (2014): Zn and Fe biofortification: The right chemical environment for human bioavailability. Plant Science, 225, 52–57.

DHALIWAL S.S., SANDHU A.S., SHUKLA A.K., SHARMA V., KUMAR B., SINGH R. (2020): Bio-fortification of oats fodder through zinc enrichment to reduce animal malnutrition. Journal of Agricultural Science and Technology A, 10, 98-108. doi: 10.17265/2161-6256/2020.02.006

DRAGICEVIC V., DOLIJANOVIĆ Z., JANOSEVIC B., BRANKOV M., STOILJKOVIC M., DODEVSKA M., SIMIC M. (2021): Enhanced nutritional quality of sweet maize kernel in response to cover crops and bio-fertilizer. Agronomy, *11*, 5, 981. https://doi.org/10.3390/agronomy11050981

DRAGIČEVIĆ V., KOVAČEVIĆ D., SREDOJEVIĆ .S, DUMANOVIĆ Z., MLADENOVIĆ DRINIĆ S. (2010): The variation of phytic and inorganic phosphorus in leaves and grain in maize populations. Genetika, 42, 555-563.

DRAGIČEVIĆ V., MLADENOVIĆ DRINIĆ S., STOJILJKOVIĆ M., FILIPOVIĆ M., DUMANOVIĆ Z. (2013): Variability of factors that affect availability of iron, manganese and zinc in maize lines. Genetika, 45, 3, 907-920.

DRAGICEVIC V., MLADENOVIC-DRINIC S., STOJILJKOVIC M., FILIPOVIC M., NIKOLIC B., BABIC V., KRAVIC N. (2016): Maize inbreds from different heterotic groups as favorable sources for increased potential bioavailability of magnesium, iron, manganese and zinc. Chilean Journal of Agricultural Research, 76, 2, 213-219.

DRAGIČEVIĆ V., NIKOLIĆ B., WAISI H., STOJILJKOVIĆ M., ĐUROVIĆ S., SPASOJEVIĆ I., PERIĆ V. (2015a): Alterations in mineral nutrients in soybean grain induced by organo-mineral foliar fertilizers. Chemical and Biological Technologies in Agriculture, 2, 12.

DRAGICEVIC V., OLJACA S., STOJILJKOVIC M., SIMIC M., DOLIJANOVIC Z., KRAVIC N. (2015b): Effect of the maize and soybean intercropping system on the potential bioavailability of magnesium, iron and zinc. Crop & Pasture Science, 66, 1118-1127.

DRAGIČEVIĆ V.D., NIKOLIĆ B.R., RADOSAVLJEVIĆ M.M., ĐURIĆ N.A., DODIG D.B., STOILJKOVIĆ M.M., KRAVIĆ N.B. (2016b): Barley grain enrichement with essential elements byagronomic biofortification. Acta Periodica Technologica, 47, 1-9. DOI: 10.2298/APT1647001D

ĐUROVIĆ S., DRAGIČEVIĆ V., WAISI H., PAGNACCO M., LUKOVIĆ N., KNEŽEVIĆ-JUGOVIĆ Z., NIKOLIĆ B. (2019): Enhancement of antioxidant activity and bioactive compound contents in yellow soybean by plant-extract-based products. Archives of Biological Sciences, 71, 3, 425-434. https://doi.org/10.2298/ABS190123024D

ECKHARD G., HORST W.J., NEUMANN E. (2012): Adaptation of plants to adverse chemical soil conditions. In: Marschner P (Eds.) Marschner's Mineral Nutrition of Higher Plants (Third Edition) pp: 409-472.

EL–SIRAFY Z.M., WOODARD H.J., EL–NORJAR E.M. (2006): Contribution of biofertilizers and fertilizer nitrogen to nutrient uptake and yield of Egyptian winter wheat. Journal of Plant Nutrition, 29, 587–599.

FANG Y., WANG L., XIN Z., ZHAO L., AN X., HU Q. (2008): Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients concentration and yield of rice grain in China. Journal of Agricultural and Food Chemistry, 56, 2079–2084.

FARDET A., ROCK E. (2020): Ultra-Processed foods and food system sustainability: What are the links? Sustainability, 12, 6280. doi:10.3390/su12156280

FERNANDO N., PANOZZO J., TAUSZ M., NORTON R., FITZGERALD G., SENEWEERA S. (2012): Rising atmospheric CO_2 concentration affects mineral nutrient and protein concentration of wheat grain. Food Chemistry, 133, 1307–1311.

GARVIN D.F., WELCH R.M., FINLEY W. (2006): Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. The Journal of the Science of Food and Agriculture, 86, 2213–2220.

GÓMEZ M.I., BARRETT C.B., RANEY T., PINSTRUP-ANDERSEN P., MEERMAN J., CROPPENSTEDT A., CARISMA B., THOMPSON B. (2013): Post-green revolution food systems and the triple burden of malnutrition. Food Policy, 42, 129-138. https://doi.org/10.1016/j.foodpol.2013.06.009.

GONZÁLEZ-GUERRERO M., MATTHIADIS A., SÁEZ Á., LONG T.A. (2014): Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. Frontiers in Plant Science, 5, Article 45.

GUERINOT M.L., SALT D.E. (2001): Fortified foods and phytoremediation. Two sides of the same coin. Plant Physiology, 125, 164-167.

GUPTA H.S., HOSSAIN F., MUTHUSAMY V. (2015): Biofortification of maize: An Indian perspective. Indian Journal of Genetics, 75, 1-22.

HACQUARD S., GARRIDO-OTER R., GONZÁLEZ A., SPAEPEN S., ACKERMANN G., LEBEIS S., MCHARDY A.C., DANGL J.L., KNIGHT R.,

LEY R., SCHULZE-LEFERT P. (2015): Microbiota and host nutrition across plant and animal kingdoms. Cell Host & Microbe, 17, 5, 603-616. https://doi.org/10.1016/j.chom.2015.04.009.
HAO H.L., WEI Y.Z., YANG X.E., FENG Y., WU C.Y. (2007): Effects of different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and grain quality in rice (*Oryza sativa*). Rice Science, 14, 289–294.
HART J.J., NORVELL W.A., WELCH R.M., SULLIVAN L.A., KOCHIAN L.V. (1998): Characterization of zinc uptake, binding, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiology, 118, 219–226.
HAYES B.J., LEWIN H.A., GODDARD M.E. (2013): The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. Trends in Genetics, 29, 4, 206-214. https://doi.org/10.1016/j.tig.2012.11.009.
HOCQUETTE J.F. (2010): Endocrine and metabolic regulation of muscle growth and body composition in cattle, Animal, 4, 11, 1797-1809. https://doi.org/10.1017/S1751731110001448.
HUSSAIN A., ZAHIR Z.A., DITTA A., TAHI M.U., AHMAD M., MUMTA M.Z., HAYAT K., HUSSAIN S. (2020): Production and implication of bio-activated organic fertilizer enriched with zinc-solubilizing bacteria to boost up maize (*Zea mays* L.) production and biofortification under two cropping seasons. Agronomy, 10, 39.
IMPA S.M., GRAMLICH A., TANDY S., SCHULIN R., FROSSARD E., JOHNSON-BEEBOUT S.E. (2013): Internal Zn allocation influences Zn deficiency tolerance and grain Zn loading in rice (*Oryza sativa* L.). Frontiers in Plant Science, 4, 534.
JANOŠEVIC B., DOLIJANOVIC Z., DRAGICEVIC V., SIMIC M., DODEVSKA M., DJORDJEVIC S., MORAVCEVI, Dj., MIODRAGOVIC R. (2017): Cover crop effects on the fate of N in sweet maize (*Zea mays* L. *saccharata* Sturt.) production in a semiarid region. International Journal *of* Plant Production,11, 285-294.
KEBREAB E., HANSEN A.V., STRATHE A.B. (2012): Animal production for efficient phosphate utilization: from optimized feed to high efficiency livestock. Current Opinion in Biotechnology, 23, 6, 872-877. https://doi.org/10.1016/j.copbio.2012.06.001.
KEDING G.B., SCHNEIDER, K., JORDAN I. (2013): Production and processing of foods as core aspects of nutrition-sensitive agriculture and sustainable diets. Food Security, 5, 825–846.
LEHMANN A., RILLIG M.C. (2015): Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops - A meta-analysis. Soil Biology and Biochemistry, 81, 147-158.

LIN Y., YE G., KUZYAKOV Y., LIU D., FAN J., DING W. (2019): Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and keystone taxa. Soil Biology and Biochemistry, 134, 187-196. https://doi.org/10.1016/j.soilbio.2019.03.030.

LOLADZE I. (2002): Rising atmospheric CO2 and human nutrition: toward globally imbalanced plant stoichiometry? Trends in Ecology & Evolution, 17, 457-461.

MASUDA H., KOBAYASHI T., ISHIMARU Y., TAKAHASHI M., AUNG M.S., NAKANISHI H., MORI S., NISHIZAWA N.K. (2013): Iron-biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. Frontiers in Plant Science, 4, 1-12.

MCKENZIE F.C., WILLIAMS J. (2015): Sustainable food production: constraints, challenges and choices by 2050. Food Security, 7, 221–233. https://doi.org/10.1007/s12571-015-0441-1

MESAROVIĆ J., SRDIĆ J., MLADENOVIĆ-DRINIĆ S., DRAGIČEVIĆ V., SIMIĆ M., BRANKOV M., MILOJKOVIĆ OPSENICA D. (2019): Evaluation of the nutritional profile of sweet maize after herbicide and foliar fertilizer application. Journal of Cereal Science, 87, 132-137. https://doi.org/10.1016/j.jcs.2019.03.017

MLADENOVIĆ DRINIĆ S., RISTIĆ D., SREDOJEVIĆ S., DRAGIČEVIĆ V., IGNJATOVIĆ MICIĆ D., DELIĆ N. (2009): Genetic variation of phytate and ionorganic phosphorus in maize population. Genetika, 41, 107-115.

MYERS S.S., ZANOBETTI A., KLOOG I., HUYBERS P., LEAKEY A.D.B., BLOOM A.J., CARLISLE E., DIETTERICH L.H., FITZGERALD G., HASEGAWA T., HOLBROOK N.M., NELSON R.L., OTTMAN M.J., RABOY V., SAKAI H., SARTOR K.A., SCHWARTZ J., SENEWEERA S., TAUSZ M., USUI Y. (2014): Increasing CO_2 threatens human nutrition. Nature, 510, 139–142.

NAQVI S., ZHU C., FARRE G., RAMESSAR K., BASSIE L., BREITENBACH J., CONESA D.P., ROS G., SANDMANN G., CAPELL T., CHRISTOU P. (2009): Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proceedings of the National Academy of Sciences of the United States of America, 106, 7762–7767.

OIKEH S.O., MENKIR A., MAZIYA-DIXON B., WELCH R.M., GLAHN R.P., GAUCH J.R.G. (2004): Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. The Journal of Agricultural Science, 142, 543–551.

PARIZA M.W., COOK M. (2010): Determining the safety of enzymes used in animal feed. Regulatory Toxicology and Pharmacology, 56, 3, 332-342. https://doi.org/10.1016/j.yrtph.2009.10.005.

RABOY V. (2013): The future of crop breeding for nutritional quality. The SABRAO Journal of Breeding and Genetics, 45, 100-111.

ROSE T.J., IMPA S.M., ROSE M.T., PARIASCA-TANAKA J., MORI A., HEUER S., JOHNSON-BEEBOUT S.E., WISSUWA M. (2013): Enhancing phosphorus and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. Annals of Botany, 112, 331–345.

ROUPHAEL Y., CARDARELLI M., SCHWARZ D., FRANKEN P., COLLA G. (2012): Effects of drought on nutrient uptake and assimilation in vegetable crops. In: Aroca R. (eds.) Plant Responses to Drought Stress. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-32653-0_7

SCHIAVON M., PILON-SMITS E.A.H. (2017): Selenium biofortification and phytoremediation phytotechnologies: A Review. Journal of Environmental Quality, 46, 1, 10-19. https://doi.org/10.2134/jeq2016.09.0342

SHIVAY Y., PRASAD R. (2012): Zinc-coated urea improves productivity and quality of basmati rice (*Oryza sativa* L.) under zinc stress condition. Journal of Plant Nutrition, 35, 928–951.

ŠIMIĆ D., MLADENOVIĆ DRINIĆ S., ZDUNIĆ Z., JAMBROVIĆ A., LEDENCAN T., BRKIĆ J., BRKIĆ A., BRKIĆ I. (2012): Quantitative trait loci for biofortification traits in maize grain. Journal of Heredety, 103, 47-54.

SLAMET-LOEDIN I.H., JOHNSON-BEEBOUT S.E., IMPA .S, TSAKIRPALOGLOU N. (2015): Enriching rice with Zn and Fe while minimizing Cd risk. Frontiers in Plant Science, 6, 121.

SOETAN K.O., OLAIYA C.O., OYEWOLE O.E. (2010): The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science, 4, 200-222.

SUWARNO W.B., PIXLEY K.V., PALACIOS-ROJAS N., KAEPPLER S.M., BABU R. (2015): Genome-wide association analysis reveals new targets for carotenoid biofortification in maize. Theoretical and Applied Genetics, 128, 851-864.

TIONG J., MCDONALD G., GENC Y., SHIRLEY N., LANGRIDGE P., HUANG C.Y. (2015): Increased expression of six *ZIP* family genes by zinc (Zn) deficiency is associated with enhanced uptake and root-to-shoot translocation of Zn in barley (*Hordeum vulgare*). New Phytologist, 207, 1097-109.

TIRADO M.C., CLARKE R., JAYKUS L.A., MCQUATTERS-GOLLOP A., FRANK J.M. (2010): Climate change and food safety: A review. Food Research International, 43, 1745–1765.

TITCOMB T.J., TANUMIHARDJO S.A. (2019): Global Concerns with B Vitamin statuses: Biofortification, fortification, hidden hunger, interactions, and toxicity. Comprehensive Reviews in Food Science and Food Safety, 18, 6, 1968-1984. https://doi.org/10.1111/1541-4337.12491

VIOLANTE A., COZZOLINO V., PERELOMOV L., CAPORALE A.G., PIGNA M. (2010): Mobility and bioavailability of heavy metals and metalloids in soil environments. Journal of Soil Science and Plant Nutrition, 10, 268 – 292.
WANG X., de SOUZA M.F., LI H., TACK F.M.G., OK Y.S., MEERS E. (2021): Zn phytoextraction and recycling of alfalfa biomass as potential Zn-biofortified feed crop. Science of The Total Environment, 760, 143424. https://doi.org/10.1016/j.scitotenv.2020.143424.
WELCH R.M. (2005): Biotechnology, biofortification, and global health. The Food and Nutrition Bulletin, 26, 419-421.
WELCH R.M. (2008): Linkages between trace elements in food crops and human health. In Micronutrient Deficiencies in Global Crop Production; Alloway, I.B.J., Ed.; Springer: London, UK, pp. 287–309.
WELCH R.M., GRAHAM R.D. (2005): Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. The Journal of Trace Elements in Medicine and Biology,18, 299-307.
WHITE P.J., BROADLEY M.R. (2009): Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytologist, 182, 49–84.
WU Z., BAÑUELOS G.S., LIN Z.Q., LIU Y., YUAN L., YIN X., LI M. (2015): Biofortification and phytoremediation of selenium in China. Frontiers in Plant Science, 6, 136.
XIONG H., KAKEI Y., KOBAYASHI T., GUO X., NAKAZONO M., TAKAHASHI H., NAKANISHI H., SHEN H., ZHANG F., NISHIZAWA N.K., ZUO Y. (2013): Molecular evidence for phytosiderophore-induced improvement of iron nutrition of peanut intercropped with maize in calcareous soil. Plant, Cell and Environment, 36, 1888–1902.
ZAREI I., SOHRABI Y., HEIDARI G.R., JALILIAN A., MOHAMMADI K. (2012): Effects of biofertilizers on grain yield and protein content of two soybean (*Glycine max* L.) cultivars. African Journal of Biotechnology, 11, 7028–7037.
ZENG N., ZHAO F., COLLATZ G. KALNAY E., SALAWITCH R.J., WEST T.O., GUANTER L. (2014): Agricultural Green Revolution as a driver of increasing atmospheric CO_2 seasonal amplitude. Nature, 515, 394–397. https://doi.org/10.1038/nature13893

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

ALFALFA AND RED CLOVER AS A PROTEIN SOURCE FOR RUMINANTS

Jordan Marković, Jasmina Milenković, Snežana Anđelković

Institute for Forage Crops Kruševac, 37251 Globoder
Corresponding author: Jordan Marković, jordan.markovic@ikbks.com
Invited paper

Abstract: High level of milk protein, increased dietary costs, as well as care for the environment have made nitrogen utilization a central component in balancing ruminant meals. Excess crude protein in meals can lead to unnecessary dietary costs, without resulting in increased milk and milk protein production. In addition, most of the excess nitrogen is excreted in the urine and is an ecologically labile form. On the other hand, poor amino acids balance will limit milk protein yield, making production more expensive than overeating. Balancing protein according to metabolic needs, with an adequate ratio of rumen undegradable protein and rumen degradable protein, and without overeating will have positive effects not only on meal price and profitability, but also on the environment. More efficient utilization of nutrients requires knowledge of how different fractions of nutrients pass through the digestive tract. Grass and legumes contain the largest and most variable part of non-protein nitrogen compounds. Although, a number of non-protein nitrogen compounds and true protein together represent crude protein, it is clear that the nutritional value of crude protein in ruminant feeds is better explained based on their rate and extent of rumen degradation. There are a number of factors that affect the amount of crude protein that will be degraded in the rumen. Legumes are high quality forages rich in protein, but these proteins are intensively degraded in the rumen. Red clover proteins are more slowly degraded than alfalfa protein.

Key words: alfalfa, red clover, protein degradability

Introduction

Alfalfa and red clover are the main source of protein *(Marković et al., 2010; 2011; 2012a; 2012b)*, and are the main constituents of ruminant meals. They occupy this place due to the ability of biological fixation of nitrogen and due to their high nutritional value. These legumes are important because they significantly reduce production costs – less use of nitrogen mineral fertilizers and a smaller ratios of concentrated nutrients in ruminant meals. These benefits and the possibility of feeding farm animals based on diets on forage crops have attracted the attention of numerous researchers *(Buxton et al., 1985; Broderick and Buxton, 1991; Broderick, 1995; Rochon et al., 2004; Peyraud et al., 2009).*

In ruminant nutrition, regulating the degradation of structural and non-structural carbohydrates and protein reduces unnecessary losses, primarily protein, which is very important in a sustainable production system. The place of digestion and the ratio in which volatile fatty acids and lactic acid are formed largely determine the rate of degradability and the characteristics of fermentation in the rumen. Both of these characteristics are important because they directly affect the type of nutrients that will be available to the animals. In the modern nutrition system, detailed information on the rate of degradation and the nature and type of fermentation processes of all nutrient components are becoming increasingly important *(Yu et al., 2004)*. Numerous studies have shown that the yield and nutritional value of forages depend on the stage of development, the forage species *(Jung and Allen, 1995; Ayres et al., 1998; Elizalde et al., 1999; Ferdinandez and coulman, 2001; Lyon et al., 2001)* or varieties *(Tremblay et al., 2000; 2002; 2003)*, soil type *(Aumont and Salas, 1996)* and climatic conditions *(Mathison et al., 1996)*.

In addition to the fact that legumes are characterized by high nutritional value, their proteins are subject to rapid and intensive degradation in the rumen. Consequently, the rate of protein degradation in the rumen directly affects the efficiency of nitrogen utilization by animals *(Broderick, 1995)*. Some authors indicate that both rumen microorganisms and proteases in plant material cause inefficient utilization of nitrogen in animals *(Kingston-Smith et al., 2003)*. Generally speaking, non-utilization of nitrogen is the result of an imbalance between protein and carbohydrates in ruminant meals, which increases the risk of inadequate nitrogen utilization and its excretion into the environment *(Tamminga, 2006)*. To avoid these losses and contamination of the environment, meals must be formulated to satisfy, but not exceed the amounts of nitrogen necessary for microorganisms growth, as well as a sufficient amount of amino acids necessary for the normal functions of the animal body *(Schwab et al., 2005)*. Modern nutrition systems, in order to minimize nitrogen losses, include knowledge of the amount and ratio of degradable and non-degradable protein in the rumen, as well as the necessary amounts of nitrogen for sufficient development of rumen microorganisms *(Lanzas et al., 2007; 2008)*.

Crude protein fractionation was performed according to CNCPS (Cornell Net Carbohydrates and Protein System) *(Lanzas et al., 2007)*. According to this system of analysis, crude protein can be divided into 3 fractions: fraction A represents non-protein nitrogen (NPN x 6.25), fraction B represents a true protein and fraction C is unavailable protein. Fraction B is further divided into 3 subfractions (B_1, B_2 and B_3) which are assumed to have different rates of degradation in the rumen. Fraction A and B_1 dissolve in the borate-phosphate buffer and degrade very rapidly in the rumen. Fraction B_2 is insoluble in the buffer, but is soluble in the neutral detergent solution with medium rate of degradation in the rumen. The B_3 fraction is insoluble in buffer and neutral detergent solution, but is soluble in acid detergent solution. This protein fraction degraded very slowly in the rumen because it is associated with the plant cell wall. Fraction C consists of proteins that do not dissolve in acid detergent solution. This fraction contains proteins that are associated with lignin, tannins and the products of Millards reactions that are highly resistant to the action of microbiological enzymes. All of these protein fractions are present in the feeds and have an impact on animal performance *(Alzueta et al., 2001).*

Alfalfa proteins in ruminant nutrition

Alfalfa is an important source of protein for ruminants, but these proteins are often poorly utilized due to intensive degradation during ruminal fermentation. Protein degradation is a limiting factor for high quality legumes, such as alfalfa. The protein quality of alfalfa can be improved by increasing the proportion of rumen undegradable protein – RUP *(Tremblay et al., 2002*).

Rapid and intensive protein degradation leads to inefficient utilization of protein from meals. These processes may be the main limiting factors when legumes are used in the diet *(Broderick, 1995).* The most favorable situation for animals is when the proteins go directly to the small intestine where they are broken down into amino acids and assimilated. *Broderick and Buxton (1991)* found variations in protein degradability between 19 cultivars of *Medicago sativa* L. and 3 cultivars of *Medicago falcata* L. *Griffin et al. (1994)* also found a difference between alfalfa varieties, but they were contradictory during harvesting and years of investigations. *Tremblay et al. (2000)* examined differences between 27 alfalfa cultivars in rumen undegradable protein (RUP) content, degradation rate and dry matter yield and found small genetic variations between cultivars, but concluded that it was possible to combine high dry matter yields and low protein degradation rates.

In the Table 1 are presented the results obtained in the investigations conducted by *Marković et al (2014)*. The results indicate that the alfalfa cut at mid-bloom stage in the third cut had the highest rapidly degradable PA fraction of CP. On the other hand, the highest content of undegradable PC fraction, associated with the lignin and cell wall was observed at full bloom stage in the fourth cut.

Table 1. Content of crude protein fractions in alfalfa, *cv* K 28 by CNCPS, g kg^{-1} CP *(Marković et al., 2014)*

Cut	PA	PB	PB_1	PB_2	PB_3	PC
I	346.3^{c}	597.7^{a}	0.0^{b}	549.5^{a}	48.1^{a}	55.9^{d}
II	323.8^{c}	585.7^{a}	19.3^{a}	536.1^{a}	29.9^{b}	90.7^{b}
III	503.1^{a}	426.3^{c}	0.0^{b}	419.4^{b}	7.0^{c}	70.4^{c}
IV	370.8^{b}	494.3^{b}	0.0^{b}	434.5^{b}	59.9^{a}	134.7^{a}

PA – non-protein nitrogen; PB_1 – rapidly degraded crude protein; PB_2 – intermediately degraded crude protein; PB_3 – slowly degraded crude protein; PC – bound, unavailable crude protein Different letters denote significantly different means ($P< 0.01$)

In the investigation conducted by *Hoffman et al. (1993)* the content of these protein fractions were examined at the late vegetative stage, late budding and mid-flowering stages of growth in alfalfa and red clover. Alfalfa was found to contain 28.5, 41.1 and 30.8% of PB fraction, respectively, whereas red clover contained 42.2, 49.7 and 53.8% of PB fraction, respectively. *Yari et al. (2012)* reported that during the day PA fraction had a tendency to decrease, and PB fraction tends to increase. These authors concluded that during the day alfalfa leaves use photosynthetic products to convert PA components into true protein.

In the investigation conducted by *Michaud et al. (2001)* the PB_2 fraction in the whole alfalfa plant was 49.4% CP, in leaves 62.5% CP and in the stem 40.5% CP. It can be concluded that the proportion of leaves in DM of alfalfa can significantly affect the content of PB_2 fraction of crude protein. It indicates that with the growth and development of leguminous plants the degradability of protein decreases with a decrease in CP content and probably other quality parameters.

Hoffman et al. (1993) found that alfalfa protein degraded more rapidly than red clover protein. A negative correlation was found between the degradability of alfalfa dry matter and the proportion of rumen undegradable protein. On the other hand, *Yari et al. (2012)* reported that PB_2 fraction had lower values at the beginning of alfalfa flowering compared to the budding stage of plant development. The same authors indicated that the PB_2 was the largest protein fraction in alfalfa, whereas *Yu et al. (2003)* found out that the largest alfalfa protein fraction was PB_3. *Tremblay et al. (2002)* found the proportion of this fraction in the first alfalfa cut was 494.0 g kg^{-1} CP.

Red clover proteins in ruminant nutrition

There is an increased interest in studying those leguminous species that reduce proteolytic processes during ensiling and reduce protein degradation in the rumen. It is important to mention that leguminous species may contain compounds that can reduce protein degradability. Leguminous species that contain protein and tannin complexes, such as birdsfoot trefoil, have the property that their proteins are less degradable, compared to species that do not contain tannins, such as alfalfa *(Julier et al., 2003)*. In contrast to alfalfa, red clover has a very low level of condensed tannins *(Jackson et al., 1996; Grabber, 2009)*, but the proportion of degradable protein is lower than in alfalfa.

This property of red clover can be explained by the presence and activity of polyphenol oxidase in red clover *(Broderick et al., 2001; 2004)*. Polyphenol oxidases are enzymes that catalyze the hydroxylation of monophenol to ortho-diphenol and the oxidation of ortho-diphenol to ortho-quinone *(Matheis and Whitaker, 1984)*. The quinones are strong oxidants and very strong electrophiles, which, passing through various biochemical reactions, give numerous secondary products. Orthoquinones can react with each other or with proteins and amino acids *(Bittner, 2006; Parveen et al., 2010)*. These ortho-quinone reactions with proteins are thought to lead to reduced proteolytic processes in red clover silage *(Sullivan and Hatfield, 2006)*. In contrast, the effects of more efficient nitrogen utilization, such as higher milk protein content and lower nitrogen excretion have not been reported in recent studies in cows fed fresh red clover with varying degrees of polyphenol oxidase activity *(Lee et al., 2009)*.

Table 2 illustrates the quantitative effect of a variety of alfalfa and red clover taken at mid-bloom stage in the first cut on the protein fractions by CNCPS *(Marković et al., 2015a)*. Although high variability between alfalfa and red clover cultivars, mean values indicated that the slowly degradable PB_3 fraction associated with the plant cell wall and the intermediately degradable PB_2 fraction were higher in red clover than in alfalfa, whereas mean values for PA fraction was higher in alfalfa than in red clover.

Table 2. Content of CP fraction in alfalfa and red clover by CNCPS, g kg^{-1} CP *(Marković et al., 2015a)*

Cultivars	PA	PB_1	PB_2	PB_3	PC
K – 28	429.2^{a}	68.7^{a}	271.0^{b}	137.2^{a}	93.6^{b}
G + 13R + CZ	421.6^{b}	33.5^{b}	410.6^{a}	26.6^{b}	107.6^{a}
Mean	**425.4**	**51.1**	**340.8**	**81.9**	**100.6**
K - 32	365.7^{ns}	25.3^{a}	447.3^{a}	72.4^{b}	89.7^{b}
K – 39	359.7^{ns}	0.0^{b}	399.4^{b}	199.2^{a}	115.4^{a}
Mean	**362.7**	**12.7**	**423.4**	**135.8**	**102.6**

K 28 – alfalfa, Serbian cv, G + 13R + CZ – alfalfa, American cv; K 32 – tetraploid red clover; K 39 – diploid red clover; PA – non-protein nitrogen; PB_1 – rapidly degraded crude protein; PB_2 – intermediately degraded crude protein; PB_3 – slowly degraded crude protein; PC – bound, unavailable crude protein; Different letters denote significantly different means ($P< 0.05$)

Krawutschke et al. (2013) in three-year trials of alfalfa and red clover found that the rate of PA fraction increased during the spring growing season in all examined years. In these years, during the spring season, the rate of this fraction ranged from 144.0 to 602.0 g kg^{-1} CP. In contrast, in the summer-autumn period, the PA fraction decreased with growth and development. The decrease in the rate of PA fraction from the beginning of flowering and during development is probably a consequence of the fact that this type of compound is less assimilated, and on the other hand, most of the assimilated has already undergone metabolic changes. When the plants begin to bloom, the processes of remobilization of the accepted nitrogen into the generative parts of plants are activated, and as a consequence of such processes the rate of PA protein fraction decreases, and after the beginning of flowering, the PA fraction starts to decrease.

Marković et al. (2015b) investigated the content of crude protein and protein fractions of tetraploid and diploid red clover harvested at mid-bloom stage of growth in the first, second and third cut. These authors found out that tetraploid cultivar of red clover was higher in the content of soluble protein and non-protein nitrogen, but lower in the content of neutral detergent insoluble crude protein and true protein, indicating large and potential differences in rumen CP degradation characteristics between tetraploid and diploid red clover cultivars. From this point of view, the most important considerations of feed CP chemistry are the concentration of NPN and the true protein, and the physical and chemical characteristics of proteins that comprise the true protein fraction of the feedstuffs.

Krawutschke et al. (2012) pointed out that red clover is less susceptible to proteolysis than alfalfa, which is associated with polyphenol oxidase activity in red clover. These authors established values for this fraction of crude protein in a green matter of red clover from 302.6 to 316.3 g kg^{-1} CP, while in dry matter these values ranged from 321.5 to 335.4 g kg^{-1} CP. In red clover silage, these values ranged from 258.0 to 263.0 g kg^{-1} CP harvested in the first cut, 240 to 247.0 g kg^{-1} CP harvested in the second cut, and from 251.0 to 274.0 g kg^{-1} CP harvested in the third cut.

Leaf and stem dry matter digestibility and ruminal protein degradability

The optimal ratio of rumen degradable protein and rumen undegradable protein in forages and increasing dry matter digestibility are the two most important criteria for improving the quality of legumes. *Broderick (1995)* reported that the proportion of rumen undegradable protein was higher in the leaves than in the stems, and that the proportion of these protein fractions varies depending on the species and genotypes. Because 2/3 of the protein is in the leaves, *Broderick and Buxton (1991)* concluded that selection based on leaf protein can give much better results. In addition, genetic variability in leaf to stem ratio between genotypes may also affect the proportion of rumen undegradable protein throughout the plants.

The nutritional value of alfalfa can also be improved by increasing the digestibility of dry matter. Digestibility decreases with advancing plant development due to an increase in the proportion of cell walls in the stems, a decreae in the digestibility of the stem, and the proportion of leaves.

Digestibility is one of the most important characteristics of forages. The digestibility of the stem in the spring ranged from 547 to 579 g kg^{-1} DM, while in the summer it was from 536 to 563 g kg^{-1} DM *(Tremblay et al., 2002).* The results of those studies confirmed the observation that selection for better digestibility can be achieved by selection for better digestibility of the stem, but the interaction between dry matter yield and nutritional value can not be ignored, because yield is a significant factor in the utilization of any nutrient. The ratio of the stem in the dry matter yield and nutritive value of stem during the summer are generally negatively correlated.

A truly comprehensive measure of forage quality should not only predict the quantity of digestible energy and protein consumed, but should also measure the synchrony between energy and protein fermentation in the rumen. Two major voids exist in the literature: fermentation patterns of different forage carbohydrates, and proteolytic degradation patterns of forage proteins. Our future goal should be to optimize the nutritional value of forage-based diets for ruminants. To achieve this goal, it is necessary to focus on maximizing microbial efficiency. Once achieved, greater animal productivity should result through increases in forage intake and digestion *(Paterson et al., 1994).*

Conclusions

Knowing the proportion and rate of degradable protein in the rumen is very important when formulating meals for dairy cows. Protein degradability depends on the type of forages, the stage of development, the method of preservation and the season of investigation. The degradability of protein from forages in the rumen depends primarily on the degradation of individual protein in the rumen, as well as on the passage of all protein through the rumen. Both of these factors depend on protein are linked to other nutrient components and peptide bonds are accessible to enzymes.

Excess nitrogen in the diet can lead to soil and water pollution. To avoid environmental contamination, animal meals must be formulated to satisfy, but not exceed the amounts of nitrogen necessary for the growth of microorganisms, as well as the sufficient amount of amino acids necessary for the normal functioning of the animal. Modern nutrition systems, in order to minimize nitrogen losses, imply knowledge of the essential degradable and undegradable protein, as well as the necessary amounts of nitrogen for sufficient development of microorganisms.

According to the original scheme, high degradability of the PB_2 fraction and almost complete degradation of soluble PA and PB_1 fractions are predicted. For many nutrients, fraction B_2 is the largest protein fraction. In addition, fraction PB_1 makes up a very small fraction of completely soluble protein, and most of the soluble protein belong to fraction A, which is thought to be immediately and almost completely converted to ammonia.

Further improvements in protein degradation characteristics are required in order to develop a higher quality alfalfa cultivar.

Selecting alfalfa plants based on protein structure or protein composition might offer new ways to develop alfalfa with a reduced ruminal degradation rate.

Proanthocyanidins are also known to reduce the rate and extent of ruminal protein degradation.

Acknowledgements

This research was financed by the Ministry of Education, Science and Technological development of Republic of Serbia, project ref. Number 451-03-9/2021-14/200217.

Alfalfa and red clover as a protein source for ruminants

Jordan Marković, Jasmina Milenković, Snežana Anđelković

Rezime

Visok nivo proteina u mleku, povećani prehrambeni troškovi, kao i briga za životnu sredinu učinili su upotrebu azota centralnom komponentom u balansiranju obroka za preživare. Prekomerna količina sirovih proteina u obrocima može dovesti do nepotrebnih prehrambenih troškova, a da ne rezultira povećanom proizvodnjom mleka i mlečnih proteina. Pored toga, većina viška azota se izlučuje urinom i predstavlja ekološki labilan oblik. S druge strane, loša ravnoteža aminokiselina ograničiće prinos mlečnih proteina, čineći proizvodnju skupljom od prekomernog konzumiranja hrane. Uravnotežavanje proteina u skladu sa metaboličkim potrebama, sa odgovarajućim odnosom proteina koji se ne može razgraditi u buragu i proteina koji se razgrađuje u buragu, i bez prekomernog konzumiranja hrane imaće pozitivne efekte ne samo na cenu obroka i isplativost, već i na životnu sredinu. Efikasnije korišćenje hranljivih sastojaka zahteva znanje o tome kako različite frakcije hranljivih sastojaka prolaze kroz probavni trakt. Trave i mahunarke sadrže visok udeo neproteinskih azotnih jedinjenja, čija količina zavisi od brojnih faktora. Iako neproteinska azotna jedinjenja i pravi proteini zajedno predstavljaju sirove proteine, jasno je da se hranljiva vrednost sirovih proteina u hrani za hranu preživara bolje objašnjava na osnovu njihove brzine i stepena razgradnje u buragu. Brojni su faktori koji utiču na količinu sirovih proteina koji će se razgraditi u buragu. Mahunarke su visokokvalitetna krma bogata proteinima, ali se ovi proteini intenzivno razgrađuju u buragu. Proteini crvene deteline sporije se razgrađuju od proteina lucerke.

Ključne reči: lucerka, crvena detelina, razgradivost proteina

References

ALZUETA C., CABALLERO R., REBOLĖ A., TRAVIŇO J., GIL A. (2001): Crude protein fractions in common vetch (*Vicia sativa* L.) fresh forage during pod filling. Journal of Animal Sciences, 79, 2449-2455.

AUMON, G., SALAS M. (1996): Effect of maturity stage, cultivar, nitrate fertilization, soil type on mineral content of sugarcane. Journal of Agriculture, 80, 37-46.

AYRES J. F., NANDRA K. S., TURNER A. D. (1998): A study of the nutritive value of white clover (*Trifolium repens* L.) in relation to different stages of phonological maturity in the primary growth phase in spring. Grass and Forage Science, 53, 250-259.

BITTNER S. (2006): When quinones meet amino acids: chemical, physical and biological consequences. Amino acids, 30, 205-224.

BRODERICK G. A., ALBRECHT K. A., OWENS V. N., SMITH R. R. (2004): Genetic variation in red clover for rumen protein degradability. Animal Feed Science and Technology, 113, 157-167.

BRODERICK G. A., WALGENBACH R. P., MAIGNAN S. (2001): Production of lactating dairy cows fed alfalfa or red clover silage at equal dry matter or crude protein contents in the diet. Journal of Dairy Science, 84, 1728-1737.

BRODERICK G. A. (1995): Desirable characteristics of forage legumes for improving protein utilization in ruminants. Journal of Animal Science, 73, 2760-2773.

BRODERICK G. A., BUXTON D. R. (1991): Genetic variation in alfalfa for ruminal protein degradability. Canadian Journal of Plant Science, 71, 755-760.

BUXTON D. R., HORNSTEIN J. S., WEDIN W. F., MARTEN G. C. (1985): Forage quality in stratified canopies of lucerne, birdsfoot trefoil and red clover. Crop Science, 25, 273-279.

ELIZALDE J. C., MERCHEN N. R., FAULKNER D. B. (1999): Fractionation of fiber and crude protein in fresh forages during spring growth. Journal of Animal Scienece, 77, 476-484.

FERDINANDEZ Y. S. N., COULMAN B. E. (2001): Nutritive values of smooth bromegrass, meadow bromegrass and meadow x smooth bromegrass hybrids for different plant parts and growth stages. Crop Sciuence, 41, 473-478.

GRABBER J. H. (2009): Protein fractions in forage legumes containing protein-binding polyphenols: freeze drying *vs* conservation as hay or silage. Animal Feed Science and Technology, 151, 324-329.

GRIFFIN T. S., CASSIDA K. A., HASTERMAN O. B., RUST S. R. (1994): Alfalfa maturity and cultivars effects on chemical and in situ estimates of protein degradability. Crop Science, 23, 1654-1661.

HOFFMAN P. C., SIENERT S. J., SHAKER R. D., WELCH D. A., COMBS D. A. (1993): In situ dry matter, protein and fiber degradation of perennial forages. Journal of Dairy Science, 76, 2632-2643.

JACKSON F. S., WENABB W. C., BERRY T. N., FAO Y. L., PETERS J. S. (1996): The condensed tannin content of a range of subtropical and temperate forages and the reactivity of condensed tannin with ribulose 1-5-bis-phosphate carboxylase (Rubisco) protein. Journal of Animal Food and Agriculture, 72, 483-492.

JULIER B., GUINES F., EMILE J. C., HUYGHE C. (2003): Variation in protein degradability in dried forage legumes. Animal Research, 52, 401-412.

JUNG H. G., ALLEN M. S. (1995): Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science, 73, 2774-2790.

KINGSTON-SMITH A. H., BOLLARD A. L., ARMSTEAD I. P., THOMAS B. J., THEODOROU M. K. (2003): Proteolysis and cell death in clover leaves in induced by grazing. Protoplasma, 220, 119-129.

KRAWUTSCHKE M., KLEEN J., WEIHER N., LAGES R., TAUBE F., GIERUS M. (2013): Changes in crude protein fractions of forage legumes during the spring growth and summer regrowth period. Journal of Agricultural Science, 151, 72-90.

KRAWUTSCHKE M., WEIHER N., GIERUS M., THAYSEN J., TAUBE F. (2012): The effect of cultivar on crude protein fraction of fresh, wilted and ensiled red clover. Grassland Science in Europe, 16, 256-258.

LANZAS C., BRODERICK G. A., FOX D. G. (2008): Improved feed protein fractionation schemes for formulating rations with the Cornell Net Carbohydrate and Protein System. Animal Feed Science and Technology, 91, 4881-4891.

LANZAS C., TEDESCHI L. O., SEO S., FOX D. G. (2007): Evaluation of protein fractionation systems used in formulating rations for dairy cattle. Journal of Dairy Science, 90, 507-521.

LEE M. R., THEOBALD V. J., TWEED J. K. S., WINTERS A. L., SCOLLAN N. D. (2009): Effect of feeding fresh or conditioned red clover on milk fathy acids and nitrogen utilization in lactating dairy cows. Journal of Dairy Science, 92, 1136-1147.

LYON D. J., BALTENSPERGER D. D., SILES M. (2001): Wheat grain and forage yields are affected by planting and harvest dates in the central Great Pains. Crop Science, 41, 488-492.

MARKOVIĆ J., ŠTRBANOVIĆ R., TERZIĆ D., POJIĆ M., VASIĆ T., BABIĆ S. (2010): Relative feed value of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) at different steg of growth. Proceedings of XII International Symposium on Forage Crops of Republic of Serbia, 26-28 May, Kruševac-Serbia, Biotechnology in Animal Husbandry, spec. issue, 26 (2), 469-474.

MARKOVIĆ J., ŠTRBANOVIĆ R., TERZIĆ D., STANISAVLJEVIĆ R., ĐOKIĆ D., VASIĆ T., ANĐELKOVIĆ B. (2011): Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the stage of growth. Proceedings of the 3th International Congress „New Perspectives and Challenges of Sustainable Livestock Production“, 5-7 October, Belgrade, Serbia, Biotechnology in Animal Husbandry, 27 (4), 1563-1569.

MARKOVIĆ J., GRUBIĆ G., TERZIĆ D., DINIĆ B., BLAGOJEVIĆ M. (2012a): Quality and production ability of diploid and tetraploid red clover cultivars. Proceedings of the 1st International Symposium on Animal Science, 8-10 November, Belgrade, Serbia, 504-510.
MARKOVIĆ J., ŠTRBANOVIĆ R., PETROVIĆ M., DINIĆ M., BLAGOJEVIĆ M., MILIĆ D., SPASIĆ N. (2012b): Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the cut, stage of growth and cultivar. Agroznanje, 13 (1), 31-38.
MARKOVIĆ J., DINIĆ B., TERZIĆ D., BLAGOJEVIĆ M., ANĐELKOVIĆ B., ZORNIĆ V., VASIĆ T. (2014): Effect of consecutive cut and vegetation stage on CNCPS protein fraction in alfalfa (*Medicago sativa* L.). Proceedings of the International Symposium on Animal Science, 23-25 September, Belgrade, Serbia, 290-294.
MARKOVIĆ J., DINIĆ B., BLAGOJEVIĆ M., ANĐELKOVIĆ B., BABIĆ S., PETROVIĆ M., TERZIĆ D. (2015a): Effects of alfalfa and red clover cultivars on protein fractions by CNCPS system of analyzis. Proceedings of the 4th International Congress, New Perspectives and Challenges of Sustainable Livestock Production, October 7-9, Belgrade, Serbia, 796-802.
MARKOVIĆ J., DINIĆ B., VASIĆ T., MILENKOVIĆ J., ANĐELKOVIĆ S., TERZIĆ D., BLAGOJEVIĆ M. (2015b): Characteristics of red clover dry matter for improving nitrogen utilization in ruminants. Book of Proceedings of Sixth International Agricultural Symposium „Agrosym 2015“, 15-18 October, Jahorina, Bosnia and Herzegovina, 1659-1664.
MATHEIS G., WHITAKER J. R. (1984): Modifications of proteins by polyphenol oxidase and peroxidase and their products. Journal of Food Biochemistry, 8, 137-162.
MATHISON R. D., SHEAFFER C. C., RABAS D. L., SWANSON D. R., HALGERSON J. H. (1996): Early spring cipping and herbicide treatments delay alfalfa maturity. Journal of Production and Agriculture, 9, 505-509.
MICHAUD R., TREMBLAY G. F., BELANGER G., MICHAUD J. (2001): Crude protein degradation in leaves and stems of alfalfa (*Medicago sativa* L.). In: Delgado I. (Ed.) lloveras, J. (Ed.) Quality in Lucerne and medics for animal production Zaragoza, CIHEAM, 211-214.
PATERSON J. A., BELYEA R. L. BOWMAN J. P., KERLEY M. S., WILLIAMS J. E. (1994): The impact of forage quality and supplementation regimen on ruminant animal intake and performance. In: Forage quality, evaluation and utilization (eds. Fahey, G., Collins, M., Mertens, D. R.,

Masser, L. E.). American Society of Agronomy, Inc. Madison, Wisconsin, USA.

PARVEEN I., THREADGILL M. D., MOORBY J. M., WINTERS A. (2010): Oxidative phenols in forage crops containing polyphenol oxidase enzymes. Journal of Agricultural and Food Chemistry, 58, 1371-1382.

PEYRAUD J. L., LE GALL A., LUSCHER A. (2009): Potential food production from forage legume based systems in Europe: on overview. Irish Journal of Agriculture and Food Research, 48, 115-135.

ROCHON J. J., DOYLE C. J., GREEF J. M., HOPKINS A., MOLLE G., SITZIA M., SCHOLEFIELD D., SMITH C. J. (2004): Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. Grass Forage Science, 59, 197-214.

SCHWAB C. G., HUHTANEN P., HUNT C. W., HVELPLUND T. (2005): Nitrogen requirements in cattle. P. 13-70. In: Nitrogen and phosphorus nutrition (eds. Pfeffer, C. E. and Hristov, A.), CABI Publishing, Wallingford, UK.

SULLIVAN M. L., HATFIELD R. D. (2006): Polyphenol oxidase and o-diphenols inhibit postharvest proteolysis in red clover and alfalfa. Crop Science, 46, 662-670.

TAMMINGA S. (2006): The effect of the supply of rumen degradable protein and metabolizable protein on negative energy balance and fertility in dairy cows. Animal Reproduction Science, 96, 227-239.

TREMBLAY G. F., MICHAUD R., BĖLANGER G. (2003): Protein fractions and ruminal undegradable proteins in alfalfa. Canadian Journal of Plant Science, 83, 555-559.

TREMBLAY G. F., BĖLANGER G., MCRAE K. B., MICHAUD R. (2002): Leaf and stem dry matter digestibility and ruminal undegradable proteins of alfalfa cultivars. Canadian Journal of Plant Science, 82, 383-393.

TREMBLAY G. F., MICHAUD R., BĖLANGER G., MCRAE K. B., PETIT H. V. (2000): *In vitro* ruminal undegradable proteins of alfalfa cultivars. Canadian Journal of Plant Science, 80, 315-325.

YARI M., VALIZADEH R., NASERIAN A. A., GHORBANI G. R., MOGHADDAM P.R., JONKER A., YU P. (2012): Botanical traits, protein and carbohydrate fractions, ruminal degradability and energy contents of alfalfa hay harvested at three stages of maturity and in the afternoon and morning. Food Science and Technology, 172, 162-170.

YU P., CHRISTENSEN D. A., MCKINNON J. J. (2004): In situ rumen degradation kinetics of timothy and alfalfa as affected by cultivar and stage of maturity. Canadian Journal of Animal Science, 84, 255-263.
YU P., CHRISTENSEN D. A., MCKINNON J. J., MARKERT J. D. (2003): Effect of variety and maturity stage on chemical composition, carbohydrate and protein subfractions, in vitro rumen degradability and energy values of timothy and alfalfa. Canadian Journal of Animal Sciences, 83, 279-290.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

INOCULATION OF MAIZE WITH PGPR

Violeta Mandić[1], Snežana Đorđević[2], Zorica Bijelić[1], Vesna Krnjaja[1], Aleksandar Simić[3], Marija Gogić[1], Maja Petričević[1]

[1]Institute for Animal Husbandry Belgrade-Zemun, Belgrade, Republic of Serbia
[2]Biounik D.O.O. Belgrade-Zemun, Belgrade, Republic of Serbia
[3]Faculty of Agriculture, University of Belgrade, Belgrade-Zemun, Republic of Serbia
Corresponding author: Violeta Mandić, violeta_randjelovic@yahoo.com
Invited paper

Abstract: Maize is a highly productive crop intended for human and animal nutrition, and industrial processing. The standard agrotechnical measures are used in its production. However, the utilization of bacterial inoculants is becoming more popular due to their effect on increased soil quality, plant performance, and yield while preserving and enhancing the agroecosystem. In addition, bacterial inoculants can be applied together with pesticides used in seed treatments. Our earlier results showed that the inoculation of maize seed with plant-growth-promoting rhizobacteria (PGPR) increased the number of diazotrophs bacteria in the rhizosphere, morphological and productive traits, and yield of maize. Accordingly, seed inoculation may represent an important strategy to improve commercial maize production and reduce the environmental impact of maize production processes.

Key words: N-fixing bacteria, nitrogen, maize, seed inoculation, yield

Introduction

Maize (*Zea mays* L.) is the most important crop in Serbia. In 2019, it was cultivated in a 962.000 ha area with a production of 7.3 million tons and with an average yield of 7.6 t ha^{-1} *(Statistical Yearbook of the Republic of Serbia, 2020)*. The average maize yield in Serbia is considered still very low (for 27.6%) compared to the USA average (10.5 t ha^{-1}), the largest maize producer in the world *(FAO, 2021)*. The maize grain yield depends on the performance of the hybrid, characteristics of soil, applied agrotechnical measures, and climatic factors *(Mandić et al., 2013; Mandić et al., 2020)*. Mostly the farmers in Serbia use available superior maize genotypes of domestic and foreign selection in combination with appropriate usual production technology. However, the use of microbial seed inoculation in maize production is not common practice, as is the case with soybeans. In Serbia, seed inoculation of maize and other non-leguminous crops is not as popular as in developed countries. On the other hand, many countries, such as EU members, USA, China, India, and Japan are trying to increase the use of microbiological inoculants through legislation and incentives for agriculture. The use of biofertilisers can increase root microbiome, especially free-living nitrogen (N)-fixing bacteria in the rhizosphere, soil fertility, yield, and biomass of maize, and reduce the use of expensive artificial N fertilizers *(Mandić, 2011)*. This is very important because artificial N fertilizers are known to adversely affect biological balance, soil quality, plant productivity, and ecological balance *(Naher et al., 2016; Vejan et al., 2016)*. Thus, the inoculation of maize seed with *Azospirillum brasilense* reduces the amount of artificial N fertilizers by 25% *(Fukami et al., 2016)*. The mismanagement of artificial N fertilizers contributes to its loss from agroecosystems due to ammonia volatilization, nitrate leaching, and denitrification *(Cui et al., 2010)*. The increase in reactive nitrogen in the atmosphere, soils, and water is expected to come in the near future *(Sutton et al., 2011)*. In general, the chemical N fertilizers do have hidden dangers and represent health hazards to both humans and animals *(Alori and Babalola, 2018)*. Accordingly, the use of biofertilizers is one of the basic and best strategies to reduce the use of synthetic N fertilizers and preserve the environment.

In the present study, we will consider only the effect of inoculation of seed with an individual strain and in a consortium of PGPR. The effect of PGPR mutants on maize growth will not be considered.

PGPR in maize and biological N fixation

The beneficial rhizobacteria are called plant growth-promoting bacteria (PGPR). In addition to symbiotic N-fixing bacteria which are associated with legumes, various PGPR are associated with non-leguminous plants, especially with cereal grasses. The bacteria responsible for N fixation are called diazotrophs. These bacteria reside at the surface or interior of the root of non-legume crops *(de Bruijn, 2015)*.

PGPR such as *Bacillus*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Streptomyces*, *Enterobacter*, *Rhizobium*, *Agrobacterium*, *Arthrobacter*, *Achromobacter*, *Micrococcus*, *Pantoea*, and *Serratia* are present in the rhizosphere of different crops *(Olanrewaju and Babalola, 2019; Verma et al., 2019)*. Their strains are good for the improvement of the growth and productivity of crops in a sustainable way.

The PGPR in biofertilizers improves plant growth primarily by synthesizing phytohormones (indole-3-acetic acid, gibberellic acid, cytokinins, and ethylene), fixing atmospheric N, and solubilising phosphates and other mineral nutrients *(Kudoyarova et al., 2015)*. PGPR has a role in the biocontrol of pathogenic microorganisms and the prevention of the development of plant diseases because of produced bacteriocins and antibiotics *(Beneduzi et al., 2012)*. Example, *Bacillus* sp. strain B25 is a biocontrol agent of maize pathogen *Fusarium verticillioides (Douriet-Gámez et al., 2018)*. The riboflavin, thiamin, auxin, and gibberellin of *Azotobacter* may accelerate seed germination and improve the control of maize plant diseases *(Baral et al., 2013)*.

Also, PGPR produce extracellular enzymes which decompose organic and non-organic matter in the soil increasing its fertility *(Alexander, 1977)*. *Ahemad and Kibret (2014)* and *Olanrewaju et al. (2019)* emphasize that the PGPR help maize production by nitrogen fixation, increasing the availability of phosphorus, and control pests with different mechanisms. The root system uptake properties improve when the PGPR colonize it allowing easier adsorption of ion nitrate, solubilization of phosphate, and chelation of iron *(Islam et al., 2009)*. Furthermore, the inoculation of maize with a PGPR improves plant tolerance to water deficit *(Pereira et al., 2020)*, high salt concentration *(Chen et al., 2016)*, and high concentrations of heavy metals *(Hassan et al., 2014)*.

Nitrogen input from the air (N_2) through the process of biological N_2-fixation in agricultural soils is about 50-70 million tonnes of N per year *(Matiru and Dakora, 2004; Herridge et al., 2008)*. N_2-fixing bacteria synthesize enzyme nitrogenase which converts inert N_2 into biologically useful NH_3. These bacteria isolated from root, stem, and leaf tissues of maize belong to different genera *Pantoea*, *Pseudomonas*, *Rhanella*, *Herbaspirillum*, *Azospirillum*, *Rhizobium* (*Agrobacterium*), and *Brevundimonas*, and may contribute to biological nitrogen fixation which ranges from 12 to 33% *(Montañez et al., 2009)*. The bacteria can fix from 30 to 90 kg N ha^{-1}, and even up to 150 kg N ha^{-1} *(Milošević et al., 1994)*. *Van Deynze et al. (2018)* point that the atmospheric N fixation contributes to 29–82% of the N nutrition of maize. *Kuan et al. (2016)* report that the N_2-fixing bacteria are able to fix N from the air up to 304 mg N $plant^{-1}$. *Baral et al. (2013)* concluded that the *Azotobacter* fixes about 20 kg N ha^{-1} $year^{-1}$.

Zahir et al. (2004) report that the fresh biomass and weight of the ear of maize are significantly higher in treatment with inoculation of seed with *Azotobacter* sp. compared to the control. *Adjanohoun et al. (2011)* show that the *Azospirillium lipoferum*, *Pseudomonas fluorescens*, and *Pseudomonas putida* isolated from the rhizosphere of maize significantly increase grain yield compared to *Pseudomonas aeruginosa*, *Bacillus coagulans*, *Bacillus thurengensis*, *Bacillus pumilus*, *Bacillus poly*, *Bacillusfimosus*. Therefore they can be used to produce biofertilizers for maize. Similarly, *Kang et al. (2010)* demonstrate that the *Bacillus* spp., *Pseudomonas* spp., and *Azospirillum lipoferum* improve plant growth and maize yield. *Nezarat and Gholami (2009)* show that the inoculation of maize seed with strains of *P. putida* (R-168 and DSM291) and *P. fluorescens* (R-93 and DSM 50090) significantly increases germination and growth of maize compared to an uninoculated seed. Also, *Biari et al. (2008)* find that the *Azospirillum lipoferum* DSM 1691, *Azospirillum brasilense* DSM 1690, *Azotobacter chroococcum* DSM 2286 increase yield and growth parameters of maize. According to *Olanrewaju and Babalola (2019)*, the six indigenous isolates (A1 - *Bacillus subtilis*, A18 and A29 - *Pseudomonas* sp., NWU4 - *Streptomyces globisporus*, NWU14 - *Streptomyces griseoflavus,* and NWU198 - *Streptomyces heliomycini*) and their consortium of two or three organisms, enhance maize growth, but the effect of the consortia was greater. *Azospirillum brasiliense* increases the grain yield of maize *(Lana et al, 2012; Oliveira, 2018)*. *Arruda et al. (2013)* have isolated 292 bacterial isolates from five maize crop areas and concluded that the dominant genera were *Klebsiella* and *Burkholderia*, but strains, identified as *Achromobacter*, *Burkholderia*, and *Arthrobacter*, were effective as PGPR in two investigated maize cultivars. *Akhtar et al. (2018)* report that the inoculation of maize seed with a consortium of beneficial bacteria significantly increases root length and biomass and improves root architecture. Therefore, the root absorbs N, P, and K better due to the solubilization of nutrients *(Ranjan et al., 2013)*. The inoculation with strains of *Azotobacter* and *Azospirillum* significantly increased the dry matter accumulation and grain yield of maize *(Naserirad et al., 2011; Sharifi et al., 2011)*. The inoculation treatment of seeds with consortia of six PGPR strains (*Pseudomonas putida* strain R-168, *P. fluorescens* strain R-93, *P. fluorescens* DSM 50090, *P. putida* DSM291, *Azospirillum lipoferum* DSM 1691, and *A. brasilense* DSM 1690) increases nitrogen content in the rhizosphere, germination, plant height, 100-grain weight, number of seed per ear, leaf area, grain yield and quality *(Gholami et al., 2009)*. *Hungria et al. (2010)* have found increases of up to 30 % in the grain yields of maize, inoculated with *A. brasilense* due to improved N nutrition and increased nutrient absorption. Inoculation of maize with strains *A. brasilense* Az39 increases yield from 13 to 33% *(Cassán and Diaz-Zorita, 2016).*

It must be pointed out that the species and strains of PGPR colonize differently the roots of maize hybrids and influence soil biogeny (*Gałązka et al., 2017; Walters et al., 2018)*. That primarily depends on root exudates of which are under host-genetic control *(Bulgarelli et al., 2013)*. For that reason, it is necessary to select strains of PGPR according to the plant genotype.

The inoculation of maize with PGPR in Serbia

Previous researches on chernozem in the Srem District (Vojvodina province, northern Serbia) shows the significant effect of the inoculation of rhizobacteria on maize growth and yield. *Mandić et al. (2016)* report that the maize seed inoculated with consortia of *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* has significantly increased nitrogen content, the total number of microorganisms, number of azotobacter and aminoheterotrophs in the soil and grain yield of Serbian maize hybrid ZP 684 during 2006-2008. Also, maize seed inoculation with *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* has increased the stem diameter, grain yield, and rain-use efficiency, and decreased the stem lodging and percentage of barren plants in maize hybrid Dijamant 6 belong to FAO 600 maturity group (*Mandić et al., 2018a)*. Accordingly, the seed bacterial inoculation reduces the risk of stem lodging due to the accumulation of soluble solids in the stem *(Fancelli and Dourado Neto, 2000)*. It is known that the lodging of plants ranges from 5% to 20% annually worldwide *(Flint-Garcia et al., 2003)* with losses of grain yield from 40% *(Ransom, 2005)* to 75% *(Van Dyk, 2001)*. Thus, bacterial inoculation of maize seed can reduce the risk of lodging, especially at high plant densities, and contribute to high grain yields. *Mandić et al. (2018b)* find that the higher nitrogen amount, total number of microorganisms, number of azotobacters, number of aminoheterotrophs, number of oligotrophic and grain yield of two maize hybrids NS 6010 and Dijamant 6 are obtained in the treatment with inoculation of seed with *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* than in untreated treatment. Also, the individual strains of *Azotobacter chroococcum* and *Bacillus megaterium* and their combination increased nitrogen content in the rhizosphere of two maize hybrids PKB-509 and Srećko-5 *(Hajnal et al., 2001)*. The consortia of *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megaterium* and *Bacillus suptilis (Govedarica et al., 2002)*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megatherium*, and *Bacillus subtilis (Cvijanović et al., 2007)* and *Azotobacter chroococcum* and *Bacillus megaterium (Hajnal and Govedarica, 2004)* significantly increase microbial activity in the soil and maize production in Vojvodina Province. Similarly, *Jarak et al. (2011)* find that the *Azotobacter chroococcum* significantly increases microbiological activity, early plant growth, and grain yield of maize. In essence, the higher number and biomass of soil microorganisms increase microbial activity, mineralization of organic matter, and concentration of mineral nutrients available for maize plants *(Mandić, 2011; Mrkovački et al., 2012)*. In addition, according to *Govedarica et al. (1999)* and *Bjelić et al. (2010)* the total number of microorganisms and the number of azotobacters increases in the course of the growing season. *Bjelić et al. (2010)* concluded that the *Azotobacter chroococcum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* applied individually and in mixture increased the number of microorganisms in the rhizosphere and height and weight of maize plants. *Jarak et al. (2012)* found that the individual strains *Pseudomonas sp*. Q4b, *Bacillus sp*. Q5a and *Azotobacter chroococcum* strain 8 and their combinations increased the number of azotobacters, pseudomonads, and aerobic spore-forming bacteria, plant height, dry weight, and yield of maize compared to control. These authors got the highest yield in the treatment with a mixture of three strains. *Govedarica et al. (1992)* find that the mixture of two strains of *Beijerinckia derx* increases the yield of maize hybrid NSSC-606, while one strain of *Azotobacter chroococcum* increases the yield of maize hybrid PKB-624.

In general, these results demonstrate that the PGPR can improve maize production, microbial abundance in the rhizosphere, and soil fertility because they increase the content of available nitrogen in the soil. However, it is necessary to constantly expand knowledge about the mutual interactions of plants and diazotrophic bacteria.

Commercialization of bacterial inoculants

The cited researches report that the use of PGPR bacteria in the cultivation of maize can be beneficial to improve yield. All this has led to the development of biofertilizers or bio-inoculants. Biofertilizers are eco-friendly organic agro-input intended to improve the growth and yield of crops and soil health and thus are considered as the best alternative to synthetic fertilizers. The form of the inoculant depends on the carrier and can be solid or liquid. There is a wide range of commercial preparations (monovalent, bivalent, and polyvalent) for the inoculation of maize seeds. In Europe and South America, the most represented is the monovalent inoculum containing *Azospirillum* sp. *(Dobbelaere et al., 2001)*. The genus *Azospirillum* is able to improve the grain yield of maize, wheat, and rice *(Steenhoudt and Vanderleyden, 2000)*. *Santos et al. (2019)* state that about 70 million doses of *A. brasilense* inoculants, for maize and wheat, are sold annually in Brazil. Nowadays, global interest has increased for the development of new microbiological inoculants, the identification of new strains, and new inoculation methods. Scientists are constantly making great innovative efforts to find new microbiological inoculants and this is an inexhaustible area for investing knowledge, techniques, and skills to improve crop yields. China has registered 800 patents related to inoculation, while India has about 100 *(Santos et al., 2019)*. There is a tendency to discover new inoculants with a wide range of applications consisting of a consortium of bacteria.

Conclusions

In the near future, the development of agriculture must be based on the application of crop cultivation technology and plant breeding that contribute to better establishment of ecological balance and stability of natural resources in the agroecosystem. In doing so, the method of growing crops must be based on an economically effective basis. Biofertilization fits into this concept. The components of microbiological fertilizers are various bacteria, mostly from the group of N-fixing bacteria that deliver nitrogen to plants (*Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and others), but also translate inaccessible phosphorus, potassium, iron, and sulfur into an accessible form for plants. In addition, these bacteria synthesize plant hormones (auxin and gibberellins) affecting plant growth and induce plant resistance and increase the free-living root microbiome. Accordingly, the application of microbiological fertilizers containing mixed populations of microorganisms can improve the growth and development of plants through the supply of plants with essential nutrients while increasing yields and preserving the environment, and producing safe food.

Inokulacija kukuruza sa PGPR

Violeta Mandić, Snežana Đorđević, Zorica Bijelić, Vesna Krnjaja, Aleksandar Simić, Marija Gogić, Maja Petričević

Rezime

Kukuruz je visokoproduktivni usev namenjen za ishranu ljudi i životinja i industrijsku preradu. U njegovoj proizvodnji koriste se standardne agrotehničke mere. Međutim, upotreba bakterijskih inokulanata postaje sve popularnija zbog povećanja kvaliteta zemljišta, performansi biljaka i prinosa. Pored toga, bakterijski inokulanti se mogu primeniti zajedno sa pesticidima koji se koriste u tretiranju semena. Naši raniji rezultati pokazali su da inokulacija semena kukuruza sa rizobakterijama koje podstiču rast biljaka (PGPR) povećava brojnost diazotrofa u rizosferi, morfološke i produktivne osobine i prinos kukuruza uz očuvanje i unapređenje agroekosistema. Shodno tome, inokulacija semena može predstavljati važnu strategiju za poboljšanje komercijalne proizvodnje kukuruza i smanjuje uticaj procesa proizvodnje kukuruza na životnu sredinu.

Ključne reči: N-fiksirajuće bakterije, azot, kukuruz, inokulacija semena, prinos

Acknowledgements

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

References

ADJANOHOUN A., ALLAGBE M., NOUMAVO P., GOTOECHAN-HODONOU H., SIKIROU R., DOSSA K., GLELEKAKAÏ R., KOTCHONI S., BABA-MOUSSA L. (2011): Effects of plant growth promoting rhizobacteria on field grown maize. Journal of Animal and Plant Sciences, 11, 1457–1465.

AHEMAD M., KIBRET M. (2014): Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University–Science; 26, 1–20.

AKHTAR N., NAVEED M., KHALID M., AHMAD N., RIZWAN M., SIDDIQUE S. (2018): Effect of bacterial consortia on growth and yield of maize grown in *Fusarium* infested soil. Soil and Environment, 37, 35–44.

ALEXANDER M. (1977): Introduction to soil microbiology. John Willey Inc., New York, London.

ALORI E.T., BABALOLA O.O. (2018): Microbial inoculant for improving crop quality and human health. Frontiers in Microbiology, 9, 2213.

ARRUDA L., BENEDUZI A., MARTINS A., LISBOA B., LOPES C., BERTOLO F., PASSAGLIA L.M.P., VARGAS L.K. (2013): Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. Applied Soil Ecology, 63, 15–22.

BARAL B.R., ADHIKARI P., PARBATI A. (2013): Effect of Azotobacter on growth and yield of maize. SAARC Journal of Agriculture, 11, 141–147.

BENEDUZI A., AMBROSINI A., PASSAGLIA L.M. (2012): Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology, 35, 4, 1044–1051.

BIARI A., GHOLAMI A., RAHMANI H.A. (2008): growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. Journal of Biological Sciences, 8, 1015–1020.

BJELIĆ D., MRKOVAČKI N., JARAK M., JOŠIĆ D., ĐALOVIĆ I. (2010): Effect of PGPR on the early growth of maize and microbial abundance in rhizosphere. Contemporary Agriculture, 59, 3–4, 339–345.

BULGARELLI D., SCHLAEPPI K., SPAEPEN S., VER LOREN VAN THEMAAT E., SCHULZE-LEFERT P. (2013): Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology, 64, 807–838.

CASSÁN F., DIAZ-ZORITA M. (2016): Azospirillum sp. in current agriculture: from the laboratory to the field. Soil Biology and Biochemistry, 103, 117–130.

CHEN L., LIU Y., WU G., VERONICAN NJERI K., SHEN Q., ZHANG N., ZHANG R. (2016): Induced maize salt tolerance by rhizosphere inoculation of *Bacillus amyloliquefaciens* SQR9. Plant Physiology, 158, 1, 34–44.

CVIJANOVIĆ G., MILOŠEVIĆ N., JARAK M. (2007): The importance of diazotrophs as biofertilizers in the maize and soybean production. Genetika, 39, 395–404.

CUI Z., CHEN X., ZHANG F. (2010): Current nitrogen management status and measures to improve the intensive wheat-maize system in China. Ambio, 39, 5–6, 376–384.

de BRUIJN F.J. (2015): Biological nitrogen fixation. John Wiley and Sons, Inc, Hoboken, New Jersey, USA.

DOBBELAERE S., CROONENBORGHS A., THYS A. (2001): Response of agronomically important crops to inoculation with *Azospirillum*. Australian Journal of Plant Physiology, 28, 1–9.

DOURIET-GÁMEZ N.R., MALDONADO-MENDOZA I.E., IBARRA-LACLETTE E., BLOM J., CALDERÓN-VÁZQUEZ C.L. (2018): Genomic analysis of *Bacillus sp*. Strain B25, a biocontrol agent of maize pathogen *Fusarium verticillioides*. Current Microbiology, 75, 247–255.

FANCELLI A.L., DOURADO NETO D. (2000): Produção de milho. Guaíba: Agropecu{ria. p. 360.

FAO. Food and Agriculture Organisation of the United Nations, 2021. Available online: http://www.fao.org/faostat/en/#data (accessed on 28 April 2021).

FLINT-GARCIA S.A., JAMPATONG C., DARRAH L.L., MCMULLEN M.D. (2003): Quantitative trait locus analysis of stalk strength in four maize populations. Crop Science, 43, 13–22.

FUKAMI, J., NOGUEIRA, M.A., ARAUJO, R.S. HUNGRIA, M. (2016): Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. *AMB Express*, 6, 3.

GAŁĄZKA A., GAWRYJOŁEK K., GRZĄDZIEL J., FRĄC M., KSIĘŻAK J. (2017): Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques. Plant, Soil and Environment, 63, 264–270.

GHOLAMI A., SHAHSAVANI S., NEZARAT S. (2009): The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth, and yield of maize. International Journal of Agricultural and Biosystems Engineering, 1, 1, 35–40.
GOVEDARICA M., JARAK M., MILOŠEVIĆ N., MANOJLOVIĆ S. (1992): The role of microorganisms in modern plant production. A Periodical of Scientific Research on Field and Vegetable Crops, 20, 95–103.
GOVEDARICA M., JELIČIĆ Z., JARAK M., MILOŠEVIĆ N., STOJNIĆ N., RAŠKOVIĆ D., PAVLOVIĆ M. (1999): Effect of diazotrophs and P–mobilisers on microbial activity under maize. Proceeding of Research Papers PKB-INI Agroekonomik, 5, 1, 115-121.
GOVEDARICA M., MILOSEVIĆ N., JARAK M., ĐURIC S., JELIČIĆ Z., KUZEVSKI J., ĐORĐEVIĆ S. (2002): Use of biofertilizers, biostimulators and biopesticides in agriculture production. Ratarstvo i povrtarstvo/Field and Vegetable Crops Research, 37, 85–95.
HAJNAL T., GOVEDARICA M. (2004): Possibilities of biofertilizers application in maize production, Zemljište i biljka, 53, 3, 211–216.
HAJNAL T., GOVEDARICA M., JELIČIĆ Z. (2001): Effect of bacterization on the number of microorganisms and the nitrogen content in soil under maize. Acta Agriculturae Serbica, 6, 11, 77–90.
HASSAN W., BANO R., BASHIR F., DAVID, J. (2014): Comparative effectiveness of ACC-deaminase and/or nitrogen-fixing rhizobacteria in promotion of maize (*Zea mays* L.) growth under lead pollution. Environmental Science and Pollution Research, 21, 10983–10996.
HERRIDGE D.F., PEOPLES M.B., BODDEY R.M. (2008): Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil, 311, 1–18.
HUNGRIA M., CAMPO R.J., SOUZA E.M., PEDROSA F.O. (2010): Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil, 331, 413–25.
ISLAM M.R., MADHAIYAN M., DEKA BORUAH H.P., YIM W., LEE G., SARAVANAN V.S., FU Q., HU H., SA T. (2009): Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. Journal of Microbiology and Biotechnology, 19, 1213–22.
JARAK M., JELIČIĆ Z., KUZEVSKI J., MRKOVAČKI N., ĐURIC S. (2011): The use of azotobacter in maize production: the effect on microbiological activity of soil, early plant growth and grain yield. Contemporary Agriculture, 60, 1–2, 80–85.
JARAK M., MRKOVAČKI N., BJELIĆ D., JOŠIĆ D., HAJNAL–JAFARI T., STAMENOV D. (2012): Effects of plant growth promoting rhizobacteria on maize

in greenhouse and field trial. African Journal of Microbiology Research, 6, 27, 5683–5690.

KANG Y., CHENG J., MEI L., YIN S. (2010): Screening and identification of plant growth-promoting rhizobacteria. Wei Sheng Wu Xue Bao, 50, 853–861.

KUAN K.B., OTHMAN R., ABDUL RAHIM K., SHAMSUDDIN Z.H. (2016): Plant Growth-Promoting Rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. Plos One, 11, 3, e0152478.

KUDOYAROVA G.R., DODD I.C., VESELOV D.S., ROTHWELL S.A., VESELOV S.Y. (2015): Common and specific responses to availability of mineral nutrients and water. Journal of Experimental Botany, 66, 2133–2144.

LANA M.C., DARTORA J., MARINI D., HANN J.E. (2012): Inoculation with *Azospirillum*, associated with nitrogen fertilization in maize. Revista Ceres, 59, 3, 399–405.

MANDIĆ V. (2011): Genotype response of stay green maize hybrids on increased crop density. Ph.D. Thesis, University of Belgrade, Faculty of Agriculture, Belgrade, Serbia, 2011.

MANDIĆ V., BIJELIĆ Z., KRNJAJA V., SIMIĆ A., SIMIĆ M., BRANKOV M., ĐORĐEVIĆ S. (2020): Sowing and fertilization strategies to improve maize productivity. Maydica, 65, 2, 1–9.

MANDIC V., DORDEVIC S., STANOJEVIC D., BIJELIC Z., KRNJAJA V., TOMIC Z., DRAGICEVIC V. (2016): Effect of bacterial seed inoculation on nitrogen dynamics, number of bacteria in soil under maize, and maize yield. Journal of Environmental Protection and Ecology, 17, 3, 1003–1010.

MANDIĆ V., ĐORĐEVIĆ S., BIJELIĆ Z., KRNJAJA V., RUŽIĆ MUSLIĆ D., PETRIČEVIĆ M., SIMIĆ A. (2018a): Effect of intra-row spacing and seed inoculation on stem lodging, yield and rain-use efficiency of maize under different climatic conditions. Philippine Agricultural Scientist, 101, 3, 243–250.

MANDIĆ V., KRNJAJA V., DJORDJEVIĆ S., DJORDJEVIĆ N., BIJELIĆ Z., SIMIĆ A., DRAGIČEVIĆ V. (2018b): Effects of bacterial seed inoculation on microbiological soil status and maize grain yield. Maydica, 63, 3, 1–7.

MANDIĆ V., SIMIĆ A., TOMIĆ Z., KRNJAJA V., BIJELIĆ Z., MARINKOV G., STOJANOVIĆ LJ. (2013): Effect of drought and foliar fertilization on maize production. Proceedings of the 10th International Symposium Modern Trends in Livestock Production, Belgrade, Republic of Serbia, 2–4 October 2013, 416–429.

MATIRU V.N., DAKORA F.D. (2004): The potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. African Journal of Biotechnology, 3, 1–7.

MILOŠEVIĆ N., GOVEDARICA M., JARAK M., HADŽIĆ V. (1994): Inoculation technology and potential application of symbiotic and non-symbiotic diazotrophs in vegetable crop production. Contemporary Agriculture, 42, 295.
MONTAÑEZ A., ABREU C., GILL P.R. HARDARSON G., SICARDI M. (2009): Biological nitrogen fixation in maize (*Zea mays* L.) by ^{15}N isotope-dilution and identification of associated culturable diazotrophs. Biology and Fertility of Soils, 45, 253–263.
MRKOVAČKI N., JARAK M., ĐALOVIĆ I., JOCKOVIĆ Đ. (2012): Importance of PGPR application and its effect on microbial activity in the maize rhizosphere. Ratarstvo i povrtarstvo/Field and Vegetable Crops Research, 49, 335–344.
NAHER U.A., PANHWAR Q.A., OTHMAN R., ISMAIL M.R., BERAHIM Z. (2016): Biofertilizer as a supplement of chemical fertilizer for yield maximization of rice. Journal of Agriculture Food and Development, 2, 16–22.
NASERIRAD H., SOLEYMANIFARD A., NASERI R. (2011): Effect of integrated application of bio-fertilizer on grain yield, yield components, and associated traits of maize cultivars. American-Eurasian Journal of Agricultural and Environmental, 10, 271–277.
NEZARAT S., GHOLAMI A. (2009): Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize. Pakistan Journal of Biological Sciences, 12, 26–32.
OLANREWAJU O.S., BABALOLA O.O. (2019): Bacterial consortium for improved maize (*Zea mays* L.) production. Microorganisms, 7, 11, 519.
OLANREWAJU O.S., AYANGBENRO A.S., GLICK B.R., BABALOLA O.O. (2019): Plant health: Feedback effect of root exudates-rhizobiome interactions. Applied Microbiology and Biotechnology, 103, 1155–1166.
OLIVEIRA, I.J., FONTES, J.R.A., PEREIRA, B.F.F., Muniz A.W. (2018): Inoculation with *Azospirillum brasiliense* increases maize yield. Chemical and Biological Technologies in Agriculture, 5, 6.
PEREIRA S.I.A., ABREU D., MOREIRA H., VEGA A., CASTRO P.M.L. (2020): Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (*Zea mays* L.) under water deficit conditions. Heliyon, 6, e05106.
RANJAN A., RAJAN M., MAHALAKSHMI, SRIDEVI M. (2013): Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem, Tamil Nadu, India. International Journal of Nutrition, Pharmacology, Neurological Diseases 3, 1, 29–33.
RANSOM J. (2005): Lodging in cereals: Crop and Pest report. Plant Science, 9, 1–4.

SANTOS M.S., NOGUEIRA M.A., HUNGRIA M. (2019): Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. AMB Express, 9, 205.
SHARIFI R.S., KHAVAZI K., GHOLIPOURI A. (2011): Effect of seed priming with plant growth promoting rhizobacteria (PGPR) on dry matter accumulation and yield of maize (*Zea mays* L.) hybrids. Journal of Food, Agriculture and Environment, 9, 393–397.
STATISTICAL YEARBOOK OF THE REPUBLIC OF SERBIA, 2020
STEENHOUDT O., VANDERLEYDEN J. (2000): *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiology Reviews, 24, 487–506.
SUTTON M.A., HOWARD C.A., ERISMAN J.W., BILLEN G., BLEEKER A., GRENNFELT P., VAN GRINSVEN P., GRIZZETTI B. (2011): The European Nitrogen Assessment: Sources, Effects and Policy Perspectives (Eds.). Cambridge University Press.
VAN DEYNZE A., ZAMORA P., DELAUX P.-M., HEITMANN C., JAYARAMAN D., RAJASEKAR S., GRAHAM, D., MAEDA J., GIBSON D., SCHWARTZ K.D., et al. (2018): Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. Plos Biology, 16, e2006352.
VAN DYK J. (2001): Corn lodging sets. In: integrated crop management: plant diseases. Department of Entomology, Iowa State University Press, Ames, Iowa.
VEJAN P., ABDULLAH R., KHADIRAN T., ISMAIL S., BOYCE A.N. (2016): Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. Molecules, 21, 573.
VERMA M., MISHRA J., ARORA N.K. (2019): Plant growth-promoting rhizobacteria: diversity and applications. In: Sobti R., Arora N., Kothari R. (eds) Environmental biotechnology: for sustainable future. Springer, Singapore.
WALTERS W.A., JIN Z., YOUNGBLUT N., WALLACE J.G., SUTTER J., ZHANG W., GONZÁLEZ-PEÑA A., PEIFFER J., KOREN O., SHI Q., KNIGH R., GLAVINA DEL RIO T., TRINGE S.G., BUCKLER E.S., DANGL J.L., LEY R.E. (2018): A large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proceedings of the National Academy of Sciences, 115, 7368–7373.
ZAHIR A.Z., ARSHAD M., FRANKENBERGER W.T. (2004): Plant-growth-promoting rhizobacteria: Applications and perspectives in agriculture. Advance in Agronomy, 81, 97–168.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

MYCOTOXIN CONTAMINATION OF CEREAL GRAINS IN BULGARIA

Maya M. Ignatova, Nadezhda M. Sertova

Institute of Animal Science, Pochivka 1, 2232 Kostinbrod, Bulgaria
Corresponding author: Nadezhda M. Sertova, sertova@hotmail.com
Invited paper

Abstract: In this research, the contamination with fusariotoxins and aflatoxins in cereal grains from the 2019 harvest was presented. A total number of 84 representative grain samples of wheat (32), barley (30) and maize (22) collected from different regions of Bulgaria were analyzed by Enzyme Linked Immunisorbent Assay (ELISA) method. The highest detected concentration of zearalenone was in maize grain samples (168 µg/kg), while in barley and wheat grain samples it was 156.78 µg/kg and 143 µg/kg, respectively. Deoxynivalenol was found in maize and barley grain samples with concentrations of 2860 µg/kg and 2290 µg/kg**,** respectively, while it was not detected in wheat grain samples. The highest concentration of fumonisins was in wheat samples 971 µg/kg followed by barley (738 µg/kg) and maize samples (520 µg/kg). The highest concentration of aflatoxins was 11.55 µg/kg in the barley grain sample, then 10.40 µg/kg in wheat and 9.90 µg/kg in maize grain samples. The concentrations of detected mycotoxins were bellow of maximum limits referenced by the European regulations.

Key words: mycotoxins, wheat, barley, maize, ELISA

Introduction

Mycotoxins are particularly dangerous contaminants that can be found during the growth, vegetation, ripening, harvesting and storage of cereals. The problem of the presence of mycotoxins in feed, despite being treated for years, remains global. The mycotoxins are a serious environmental and health problem, both for the whole world and for Europe.

In Bulgaria the cereal grains production has a leading share. The agricultural sector is very important in the economy of the country. The major feed crops are maize, wheat, barley and sunflower.

Maize is traditionally a feed grain and this continues to be an important use. Barley is a common feed grain for poultry in most European countries and

Canada; it supplies the major portion of energy and nutrients in poultry diets (*Rosentrater and Evers, 2018*).
Gruber-Dorninger et al.(2019) investigated samples collected from 100 countries from Europe, Asia and Africa. They have reported that 88% of the samples were contaminated with at least one mycotoxin. Large fractions of samples from Sub-Saharan Africa, Southeast Asia, and South Asia were contaminated with AFB_1 concentrations exceeding the EU maximum limit for the most resistant species, indicating a threat for animal and human health.

Mycotoxin occurrence showed distinct regional trends and climate was a key determinant governing these trends. To prevent negative effects on animals and consumers, many countries regulate mycotoxin concentrations in feed. Mycotoxins contaminating animal feed can exert toxic effects in animals and be transferred into animal products. Therefore mycotoxin concentrations in feed should be continuously monitored to support risk assessment *(Gruber-Dorninger et al., 2019).*

The most common mycotoxins are aflatoxins (e.g., aflatoxin B_1; AFB_1), fumonisins, zearalenone, type B trichothecenes (e.g., deoxynivalenol), type A trichothecenes (e.g., T-2 toxin; T-2), and ochratoxin A (OTA). The widespread mycotoxins produced by *Fusarium* spp. are fumonisins, zearalenone (ZEA) and trichothecenes (deoxynivalenol, nivalenol and T-2 toxin). Suitable substrates for their accumulation are crops as maize, wheat, barley, rye, rice and oats (*Goyarts et al., 2007).*

ZEA is a mycotoxin which is produced by *Fusarium* species and has the ability to show strong estrogenic activity (*Zinedine et al., 2007*). It also leads to a number of diseases in animals sometimes resulting in a high rate of mortality (*Valcheva and Valchev, 2007).* This toxin was detected in feed and silage maize and as well as in food products (*Sirot et al., 2013; Bai et al., 2018*). In view of the difficulty in removing ZEA mycotoxins, monitoring of grains during the period from planting to harvest is important for the control of exposure to these toxins *(Moreno et al., 2009).*

Deoxinivalenol (DON) is known as vomitoxin because it causes vomiting when the pigs consume contaminated feed. It can lead to adverse symptoms such as feed refusal, reduced weight, diarrhea, vomiting *(Moazami and Jinao, 2009),* and neurotoxic disturbs *(Ndossi et al., 2012).*

The most important mycotoxigenic fungus associated with maize and other crops, which produces fumonisins is *Fusarium verticillioides (Okoth and Kola, 2012; Nyinawabali, 2013).* Fumonisins are found to be relatively heat stable (*Howard, 1998*) and water-soluble. The occurrence of fumonisins in grains and grain products has been a worldwide problem. *Fusarium* spp. has an impact on the economy by causing lower grain yields and economic losses (*Scherm et al., 2013; Teli et al., 2016).*

Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They occur mainly after harvest during grain storage and are called ’’storage’’ mycotoxins. Rabbits, turkeys, chickens, pigs, cows and goats are susceptible to aflatoxin contamination *(Lizárraga-Paulín, 2012). Iqbal et al. (2014)* in their study demonstrated that contaminated poultry feed with aflatoxins has a high rate of contamination in chicken and eggs in Pakistan. Aflatoxins are categorized by the International Agency for Research on Cancer as highly toxic and primary carcinogenic compounds.

Consequently, the aim of this study encompassed comparative investigations of concentrations of aflatoxins and fusariotoxins in field crops (cereals) commonly grown in Bulgaria.

Materials and Methods

A total number of 84 representative wheat, barley and maize samples freshly harvested in 2019 collected from Northwestern, Northeastern, North central, Southwestern, Southeastern and South central regions of Bulgaria were analyzed. Wheat (32), barley (30) and maize (22) were grounded, thoroughly mixed and processed using 70% methanol as solvent for extraction. The filtered samples were screened for fusariotoxins and aflatoxins by Enzyme-Linked Immunosorbent Assay (ELISA) method. For the investigations, the samples were prepared according to the instructions of the kit manufacturer (R-Biopharm). All reagents including standard solutions, conjugate solution, antibody, substrate/chromogen and stop solution must be bring at room temperature before use. Microtitre plate with 48 wells was used. Wells are coated with antibodies. Using the optical densities (OD) of the standard, the calibration curve is plotted against the concentrations of other standards, and the amount of mycotoxin in the sample is extrapolated from standard curve. The measurement was made at 450 nm. The absorbance was inversely proportional to the mycotoxin concentration in the sample. The values calculated for the standards were entered the Ridawin program, Computer Systems (ELx800 Universal Microplate Reader, BIOTEK® Instruments, Inc., USA).

The results were obtained by calculation of average value (where the average of a set of numbers was the sum of the numbers divided by the total number of values in the set) and by using formula for standard deviation:

$$\sigma = \sqrt{\frac{\Sigma (X - \mu)^2}{N}}$$

where ∑ means "sum of", x is a value in the data set, μ is the mean of the data set, and N is the number of data points.

The results (%) for all regions were calculated by dividing the number of infected samples for each region by the total number of samples (all cereal grains) from each region and multiply the value by 100.

Results and Discussion

In this research study mycotoxin contamination of cereal grain samples from the 2019 harvest was established. The results of the quantitative analyses of studied *Fusarium* mycotoxins (fusariotoxins) and aflatoxins are shown in Table 1.

Table 1. Mycotoxins contamination of wheat, barley, and maize grain samples from the 2019 harvest

Mycotoxin	Cereal	Number of samples	% of positive samples	Range	Average±SD
Zearalenone (µg/kg)	wheat	32	28	50.92-143	126.88±28
	barley	30	20	76.18-156,78	135±29
	maize	22	23	72-168	133±37
Deoxinivalenol (µg/kg)	wheat	32	0	-	-
	barley	30	10	544-2290	1560±910
	maize	22	23	287-2860	2050±1020
Fumonisins (µg/kg)	wheat	32	28	285-971	789±120
	barley	30	46	351-738	581±140
	maize	22	41	380-520	448±50
Aflatoxins (µg/kg)	wheat	32	22	8.29-10.40	9.4±0,82
	barley	30	23	2.18-11.55	9.2±3,3
	maize	22	23	7.94-9,90	8.52±0.81

By mycotoxicological analyses, the presence of aflatoxins and fusariotoxins (*Fusarium* toxins) in all of the studied samples, except DON in wheat grain samples were established. ZEA was the most frequently in wheat samples (28%), while it was 23% and 20% in maize and barley, respectively. The highest concentration of ZEA in maize was 168 µg/kg, followed by barley (156.78 µg/kg) and wheat (143 µg/kg). It could be noted that the average concentrations of ZEA were not in correlation with the percentage of mycotoxin positive samples (Table 1). The obtained concentrations of *Fusarium* toxins did not exceeded the maximum limits adopted by the European Commission, Regulation 2006/576/EC. Similar to our results, in Turkey, *Bilal et al. (2014)* have reported varied ZEA concentrations below the maximum limits in feedstuffs. Likewise, in Romania, *Galbenu et al. (2011)* have also found low concentrations of ZEA in wheat, barley and maize. It should be considered that mycotoxins contamination and concentrations in feeds can be varying according to regional climate, harvesting, storage conditions etc.

Than with the neighboring countries our results were in accordance with the previous ones obtained by *Manova and Mladenova (2009)* that on the incidence of ZEA in wheat, barley, and maize in Bulgaria all obtained ZEA value were bellow of those ones recommended in the valid EC Regulation 1881/2006 concerning foodstuffs. Nevertheless, the occurrence of ZEA for wheat and barley in the both studies intended for feeding stuffs and for foodstuffs was below the setting maximum limits as referred in EC legislations - EC Recommendation 576/2006 and EC Regulation 1881/2006. The frequency of ZEA contamination in the evaluated samples was found mainly in the North part of Bulgaria, especially in Northwestern and Northeastern regions. There the contamination was higher compare to the South part because of the weather conditions. Mainly summer rainfall, which promotes the formation of fusariotoxins (Fig. 1).

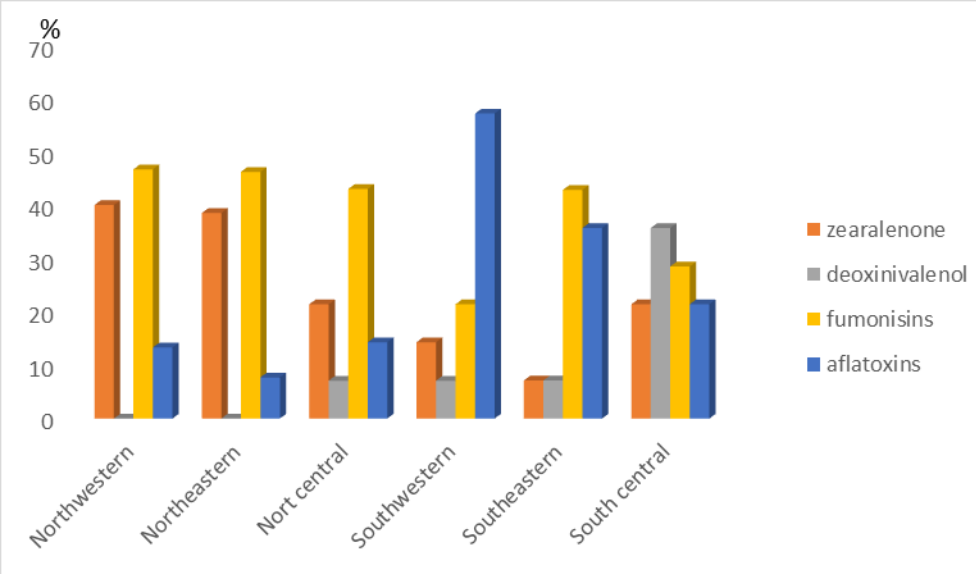


Figure 1. Mycotoxin contamination of cereal grain samples in different regions in Bulgaria

ZEA is frequently found as a natural contaminant of cereals together with DON *(Valcheva, 2000*). The high incidence of DON concentrations in the studied maize samples might be explained by the hot and humid environmental conditions during the summer season in Bulgaria. It should be noted that fusariotoxins are considered as field mycotoxins, which are formed during growth, maturation and harvesting of the grain.

The positive samples mostly originated from the South part of the country (Fig. 1). The highest concentration of 2860 µg/kg had proven in maize samples followed by barley samples of 2290 (µg/kg). The lowest value of contamination 287 µg/kg in maize samples was detected in the North part of the country. *Martos et al. (2010)* from Canada, investigated 15 samples of maize for the occurrence of DON, and found that 14 samples were positive, with a mean concentration of 1513.5 µg/kg (ranged from 574 to 4865 µg/kg). However, the spread of fungi and the production of mycotoxins in cereals may be the result of both climatic and improper storage conditions ***(****Iqbal et al., 2010*). The detected values concerning DON contamination were below the EC permitted value.

It can be mentioned that for all studied representative samples of feed materials the content of fumonisins was below 1000 µg/kg. The highest concentration of 971µg/kg was detected in wheat samples, followed by barley 739 µg/kg and maize grain samples 520 µg/kg, (Table 1).

Similar percentages of fumonisins positive samples were determined for maize (41%) and barley (46%). The lowest percentage of wheat samples was contaminated with fumonisins (28%). However, it was not established the positive correlation between the percentage of fumonisins positive samples and the concentrations of fumonisins in all studied grain samples. The average fumonisin concentration in maize samples was 448 µg/kg. The content was closed to the concentrations of 550 µg/kg reported by *Solovey et al. (1999).* On the contrary, *Vrabcheva et al. (2002)* have reported higher fumonisins concentration (up to 2100 µg/kg) in maize samples compared to our results.The presence of fumonisins in the tested samples could be attributed to the warmer and raining weather during the harvest in 2019, which favors their formation. The detected results confirm the opinion that *Fusarium* contamination was high, especially in the Northern part of the country, followed by Southeastern region (Figure 1).

Aflatoxins and fumonisins are the main contaminants of fodder crops, including maize, wheat and barley (*Woloshuk and Shim, 2013*). This was the reason why these cereals were tested for fumonisins and aflatoxins. Like fumonisins, the presence of aflatoxins was also registered in all of the studied cereal samples, with 22% positive wheat grain samples and 23% positive barley and maize grain samples (Table 1).

The lowest detected concentration of 2.18 µg/kg and the highest one of 11.55 µg/kg was proven for barley samples followed by wheat 10.40 µg/kg and maize 9.90 µg/kg. The average aflatoxin concentrations in investigated samples were closely related. The average aflatoxins concentration in the analyzed maize samples (8.52 µg/kg) was lower than the results reported by *Peker et al. (1994)* (36.83 µg/kg) and closed to results of *Oruc et al. (2006)* (10.94 µg/kg) in Turkey, and higher than the results reported by *Warth et al. (2012)* for maize with a mean value of 2.4 µg/kg.

Aflatoxins contamination was detected mostly in samples originating from the southern part of the country while in the northern part the contamination was less. It could be attributed to the climatic and storage conditions. The maximum concentration of aflatoxins found in this survey was below 20 µg/ kg as referred in EC 2003/100 on undesirable substances in animal feed.

Conclusions

It was established the occurrence of fusariotoxins and aflatoxins in all of the studied cereal grain samples while, DON was not found in wheat samples. Maize grain samples originating from North part of the country were more contaminated with ZEA compare to DON. A high percentage of ZEA contamination of cereals was observed in the northern part while, the presence of DON was registered in the southern part of the country.

Maize samples from Northern Bulgaria were more contaminated with fumonisins compared to Southern Bulgaria. The presence of fumonisins in the studied samples was more than aflatoxins. Aflatoxins were recorded in all of the representative samples originating from all collected regions especially in the southwestern. The concentrations found in this survey indicate that the presence of aflatoxins in the cereal grains was not hazard for animal health. The occurrence of the studied *Fusarium* toxins and aflatoxins in the investigated lots of samples did not exceed the maximum limits as referred in EC Recommendations.

However, continuous monitoring of grains is recommended to reduce potential risks of mycotoxin contamination in cereal crops.

Kontaminacija mikotoksinima zrna žitarica u Bugarskoj

Maya M. Ignatova, Nadezhda M. Sertova

Rezime

U ovom istraživanju prikazana je kontaminacija fusariotoksinima i aflatoksinima u zrnu žitarica iz žetve 2019. godine. Ukupan broj od 84 reprezentativna uzorka zrna pšenice (32), ječma (30) i kukuruza (22) prikupljenih iz različitih regiona Bugarske analiziran je metodom ELISA (Enzyme Linked Immunosorbent Assay) (ELISA). Najveća detektovana koncentracija zearalenona bila je u uzorcima zrna kukuruza (168 µg/kg), dok je u uzorcima zrna ječma bila 156,78 µg/kg i 143 µg/kg. Deoksinivalenol je nađen u uzorcima zrna kukuruza i ječma sa koncentracijama od 2860 µg/kg i 2290 µg/kg, respektivno, dok u uzorcima zrna pšenice nije detektovan. Najveća koncentracija fumonizina bila je u uzorcima pšenice 971 µg/kg, zatim ječma (738 µg/kg) i uzoraka kukuruza (520 µg/kg). Najveća koncentracija aflatoksina bila je 11,55 mg/kg u uzorku zrna ječma, zatim 10,40 mg/kg u pšenici i 9,90 mg/kg u uzorcima zrna kukuruza. Koncentracije otkrivenih mikotoksina bile su ispod maksimalnih granica navedenih u evropskim propisima.

Ključne reči: mikotoksini, pšenica, ječam, kukuruz, ELISA

References

BAI X., SUN C., XU J., LIU D., HAN Y., WU S., LUO X. (2018): Detoxification of zearalenone from corn oil by adsorption of functionalized GO systems. Applied Surface Science, 430, 198-207.

BILAL T., AKSAKAL H.D., SÜNNETCI S., KESER O. (2014): Detection of Aflatoxin, Zearalenone and Deoxynivalenol in Some Feed and Feedstuffs in Turkey. Pakistan Veterinary Journal, 34, 459-463.

GRUBER-DORNINGER C.H., JENKINS T., SCHATZMAYR G. (2019): Global Mycotoxin Occurrence in Feed: A Ten-Year Survey. Toxins, 11, 7, 375.

COMMISSION DIRECTIVE 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed.

COMMISSION RECOMMENDATION (EC) No 576/2006 of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

GALBENU P., DAMIESCU L., TRIF A. (2011): Zearalenone occurrence in cereal and cereal-based foodstuffs marketed in Timis County. Research Journal Agricultural Science, 43, 1, 43-49.

GREMEMELS F. J. (2005): Mycotoxicoses in Animal Health. Proceeding of the European Mycotoxin Seminar, 22nd February, Sofia, Bulgaria, 25-47.

HOWARD P. C., CHURCHWELL M. I., COUGH L. H., MARQUES M. M., DOERGE D. R. (1998): Formation of N-(carboxymethyl) fumonisin B1, following the reaction of fumonisin B1 with reducing sugars. Journal of Agricultural and Food Chemistry, 46, 3546-3557.

IQBAL S. Z., PATERSON R. R. M., BHATTI I. A., ASI M. R., SHEIKH M. A., BHATTI H. N. (2010): Aflatoxin B1 in Chilies from the Punjab Region, Pakistan. Mycotoxin Research, 26, 205-209.

IQBAL S. Z., NISAR S., ASI M.R., JINAP S. (2014): Natural incidence of aflatoxins, ochratoxin A and zearalenone in chicken meat and eggs. Food Control, 43, 98-103.

LIZÁRRAGA-PAULÍN E. G., MORENO-MARTÍNEZ E., MIRANDA-CASTRO S. P. (2011): Aflatoxins and their impact on human and animal health: An Emerging Problem. In: Aflatoxins-Biochemistry and Molecular Biology. Ed. Guevara-González R. InTech Janeza Trdine Rijeka, Croatia, 255-282.

MANOVA R., MLADENOVA R. (2009): Incidence of zearalenone and fumonisins in Bulgarian cereal production. Food Control, 20, 362-365.

MARTOS P., THOMPSON W., DIAZ G. (2010): Multiresidue mycotoxin analysis in wheat, barley, oats, rye and maize grain by high-performance liquid chromatography tandem mass spectrometry. World Mycotoxin Journal, 3, 205-223.

MOAZAMI E. F., JINAP S. (2009): Natural occurrence of deoxynivalenol (DON) in wheat based noodles consumed in Malaysia. Microchemical Journal, 93, 25-28.

MORENO E. C., GARCIA G. T., ONO M. A., VIZONI E., KAWAMURA O., HIROOKA E. Y., ONO E. Y. S. (2009): Co-occurrence of mycotoxins in corn samples from the Northern region of Parana State, Brazil. Food Chemistry, 116, 220-226.

NDOSSI D.G., FRIZZELL C.,TREMOEN N.H., FAESTE C.K., VERHAEGEN S., DAHL E., _ERIKSEN G. S, SØRLIE M, CONNOLLY L., ROPSTAD E. (2012): An in vitro investigation of endocrine disrupting effects of trichothecenes deoxynivalenol (DON), T-2 and HT-2 toxins. Toxicology Letters, 214, 268-278.

NYINAWABALI F. (2013): A Survey of fungi and mycotoxins in selected food commodities from Rwanda. MSc thesis. University of Johannesburg.

OKOTH S. A, KOLA M. A. (2012): Market samples as a source of chronic aflatoxin exposure in Kenya. African Journal of Health Sciences, 20, 1-2, 56-61.

ORUC H., CENGIZ M., KALKANLI O. (2006): Comparison of aflatoxin and fumonis in levels in maize grown in turkey and imported from the USA. Animal Feed Science Technolology, 128, 337-341.

PEKER I., ATAKUL S., AKDOGAN O., AZ D., VAHAPOGLU H. (1994): The amount of aflatoxin in different food, feedstuffs and feed in Turkey. Chimica Acta Turcica, 22, 103-109.

ROSENTRATER K. A., EVERS D. (2018): Introduction to cereals and pseudocereals and their production. In: Kent's Technology of Cereals. 5th Edition. Woodhead Publishing Series in Food Science, Technology and Nutrition, 1-76.

SCHERM B., BALMAS V., SPANU F., PANI G., DELOGU G., PASQUALI M., MIGHELI Q. (2013): Fusarium culmorum: Causal agent of foot and root rot and head blight on wheat. Molecular Plant Pathology, 14, 323-341.

SIROT V., FREMY J.M., LEBLANC J.C. (2013): Dietary exposure to mycotoxins and health risk assessment in the second French total diet study. Food and Chemical Toxicology, 52, 1-11.

SOLOVEY M. M. S., SOMOZ C., CANO G., PACIN A., RESNIK S. (1999): A survey of fumonisin, deoxynivalenol, zearalenone and aflatoxins contamination in corn-based food products in Argentina. Food Additives Contaminants, 16, 8, 325-329.

TELI B., CHATTOPADHYAY A., MEEN S. C., GANGWAR G. P., PANDEY S. K. (2016): Present status of Fusarium head blight of wheat and barley in India. In: Diseases of wheat and their management. Ed. S. Vaish, Ltd., New Delhi, Astral International, India, 79-94.

VALCHEVA A. (2000): Mycological and mycotoxicological status of wheat. Proof of Deoxinivalenol (DON) from the group of trihotecene micotoxins. Animal Science, 5/6, 93-96 (Bg).

VALCHEVA A., VALCHEV G. (2007): The Fusariotoxins Zearalenone and Deoxinivalenol as natural contaminators of some basic cereal components in the production of combined feed. Bulgarian Journal of Agricultural Science, 13, 99-104.

VRABCHEVA T., STROKA J., ANKLAM E. (2002): Occurrence of fumonisin B1 in Bulgarian maize samples determined by ELISA and TLC methods using different clean up steps. Mycotoxin Research 18, 46-56.

WARTH B., PARICH A., ATEHNKENG J., BANDYOPADHYAY R., SCHUHMACHER R., SULYOK M., KRSKA R. (2012): Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method. Journal of Agricultural and Food Chemistry, 60, 9352-9363.

WOLOSHUK CH. P., SHIM W. B. (2013): Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. Microbiology Reviews, 37,1, 94-109.

ZINEDINE A., SORIANO J. M., MOLTÓ J .C. MAÑES J. (2007): Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic Mycotoxin. Food and Chemical Toxicology, 45, 1-18.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

INFLUENCE OF CATTLE REARING CONDITIONS ON HEALTH, REPRODUCTION, GROWTH, MILK YIELD AND MEAT AND MILK QUALITY

Slavča Hristov[1], Branislav Stanković[1], Dušica Ostojić Andrić[2], Ljiljana Samolovac[2], Nevena Maksimović[2], Marko Cincović[3], Dimitar Nakov[4]

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia
[2]Institute for Animal Husbandry, Auto put 16, 11080 Belgrade-Zemun, Republic of Serbia
[3]Univerzity of Novi Sad Faculty of Agriculture, Department for Veterinary Medicine, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
[4]Ss. Cyril and Methodius University, Faculty of Agricultural Sciences and Food, 16. Makedonska brigada 3, 1000 Skopje, Republic of North Macedonia
Corresponding author: Slavča Hristov, hristovs@agrif.bg.ac.rs

Abstract: The paper describes the effects of different rearing conditions as possible stressors on health, reproduction, growth, milk yield and quality of meat and milk. It was pointed out that numerous internal and external factors of cattle affect the physiological processes, health, reproduction, growth, milk yield and quality of milk and meat as very strong stressors. They are especially important when acting immediately after parturition, i.e. in the puerperium in cows and immediately after birth in calves. In intensive cattle production, the most important complex stressors regardless of origin, and physiological conditions in which these animals are more susceptible to distress are parturition, calf birth, puerperium, intensive lactation, machine milking, oestrus, high pregnancy, dry period, grouping of animals, disturbed social relations, dehorning, castration, hoof trimming, transport, sudden changes in microclimatic conditions and feed quality, etc. Excessive disturbances before slaughtering cattle also cause a strong stress reaction. In modern housing systems, animal disturbance, immobilization of animals, restriction of movement, significant reduction of living space, strong painful stimuli, dystocia, pain during uterine prolapse, castration and acute inflammatory processes in the udder and uterus are also strong stressors. Unfavourable social relations between animals in the group are usually very strong stressors that lead to disturbances of their behavioural patterns. As a consequence of the action of numerous stressors, behavioural disorders, reduction of growth and milk production, the occurrence of metabolic and reproductive disorders, the occurrence of infectious diseases, reduction of meat and milk quality most often occur.

Key words: cattle, rearing conditions, reproduction, growth, milk yield, meat and milk quality

Introduction

Numerous internal and external factors of cattle affect the physiological processes, health, reproduction, growth, milk yield and quality of meat and milk as stressors. Many scientists have contributed to the clarification and deeper understanding of the mentioned effects *(Hristov and Bešlin, 1991; Broom and Fraser, 2015; Collier et al., 2017; Williams, 2019)*. An integrative approach relating to the biology of stress in farm animals was given by *Wiepkema (1987)*. The basic principles and implications for animal welfare in the biology of animal stress were considered in details *by Moberg and Mench (2000)*. In the meantime, a significant contribution to the consideration of the stress reaction of domestic animals in our country was given in the monograph by *Hristov and Bešlin (1991)*. Earlier, *Dantzer and Mormède (1983)* were discussed the need to reevaluate stress in farm animals. It should always be borne in mind that the occurrence of stress reactions in cattle depends primarily on the type, intensity and degree of stress-triggering factor *(Hristov and Bešlin, 1991; Hristov and Vučinić, 1991)*. According to the literature data in cattle intensive production, the most significant complex stressors are related to rearing and microclimatic conditions, nutrition, machine milking, grouping of animals, transport and many procedures of breeders *(Wiepkema, 1987; Hristov and Bešlin, 1991; Kadzere et al., 2002; Grandin, 2006; Herbut et al., 2018; Herbut et al., 2019; Williams, 2019, Benni et al., 2020)*. Physiological conditions in which these species of animals are susceptible to stress reactions are calving, calf birth, puerperium, intensive lactation, oestrus, high pregnancy, dry period, etc. *(Hristov and Bešlin, 1991; Broom and Fraser, 2015; Williams, 2019)*. In addition, inappropriate handling of animals, painful surgery procedures, restriction of movements, reduction of living space and health disorders, such as dystocia, uterine prolapse and acute inflammatory processes in the udder and uterus are also potential stressors (*Bova et al.,* 2015). The aim of the paper is to consider the most important literature data on influence of cattle rearing conditions on health, reproduction, growth, milk yield and meat and milk quality.

Influence of stressors on health, reproduction, growth, milk yield and quality of meat and milk

Dairy cattle face remarkable metabolic and physiological changes during the transition from late gestation to early lactation in preparation for calving and milk production. The intense metabolic processes are accompanied by modification of energetic metabolism and by an increase of oxygen consumption. This enhances metabolism severely, resulting in a raised production of reactive oxygen species and leading to the metabolic stress (*Contreras and Sordillo, 2011; Wathes et al., 2013*). This metabolic stress leads to an increased risk of many health conditions, including mastitis, metritis, ketosis, and displaced abomasum *(Wisnieski et al., 2019)*. The most commonly used biomarkers to monitor metabolic stress in cattle are nutrient metabolism biomarkers, including non-esterified fatty acids, beta-hydroxybutyrate and body condition score. These biomarkers represent the balance of mobilization of excess body fat tissue as a result of negative energy balance *(Sordillo and Mavangira, 2014)*.

It has been known for decades that stressors of different nature and intensity participate in the development of bovine acetonaemia *(Shaw, 1956; Radostits et al., 2006)*. A meta-analysis and review of diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cow were presented in the paper by *Raboisson et al. (2014*). Dairy cows, selected for high production, often show subclinical symptoms of acetonaemia in different housing conditions, most often in the period immediately after calving, i.e. in the puerperium, when milk production gradually increases *(Zhang et al., 2012; Zhang and Ametaj, 2020)*. There are many risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows (*Vanholder et al., 2015; Tatone et al., 2017)*. This disease is common in susceptible dairy cows that are subjected to sudden adverse stimuli from the environment. It has been established that clinically manifested acetonaemia can occur in some cows if their organism is exposed to prolonged stimulation and thus to the load on the adrenal cortex during the period of maximum milk production *(Vanholder et al., 2015; Kushwah et al., 2020)*. From research on changes in the adrenal cortex and anterior pituitary gland in highly productive dairy cows, in which clinical signs of acetonaemia were found in response to known stressors, conducted in 1950, this disease of cows has been used extensively in general studies of pituitary-adrenal function *(Zhang and Ametaj, 2020)*.

Common biological materials for the analysis of cortisol or its metabolites are blood, saliva, urine, faeces and hair *(Zhang et al., 2012; Meyer and Novak, 2012; Heimbürge et al., 2019; Zhang and Ametaj, 2020)*. In these materials, except for the hair, the measured cortisol levels represent only a retrospective timespan of a few minutes up to one or two days. Accuracy in measuring cortisol is especially important for cattle because their steroid concentration is lower compared to other animal species (*Zhang et al., 2012; Zhang and Ametaj, 2020)***.** The hair cortisol concentration is assumed to be a retrospective marker of integrated cortisol secretion and stress over longer periods *(Heimbürge et al., 2019)*.

The change in cortisol concentration in the first month of life of calves was described by *Hristov (1990)* and cortisol concentration in bovine blood serum by *Hristov et al. (1994)*. The concentration of cortisol in the blood plasma is high in calves' immediately after birth and then gradually reduced over the first two weeks. After that, it remains constant until the age of 15. The influence of some stressors on serum cortisol and glucose of calves has been considered by *Hristov et al. (1991).* It was found that the concentration of cortisol in calves increases immediately after dehorning and moving from individual to group boxes. Gut health, stress and immunity in neonatal dairy calves in terms of the host side of host-pathogen interactions were discussed by *Osorio (2020).* It is highlighted that maternal stressors during late pregnancy cannot only influence colostrogenesis but also compromise adequate intestinal development in the foetus, thus, that further limits the new-born's ability to absorb nutrients, bioactive compounds, and immunity (i.e., immunoglobulins, cytokines, and immune cells) from colostrum. In terms of animal production and neuroendocrine stress response, higher cortisol levels have negative effects on growth rate and feed efficiency and increase the fat lean ratio of carcasses. On the contrary, cortisol has positive effects on functional traits and adaptation *(Mormede and Terenina, 2012).*

Numerous studies have documented the response of the adrenal gland to the injection of ACTH in cattle *(Gwazdauskas et al., 1980; Alam et al., 1986*; *González-de-la-Vara et al., 2011)*. Regarding the rate of secretion, the researchers found a tenfold increase in corticosteroid concentrations within 2 minutes after ACTH injection. Some studies indicate that the application of corticosteroids to calves immediately after birth reduces the absorption of essential immunoglobulins by half, and also delays the endogenous production of certain types of immunoglobulins. The application of ACTH causes a greater increase in the concentration of corticosteroids in the blood plasma than the vein puncture in calves itself. These data that the puncture of the vein and the application of ACTH cause an increase in corticosteroids in the blood plasma are in line with the fact that the applied new stimuli cause a stress reaction in the body of calves *(Gwazdauskas et al., 1980; Hulbert and Moisá, 2016)*. The secretion of ACTH and cortisol is pulsatile in most species, with a pulse frequency of about 90 min, follows a diurnal cycle and is influenced by meals, physical activity, and environmental conditions (*Mormede and Terenina, 2012)*. However, accurate measurement of the activity of the adrenocortical axis is challenging *(Mormede et al., 2007)*.

A review of the physiological and productivity effects of heat stress in cattle was considered by *Farooq et al. (2010)* and *Herbut et al. (2019*), environmental parameters to assessing heat stress in dairy cattle by *Herbut et al. (2018)* and physiological and behavioural effects of heat stress in dairy cows by *Becker et al. (2020)*. Also, a review of heat stress on calves and heifers were presented in detail by

Wang et al. (2020). The impact of heat stress on milk and meat production was presented in detail in the paper by *Summer et al. (2019)*. Furthermore, a review of the impact of heat stress on the immune system in dairy cattle was presented by *Bagath et al. (2019).* Earlier, *Kadzere et al. (2002)* was considered a review of the literature on heat stress in lactating dairy cows. Also, the effect of heat stress on milk production in dairy cows was discussed by *Joksimović-Todorović et al. (2011)*. Generally, a biphasic response of the bovine adrenal gland to acute thermal stress has been established. At the beginning of the exposure, a response was established in the form of a noticeable increase in the concentration of steroids in the blood plasma. In the second phase of exposure, steroid levels dropped to below normal. The too hot environment also contributes to the appearance of reduced food intake, and thus reduced growth and milk production (*Knah et al., 2011; Baumgard and Rhoads, 2012)*. These consequences become noticeable only in a few days. The studies further established a decrease in thyroxine secretion during long-term exposure to heat in cattle. Short-term heat has been found to induce increased secretion of adrenal cortex hormones, while prolonged exposure of the body to heat for several days leads to a decrease in glucocorticosteroid secretion. Similar results were observed after the action of the same stressor for growth hormone concentration. Changes in the values of haematological parameters, as well as the number of somatic cells in milk, in response to prolonged heat stress, were determined *(Collier et al., 2017; Polsky and von Keyserlingk, 2017; Herbut et al., 2018; Herbut et al., 2019).* It has been established that heat stress has a negative impact on the immune system via cell mediated and humoral immune responses *(Becker et al., 2020).*

A review of effect of heat stress on reproductive performances of dairy cattle and buffaloes was presented by *Dash et al. (2016).* A sudden increase in the concentration of corticosteroids in the blood plasma that occurs during oestrus can be a cause for the manifestation of excitement and an increase in the metabolic activity of the organism of heifers and cows. This is possible because the endocrine changes that accompany oestrus also lead to an increase in adrenocortical activity *(Lyimo et al., 2000)*. During the summer season, animals usually suffer from summer sterility due to prevailing hot and humid conditions *(De Rensis and Scaramuzzi, 2003).* In female animal's age at puberty, oestrous signs, ovulation time, ova quality, conception rate, embryonic development, embryo size, embryo weight and hormonal balance are affected by exposure to heat stress *(Sammad et al., 2020*). The most sensitive to heat stress are growing ovarian follicles. Heat stress-induced changes in growing follicles can be expressed later as compromised maturation and developmental capacity of the ovulating oocyte (*Girma and Gebremariam, 2019*). It was concluded that this phenomenon occurs as a consequence of blockage of the secretion of the anterior pituitary gland, which explains the occurrence of abnormal estrus cycles and a high percentage of embryonic losses in cattle at high ambient

temperatures (*Diskin and Morris, 2008)*. Exposure of cows to strong heat stress leads to an increase in the rate of embryonic mortality, especially in the period immediately after mating (*Hansen, 2007; Lockwood et al., 2017)*. In males, sex hormone levels, spermatogenesis, temporary sterility, libido, ejaculate volume, macroscopic as well as microscopic semen characteristics in an ejaculate are affected, and sperm abnormalities and dead sperm increase by exposure to heat stress *(Fernandez – Novo et al., 2020; Para et al., 2020).*
Bhatt et al. (2021) a detailed review of transportation stress in livestock and its management techniques were presented. The effect of long distance transportation stress on cattle was presented in a review paper by *Damtew et al. (2018).* A review of the welfare of young calves transported by road was given by *Roadknight et al.(2021). Zavy et al. (1992)* effect of initial restraint, weaning, and transport stress on the baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes were described. The mortality rate of young calves may be particularly high after exposure to long-term transport. If the transport conditions are very unfavourable, the reaction of the adrenal gland to the present stressors occurs during the transport itself. Under these conditions, there is an almost instantaneous change in the concentration of corticosteroids, which is a reliable indicator of the stress state of the organism. In addition, it was found that the effect of transport on the concentration of mentioned corticosteroid in the blood plasma, and thus the stress caused, persists, and only after a period of 3 to 4 weeks the level of corticosteroids in the blood plasma returns to previous values. Also, it has been established that the transport of calves for 1 to 4 hours is a stronger stressor (marked by an increase in the concentration of corticosteroids in the blood plasma) than castration, dehorning and 48-hour disabling of water intake. Because transport is a strong stressor that can cause the extensive release of corticosteroids in the blood plasma, the ability of young animals to respond successfully to other strong stressors in the environment, usually bacterial and viral infections, is limited (*Damtew et al., 2018; Kukharenko and Fedorova, 2018*).
Pain at the slaughterhouse in ruminants with a focus on the neurobiology of sensitisation was described by *Mota-Rojas et al. (2021*) and pain evaluation in dairy cattle by *Gleerup et al. (2015).* In these reviews a neurobiological approach is taken to discuss the hypothesis in the light of basic science and extrapolations from existing literature on the slaughter of ruminants. A review of effects of age and method of castration on performance and stress response of beef male cattle was presented by *Bretschneider (2005).* Data of average daily gain and peak plasma cortisol concentration of calves castrated by surgical and rubber banding methods at different ages were analysed.
Effects of stocking and transport conditions on physicochemical properties of meat and acute-phase proteins in cattle were described by *Abubakar et al. (2021*). The

results revealed that the colour, pH, shear force values, water holding capacity, glycogen level, and malondilaldehyde assay concentrations in meat and acute-phase proteins were affected by both distances and stocking densities. Certain transport, including the loading density, environmental conditions, transport duration, and human factors, have caused an increased stress response, as indicated by plasma cortisol, adrenaline, glucose, or LDH levels, which have been associated with deteriorated meat quality (*Xing et al., 2019*). Excessive disturbance of cattle before slaughter leads to an increase in pH and progressive changes in meat colour in the sense previously emphasized *(Grandin, 2006)*. Studies of the frequency of meat discolouration have shown that it occurs in 30% of young bulls and only 8% of ox meat. Studies of emotional disorders in young bulls have shown that the appearance of a change in the colour of the meat can be eliminated if the bulls designated for slaughter are placed in a group not less than 48 hours before being sent to the slaughterhouse. Insemination of females in the period immediately after transport significantly reduces the rate of conception. Exposure to the new environment also causes an increase in the concentration of adrenocortical metabolites in the urine, which can be established up to 3 months. Practical experiences on long-distance transport of animals emphasize the need to do everything to help animals during the first and second day after transport because the most pronounced changes in their behaviour occur during that period *(Grandin, 2006; Weglarz, 2010; Damtew et al., 2018; Roadknight et al. 2021)*.
Further research has shown that even non-painful stimuli can cause an increase in the concentration of corticosteroids in the blood plasma of cows. For example, human contact has effects of acute stress on cows at milking. Leaving the milking machine on the udder, 15 minutes after the cessation of milk secretion, increases the concentration of corticosteroids in the blood plasma above the concentration observed in a normal machine milking. The increase in the concentration of corticosteroids in the blood plasma of cows seems to be maintained as long as the artificial milking stimulus lasts *(Rushen et al., 2001; Hopster et al., 2002).*
A personal perspective of pain and stress in cattle was given by *Bomzon (2011)* and pain evaluation in dairy cattle by *Gleerup et al. (2015*). Strong painful stimuli in cattle such as the use of an electric prodder, then dystocia, pain during uterine prolapse, surgery and acute inflammatory processes in the udder and uterus lead to a significant increase in the concentration of corticosteroids in the blood plasma. These results support the theory that the activity of the adrenal cortex is a reliable indicator of stress intensity from both the external and internal environment *(Hristov and Bešlin, 1991, Grandin, 2006; Mormède and Terenina, 2012; Herbut et al., 2018)*.
The stress and welfare of farm animals was considered by *Hristov et al. (2007)*. The most important dilemmas regarding the welfare of farm animals were

described by *Hristov et al. (2019).* General principles and good animal welfare practices on dairy cattle farms were considered by *Hristov et al. (2019).* Many factors contribute to the welfare of calves on the commercial dairy including housing and environment, rearing conditions and birth season, nutritional and health programs, handling and caretaker interactions, herd dynamics, and the common management practices of transportation, euthanasia, dehorning, and teat removal *(Stull and Reynolds, 2008; Samolovac et al., 2019).* The environment with its physical and social factors in the stages of the early development of the body of calves affects the ability of its body to react to stressors in later life. When calves are kept in pens, they show the significantly less general activity of the organism in comparison with freely kept calves, which are also enabled to suck the mother. If calves are kept in isolation for the first 12 weeks of life, after that period there is a decrease in their grouping activity compared to calves that were not kept in isolation *(Broom and Fraser, 2015; Jensen, 2018; Meagher et al., 2019).*
When calves are exposed to uncomfortable housing conditions significantly reduce their length and noticeably change their sleeping characteristics. Changes in other behavioural patterns of calves also occur in uncomfortable housing conditions *(Broom and Fraser, 2015).* Modern breeding systems are often associated with the emergence of the establishment of artificial social relations between individuals that are periodically disrupted by events such as the entry of a new dominant individual into the group. Unfavourable social relations between animals in the group are usually very strong stressors that lead to disorders of behavioural patterns. Effects of group housing of dairy calves on behaviour, cognition, performance, and health was analysed by *Costa et al. (2016*). Therefore, for example, ruminant lesions occur in calves as a result of licking the skin of other calves or there is a weaker growth of subordinates compared to the dominant calves in the group when kept on artificial teats *(Hulbert and Moisá, 2016).*
Acute effects of cow-calf separation on heart rate, plasma cortisol and behaviour in multiparous dairy cows were analysed by *Hopster et al. (1995).* Adult cattle establish stable social relations in the group in free intensive systems with adequate space and manifest minimal conflict situations. Disorders in the social status of cattle when introducing a new individual to the group can cause a decrease in the average milk production of the herd by 5%. Insufficiently adapted cows in large herds often do not maintain lactation for an average of 305 days and have a prolonged calving index *(Broom and Fraser, 2015).* Current knowledge and future directions of social stress as a cause of diseases in farm animals were described by *Proudfoot and Habing (2015*).
It is difficult to assess the significance of very pronounced changes in heart rate and blood pressure in calves during suckling, although in suckling pronounced behavioural and physiological effects stem from the rapid learning process contained in the formation of the natural mother-foetus bond in cattle resulting from an increase in

sympathetic efferent activity *(Meagher et al., 2019)*. In cows kept together with calves, corticosteroid levels in the blood plasma increase before suckling *(Murray et al., 2016*). This increase occurs even if the licking is performed to a lesser extent by the mother, with the levels of increase being lower concerning the group of cows where machine milking and giving milk to calves was applied. These studies show that a period of up to 48 hours after birth is critical for establishing a bond between a cow and a calf. When staying with calves for 24 hours, the sleeping time of the cows after weaning the calves does not change. However, if they spend 48 hours in the presence of calves, the time of sleep and rumination is reduced after weaning the calves. When the calves were separated from the cows after 26 days and placed in boxes near them for a week so that the cows could see and hear them, the time of sleeping and rumination was reduced during the first two days. After two days, the cows re-established normal sleep and rumination behaviour *(Hopster et al., 1995; Enríquez et al., 2011; Veissier et al., 2013; Broom and Fraser, 2015)*.
It has been studied how modern breeding methods can induce a stress reaction and the appearance of pathological conditions in dairy cows *(Proudfoot and Habing, 2015)*. The occurrence of foetal membrane retention has been shown to be almost twice as high in dairy cows as in fattening cows in which calves suckle the mother. It was concluded that the differences in this phenomenon occur due to "premature rupture of the placental connection", i.e. due to the separation of the mother from the calves immediately after birth. It occurs similarly in pregnant dairy cows that are kept free in pens where other animals in the group can disturb them. The removal of calves immediately after birth is another acute disturbance of the cow that can inhibit the necessary uterine contractions for the normal ejection of foetal membranes *(Broom and Fraser, 2015; Peter, 2021).*

Conclusion

Based on a detailed review of the literature data regarding the impact of cattle rearing conditions as a stressor on reproduction, growth, milk yield and quality of meat and milk, the following can be concluded:

- A large number of complex stressors can affect the occurrence of different phases of the stress response of the organism of certain categories of cattle in intensive cattle production;
- The occurrence of a stress reaction depends primarily on the type, intensity and degree of novelty of stress stimuli;
- The most significant complex stressors are related to microclimatic conditions, nutrition, machine milking, social relations, moving and regrouping of animals, transport, zootechnical procedures (dehorning, hoof trimming, marking and fixing)

and veterinary procedures (injection and oral treatment, castration and other surgical procedures);
- Physiological conditions in which these species of animals are susceptible to stress reactions are: parturition, calf birth, puerperium, intensive lactation, oestrus, high pregnancy, dry period, etc.

Uticaj uslova gajenja goveda na zdravlje, reprodukciju, prirast, mlečnost i kvalitet mesa i mleka

Slavča Hristov, Branislav Stanković, Dušica Ostojić Andrić, Ljiljana Samolovac, Nevena Maksimović, Marko Cincović, Dimitar Nakov

Rezime

U radu su opisani uticaji različitih uslova gajenja kao mogućih stresora na zdravlje, reprodukciju, prirast, mlečnost i kvalitet mesa i mleka goveda. Istaknuto je da brojni faktori iz životne sredine goveda utiču na fizološke procese, zdravlje, reprodukciju, prirast, mlečnost i kvalitet mesa i mleka kao vrlo snažni stresori. Oni su od naročito velikog značaja kada deluju neposredno posle porođaja odnosno u puerperijumu kod krava i neposredno posle rođenja kod teladi. U intenzivnoj govedarskoj proizvodnji najznačajniji kompleksni stresori nezavisno od porekla, a i fiziološka stanja u kojima su ove životinje podložnije pojavi distresa su: porođaj, rođenje, puerperijum, intenzivna laktacija, mašinska muža, estrus, visoki graviditet, zasušenje, premeštanje i grupisanje životinja, socijalni odnosi, obezrožavanje, kastracija i drugi hirurški zahvati, obrezivanje papaka, obeležavanje i fiksiranje, injekciono i peroralno tretiranje, transport, nagle promene mikroklimatskih uslova i hrane, stresori antropogene prirode i dr. Suvišna uznemiravanja ili naprezanja pre klanja goveda takođe izazivaju snažnu stresnu reakciju. U modernim sistemima držanja i smeštaja od značaja su i uznemiravanje životinja, imobilizacija životinja, ograničenje kretanja, znatno smanjenje životnog prostora u boksovima, snažni bolni nadražaji, kao primena električnog goniča, zatim distokija, bolovi pri prolapsusu uterusa, hirurški zahvati i akutni inflamatorni procesi u vimena i uterusu kao snažni stresori. Nepovoljni socijalni odnosi između životinja u grupi su obično veoma snažni stresori koji dovode do poremećaja bihejvioralnih obrazaca. Kao posledica delovanja brojnih stresora najčešće dolazi do poremečaja u ponašanju, smanjenja prirasta i proizvodnje mleka, pojave metaboličkih i reproduktivnih premećaja, pojave zaraznih bolesti i smanjenja kvaliteta mesa i mleka.

Ključne reči: goveda, uslovi gajenja, reprodukcija, porast, prinos mleka, kvalitet mesa i mleka

Acknowledgement:

This paper was created as a result of research within the "Agreement on the implementation and financing of scientific research in 2021 between the Faculty of Agriculture in Belgrade and the Ministry of Education, Science and Technological Development of the Republic of Serbia", contract record number: 451-03-9/2021-14/200116 and the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

References

ABUBAKAR A.A., ZULKIFLI I., GOH Y.M., KAKA U., SABOW A.B., IMLAN J.C., AWAD E.A., OTHMAN A.H., RAGHAZLI R., MITIN H., SAZILI, A.Q. (2021): Effects of Stocking and Transport Conditions on Physicochemical Properties of Meat and Acute-Phase Proteins in Cattle. Foods, 10(2), 252, 1-19.

ALAM M.G.S., DOBSON H., FITZPATRICK R.J. (1986): Endocrine response to different doses of ACTH in cows. British Veterinary Journal Volume 142, Issue 3, 239-245.

BAGATH M., KRISHNAN G., DEVARAJ C., RASHAMOL V.P., PRAGNA P., LEES A.M., SEJIAN V. (2019): The impact of heat stress on the immune system in dairy cattle: A review. Research in veterinary science, 126, 94-102.

BAUMGARD L.H., RHOADS R.P. (2012): Ruminant Nutrition Symposium: ruminant production and metabolic responses to heat stress. Journal of Animal Science, 90(6), 1855-1865.

BECKER C.A., COLLIER R.J., STONE A.E. (2020): Invited review: Physiological and behavioral effects of heat stress in dairy cows. Journal of Dairy Science, 103, 8, 6751-6770.

BENNI S., PASTELL M., BONORA F., TASSINARI P., TORREGGIANI D. (2020): A generalised additive model to characterise dairy cows' responses to heat stress. Animal, 14(2), 418-424.

BHATT N, SINGH N.P., MISHRA A.K., KANDPAL D., RAJNEESH J.S. (2021): A Detailed Review of Transportation Stress in Livestock and its Management Techniques. International Journal of Livestock Research, 11(1), 30-41.

BOMZON A. (2011): Pain and stress in cattle: a personal perspective. Isr. J. Vet. Med., 66, 12-20.

BOVA T.L., CHIAVACCINI L., CLINE G.F., HART C.G., MATHENY K., MUTH A.M., VOELZ B.E., KESLER D., MEMILI E. (2014): Environmental stressors influencing hormones and systems physiology in cattle. Reproductive Biology and Endocrinology, 12.1: 1-5.

BRETSCHNEIDER G. (2005): Effects of age and method of castration on performance and stress response of beef male cattle: A review. Livestock Production Science, 97(2-3), 89-100.

BROOM D.M., FRASER A.F. (2015): Domestic Animal Behaviour and Welfare. Edition: 5th ed. Publisher: Wallingford: CABI, UK, pp 472.

COLLIER R.J., RENQUIST B.J., XIAO Y. (2017): A 100-Year Review: Stress physiology including heat stress. Journal of dairy science, 100(12), 10367-10380.

CONTRERAS G.A., SORDILLO L.M. (2011): Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comparative Immunology, Microbiology and Infectious Diseases, 34, 281-289.

COSTA J.H.C., VON KEYSERLINGK M.A.G., WEARY D.M. (2016): Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. Journal of Dairy Science, 99(4), 2453-2467.

DAMTEW A., EREGA Y., EBRAHIM H., TSEGAYE S., MSIGIE, D. (2018): The effect of long distance transportation stress on cattle: a review. Biomedical Journal, 2(5), 3304-3308.

DANTZER R., MORMÈDE P. (1983): Stress in farm animals: a need for reevaluation. Journal of animal science, 57(1), 6-18.

DASH S., CHAKRAVARTY A.K., SINGH A., UPADHYAY A., SINGH M., YOUSUF S. (2016): Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. Veterinary world, 9(3), 235-244.

DE RENSIS F., SCARAMUZZI R. J. (2003): Heat stress and seasonal effects on reproduction in the dairy cow - a review. Theriogenology, 60(6), 1139-1151.

GONZÁLEZ-DE-LA-VARA R., VALDEZ R. A., LEMUS-RAMIREZ V., VÁZQUEZ-CHAGOYÁN J.C., VILLA-GODOY A., ROMANO, M.C. (2011): Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle. Canadian Journal of Veterinary Research, 75(3), 216-221.

DISKIN M.G., MORRIS D.G. (2008): Embryonic and early foetal losses in cattle and other ruminants. Reproduction in Domestic Animals, 43, 260-267.

ENRÍQUEZ D., HÖTZEL M. J., UNGERFELD R. (2011): Minimising the stress of weaning of beef calves: a review. Acta Veterinaria Scandinavica, 53(1), 1-8.

FAROOQ U., SAMAD H.A., SHEHZAD F., QAYYUM A. (2010): Physiological responses of cattle to heat stress. World Appl. Sci. J., 8, 38-43.

FERNANDEZ-NOVO A., PÉREZ-GARNELO S.S., VILLAGRÁ A., PÉREZ-VILLALOBOS N., ASTIZ S. (2020): The effect of stress on reproduction and reproductive technologies in beef cattle - A review. Animals, 10(11), 2096, 1-23.

GIRMA F., GEBREMARIAM B. (2019): Review on Effect of Stress on Production and Reproduction of Dairy Cattle. Journal of Scientific and Innovative Research, 8(1), 29-32.

GLEERUP K.B., ANDERSEN P.H., MUNKSGAARD L., FORKMAN B. (2015): Pain evaluation in dairy cattle. Applied Animal Behaviour Science, 171, 25-32.

GRANDIN T. (2006): Progress and challenges in animal handling and slaughter in the US. Applied Animal Behaviour Science, 100(1-2), 129-139.

GWAZDAUSKAS F.C., PAAPE M.J., PEERY D.A., MCGILLIARD M.L. (1980): Plasma glucocorticoid and circulating blood leukocyte responses in cattle after sequential intramuscular injections of ACTH. American journal of veterinary research, 41(7), 1052-1056.

HANSEN P.J. (2007): Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology, 68, S242-S249.

HEIMBÜRGE S., KANITZ E., OTTEN W. (2019): The use of hair cortisol for the assessment of stress in animals. General and Comparative Endocrinology, 270, 10-17.

HERBUT P., ANGRECKA S., WALCZAK J. (2018): Environmental parameters to assessing of heat stress in dairy cattle - a review. International journal of biometeorology, 62(12), 2089-2097.

HERBUT P., ANGRECKA S., GODYŃ D., HOFFMANN G. (2019): The physiological and productivity effects of heat stress in cattle–a review. Annals of animal science, 19(3), 579-593.

HOPSTER H.R.M.B., BRUCKMAIER R.M., VAN DER WERF J.T.N., KORTE S.M., MACUHOVA J., KORTE-BOUWS G., VAN REENEN C.G. (2002): Stress responses during milking; comparing conventional and automatic milking in primiparous dairy cows. Journal of Dairy Science, 85(12), 3206-3216.

HOPSTER H., O'CONNELL J.M., BLOKHUIS H.J. (1995): Acute effects of cow-calf separation on heart rate, plasma cortisol and behaviour in multiparous dairy cows. Applied Animal Behaviour Science, 44(1), 1-8.

HRISTOV S. (1990): Promena koncentracije kortizola u prvom mesecu života teladi. IX Seminar "Inovacije u stočarstvu", Univerzitet u Beogradu – Poljoprivredni fakultet, Zemun, 89-98.

HRISTOV S., BEŠLIN R. (1991): Stres domaćih životinja. Univerzitet u Beogradu – Poljoprivredni fakultet, Beograd.

HRISTOV S., BEŠLIN R., ANOJČIĆ B., VUČINIĆ M., ZVENIGORODSKA O., BOKAN LJ. (1991): The study of influence of some stressors on the serum cortisol and glucoze of calves. VIIth International Congress on animal hygiene, Leipzig, vol. I, 202-207.

HRISTOV S., ĐURĐEVIĆ Đ., GRUBIĆ G., BOGDANOVIĆ V., VIDIĆ R., BOKAN LJ. (1994): Koncentracija kortizola u krvnom serumu goveda. Vet. Glasnik, 48, 10, 853-859.
HRISTOV S., OSTOJIĆ ANDRIĆ D., STANKOVIĆ B. (2019): General principles and good animal welfare practices on dairy cattle farms. Proceedings of 12th international Symposium "Modern trends in livestock production", Belgrade, 23-38.
HRISTOV S., STANKOVIĆ B., OSTOJIĆ ANDRIĆ D., MAKSIMOVIĆ N., NAKOV D. (2019): The most important dilemmas regarding the welfare of farm animals. Journal of Agricultural Sciences, 64(4): 319-340.
HRISTOV S., VUČINIĆ M. (1991): Savremena gledišta na stresnu reakciju organizma domaćih životinja. Zbornik radova Poljoprivrednog fakulteta, 401-411.
HRISTOV S., FRATRIĆ N., KIROVSKI D., STANKOVIĆ B. (2007): Stres i dobrobit farmskih životinja. U: Rudić D. (urednik), Prva međunarodna konferencija o dobrobiti i biosigurnosti na farmama u Srbiji. “Dobrobit životinja i biosigurnost na farmama”, 87-95, Beograd.
HULBERT L.E., MOISÁ S.J. (2016): Stress, immunity, and the management of calves. Journal of Dairy Science, 99(4), 3199-3216.
JENSEN M.B. (2018): The role of social behavior in cattle welfare. In Advances in Cattle Welfare (pp. 123-155). Woodhead Publishing.
JOKSIMOVIĆ-TODOROVIĆ M., DAVIDOVIĆ V., HRISTOV S., STANKOVIĆ B. (2011): Effect of heat stress on milk production in dairy cows. Biotechnology in Animal Husbandry, 27(3), 1017-1023.
KADZERE C.T., MURPHY M.R., SILANIKOVE N., MALTZ, E. (2002): Heat stress in lactating dairy cows: a review. Livestock production science, 77(1), 59-91.
KHAN M.A., WEARY D.M., VON KEYSERLINGK M.A.G. (2011): Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. International journal of dairy science, 94.3: 1071-1081.
KUKHARENKO N., FEDOROVA A. (2018): The effect of long transportation stress on young calves born from cows and animal ecology. Ekoloji, 27.106: 293-299.
KUSHWAH N., UPADHYAY V., ROY A., SINGH M. (2020): Incidence, Causes and Treatment of Ketosis in Lactating Bovines. International Journal of Livestock Research, 10(6), 1-10.
LOCKWOOD S.A., KATTESH H.G., RHINEHART J.D., STRICKLAND L.G., KRAWCZEL P.D., WILKERSON J.B., KIRKPATRICK F.D., SAXTON A.M. (2017): Relationships among temperament, acute and chronic cortisol and testosterone concentrations, and breeding soundness during performance testing of Angus bulls. Theriogenology, 89: 140-145.

LYIMO Z.C., NIELEN M., OUWELTJES W., KRUIP T.A., VAN EERDENBURG F.J.C.M. (2000): Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology, 53(9), 1783-1795.

MEAGHER R.K., BEAVER A., WEARY D.M., VON KEYSERLINGK, M.A. (2019): Invited review: A systematic review of the effects of prolonged cow–calf contact on behavior, welfare, and productivity. Journal of Dairy Science, 102(7), 5765-5783.

MEYER J.S., NOVAK M.A. (2012): Mini review: Hair cortisol: a novel biomarker of hypothalamic - pituitary - adrenocortical activity. Endocrinology, 153, 4120-4127.

MOBERG G. P., Mench J. A. (eds.). (2000): The biology of animal stress: basic principles and implications for animal welfare. CABI.

MORMÈDE P., ANDANSON S., AUPÉRIN B., BEERDA B., GUÉMENÉ D., MALMKVIST J., MANTECA X., MANTEUFFEL G., PRUNET P., VAN REENEN C.G, RICHARD S., VEISSIER I. (2007): Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiology and Behavior, 92.3: 317-339.

MORMÈDE P., TERENINA E. (2012): Molecular genetics of the adrenocortical axis and breeding for robustness. Domestic animal endocrinology: 116–131.

MOTA-ROJAS D., NAPOLITANO, F., STRAPPINI, A., ORIHUELA, A., GHEZZI M.D., HERNÁNDEZ-ÁVALOS I., MORA-MEDINA P., WHITTAKER A. L. (2021): Pain at the Slaughterhouse in Ruminants with a Focus on the Neurobiology of Sensitisation. Animals, 11(4), 1085, 1-16.

MURRAY C.F., DUFFIELD T.F., HALEY D.B., PEARL D.L., DOUGLAS V., DEELEN S.M, LESLIE K.E. (2016): The effect of meloxicam NSAID therapy on the change in vigor, suckling reflex, blood gas measures, milk intake and other variables in newborn dairy calves. International Journal of Veterinary Sciences and Animal Husbandry, 4.1: 103, 5-14.

OSORIO J. S. (2020): Gut health, stress, and immunity in neonatal dairy calves: the host side of host-pathogen interactions. Journal of Animal Science and Biotechnology, 11(1), 1-15.

PARA I.A., DAR P.A., MALLA B.A., PUNETHA M., RAUTELA A., MAQBOOL I., MOHD A., SHAH M.A., WAR Z.A., ISHAAQ R., MALLA W.A., SHEIKH A.A., RAYEES M. (2020): Impact of heat stress on the reproduction of farm animals and strategies to ameliorate it. Biological Rhythm Research, 51:4, 616-632.

PETER A. T. (2021): Retained fetal membranes. Bovine reproduction, 629-638.

POLSKY L., VON KEYSERLINGK M.A. (2017): Invited review: Effects of heat stress on dairy cattle welfare. Journal of Dairy Science, 100(11), 8645-8657.

PROUDFOOT K., HABING G. (2015): Social stress as a cause of diseases in farm animals: current knowledge and future directions. The Veterinary Journal, 206(1), 15-21.

RABOISSON D., MOUNIÉ M., MAIGNE E. (2014): Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: a meta-analysis and review. Journal of Dairy Science, 97(12), 7547-7563.

RADOSTITS O.M., GAY C.C., HINCHCLIFF K.W., CONSTABLE P.D. (eds.). (2006): Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences.

ROADKNIGHT N., MANSELL P., JONGMAN E., COURTMAN N., FISHER A. (2021): Invited review: The welfare of young calves transported by road. Journal of Dairy Science, 104(6): 6343-6357.

RUSHEN J., MUNKSGAARD L., MARNET P.G., DE PASSILLÉ A.M. (2001): Human contact and the effects of acute stress on cows at milking. Applied animal behaviour science, 73(1), 1-14.

SAMMAD A., UMER S., SHI R., ZHU H., ZHAO X., WANG Y. (2020): Dairy cow reproduction under the influence of heat stress. Journal of Animal Physiology and Animal Nutrition, 104(4), 978-986.

SAMOLOVAC LJ., HRISTOV S., STANKOVIĆ B., MALETIĆ R., RELIĆ R., ZLATANOVIĆ Z. (2019): Influence of rearing conditions and birth season on calf welfare in the first month of life. Turk J Vet Anim Sci 43: 102-109.

SHAW J.C. (1956): Ketosis in dairy cattle. A review. Journal of Dairy Science, 39(4), 402-434.

SORDILLO L.M., MAVANGIRA V. (2014): The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim. Prod. Sci., 54, 1204-1214.

STULL C., REYNOLDS J. (2008): Calf welfare. Veterinary Clinics of North America: Food Animal Practice, 24(1), 191-203.

SUMMER A., LORA I., FORMAGGIONI P., GOTTARDO F. (2019): Impact of heat stress on milk and meat production. Animal Frontiers, 9(1), 39-46.

TATONE E.H., DUFFIELD T.F., LEBLANC S.J., DEVRIES T.J., GORDON J.L. (2017): Investigating the within-herd prevalence and risk factors for ketosis in dairy cattle in Ontario as diagnosed by the test-day concentration of β-hydroxybutyrate in milk. Journal of Dairy Science, 100(2), 1308-1318.

VANHOLDER T., PAPEN J., BEMERS R., VERTENTEN G., BERGE A. C.B. (2015): Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. Journal of Dairy Science, 98(2), 880-888.

VEISSIER I., CARÉ S., POMIÈS D. (2013): Suckling, weaning, and the development of oral behaviours in dairy calves. Applied Animal Behaviour Science, 147(1-2), 11-18.

WANG J., LI J., WANG F., XIAO J., WANG Y., YANG H., LI S., CAO Z. (2020): Heat stress on calves and heifers: a review. Journal of Animal Science and Biotechnology, 11(1), 1-8.

WATHES D.C., CLEMPSON A.M., POLLOTT G.E. (2013): Associations between lipid metabolism and fertility in the dairy cow. Reproduction, Fertility and Development, 25, 48–61.

WĘGLARZ A. (2010): Meat quality defined based on pH and colour depending on cattle category and slaughter season. Czech Journal of Animal Science, 55, (12): 548–556.

WIEPKEMA P.R. (1987): Biology of stress in farm animals: An integrative approach. van Adrichem P.W. (ed.). Dordrecht: Martinus Nijhoff.

WILLIAMS D. (2019): Stress and its effects on cattle. In Beef Cattle Science Handbook (pp. 535-538). CRC Press.

WISNIESKI L., NORBY B., PIERCE S.J., BECKER T., GANDY J.C., SORDILLO L.M. (2019): Predictive models for early lactation diseases in transition dairy cattle at dry-off. Preventive veterinary medicine, 163, 68-78.

XING T., GAO F., TUME R.K., ZHOU G., XU X. (2018): Stress Effects on Meat Quality: A Mechanistic Perspective. Comprehensive Reviews in Food Science and Food Safety, 18: 380–401.

ZAVY M.T., JUNIEWICZ P.E., PHILLIPS W.A., VONTUNGELN, D.L. (1992): Effect of initial restraint, weaning, and transport stress on baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes. American Journal of Veterinary Research, 53(4), 551-557.

ZHANG G., AMETAJ B.N. (2020): Ketosis an Old Story Under a New Approach. Dairy, 1(1), 5, 42-60.

ZHANG Z., LIU G., WANG H., LI X., WANG Z. (2012): Detection of Subclinical Ketosis in Dairy Cows. Pakistan Veterinary Journal, 32(2).

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

GASTROINTESTINAL HELMINTHS OF SHEEP BREED IN POMORAVSKI AND RASINA DISTRICT (SERBIA)

Ivan Pavlović[1], Violeta Caro-Petrović[2], Dragana Ružić Muslić[2], Jovan Bojkovski[3], Nemanja Zdravković[1], Renata Relić[4], Vukašin Stefanović[5]

[1] Scientific Veterinary Institute of Serbia, 11000 Belgrade, Serbia,
[2] Institute for Animal Husbandry, 11000 Belgrade-Zemun, Serbia
[3] University of Belgrade, Faculty of Veterinary Medicine, 11000 Belgrade, Serbia
[4] University of Belgrade, Faculty of Agriculture, 11000 Belgrade, Serbia
[5] University of Priština, Faculty of Agriculture, 38219 Lešak, Serbia
Corresponding author: Ivan Pavlović, dripavlovic58@gmail.com

Abstract: The study about gastrointestinal helminthes of sheep at central parts of Šumadija region - Pomoravski and Rasina district was started in March 2016 and finished in October 2018. During our research 937 fecal samples originated from 62 sheep flocks were collected individual at monthly intervals. A total of 57 animals we were analyzed by post-mortem examination. Determination of parasites eggs we performed by keys given by Euzeby (1981). We occured next parasite species: *Haemonchus contortus*, *Teladorsagia* (*Ostertagia*) *circumcincta*, *Ostertagia trifurcata*, *Ostertagia ostertagi*, *Ostertagia occidentalis*, *Marshallagia marshalli*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Nematodirus filicoliis*, *Nematodirus spathiger*, *Nematodirus abnormalis*, *Cooperia curticei*, *Cooperia oncophora*, *Cooperia punctata*, *Cooperia zurnabada*, *Skrjabinema ovis*, *Bunostomum trigonocephalum*, *Oesophagostomum venulosum* and *Chabertia ovina*. Most prevalence species of nematode are *Ostertagia*, *Trichostrongylus* and *Nematodirus*,species. Although most of the gastro-intestinal species appear to follow this general pattern of seasonal distribution, some variations in intensively and duration of these characteristics with different worm species occurred. Thus with *Trichostrongylus* and *Ostertagia* species infection at mature goats the spring peak was more pronounced that the autumn infection. Poliparasitismus and infection were established at all examined animals. The intensity of infection and polyparasitsm was monitored in relation to the age of sheep. It was found that in younger animals intensity of infection was lower than that of older animals.

Key words: sheep, gastrointestinal helminths, Serbia

Introduction

Sheep play an important role in providing animal protein for diet, especially for those people who live in villages. Sheep are milked and they produce the bulk milk supply, together with a large proportion of the meat that is consumed (*Petrović et al., 2021*). The method of breeding, which has been established in sheep breeding for centuries, is acquired through conditions that affect the development and maintenance of a significant number of diseases, including parasitic infections (*Vlassoff, 1982; Rose and Jacobs, 1990; Cabaret et al., 2002, Kenyon et al., 2009)*The grazing diet allows sheep constant contact with transitional hosts (oribatids, mollusks, etc.) and eggs and larval forms of parasites, so that there is no sheep that is not infected with at least one parasitic species (*Ardelanu et al., 2007*). The harmful effect of parasites is reflected in the reduction of milk yield, reduction of body weight and the quality of wool, leading to large losses in sheep production (*Karanfilovski, 1991; Pavlović et al., 2009*).

From these reasoni in mind, during aplication of project BT 31053 we started with examination of parasitic fauna of goat and sheep at warious pats of Serbia. In our paper we presented results of examination performed at Šumadija, a geographical region in the central part of Serbia. It is administratively divided into several districts - Šumadija, Pomoravski, Rasina, Podunavlje, Moravica, Kolubara and Belgrade City district. In the past period research of parasites on small ruminants are made in Podunavlje, Moravica, Kolubara and Belgrade City district (*Pavlovic et al., 2012b, 2017a, 2019*).

In our paper we presented result of examination from central Šumadija district which is well known for its rich horticulture and there is a large number of pastures suitable for growing small and larger ruminants. We examined central part of Šumadija, Pomoravski and Rasina district, areas where the largest number of sheep are bred in the Šumadija region.

Material and Methods

The study about gastrointestinal helminthes of sheep at Pomoravski and Rasina districts, part of Šumadija region, was started in March 2016 and finished in October 2018.

Šumadija is a geographical region in the central part of Serbia. It is administratively divided into Sumadija district, Pomoravski district, Rasina district, Podunavlje district, Moravica district, Kolubara district, Belgrade City District. The area used to be heavily covered with forests, hence the name (from *šuma* 'forest'). In the relief of Pomoravski and Rasina district a series of surfaces stands out, above which rise low island mountains and wide valleys cut into the surface.

The island mountains of the southern rim of the Pannonian Basin in Šumadija are Gledićke planine, Kotlenik, Juhor, Rudnik, Crni vrh, Venčac, Bukulja, Kosmaj and Avala.

Pomoravski and Rasina district has a distinct temperate continental climate. Considering the size of this area and the height differences in it (100 to 1130 m), there are significant microclimatic differences in Šumadija. Temperature fluctuations in Šumadija, as well as in the entire southern edge of the Pannonian Basin, can be significant. it even happens that some winter day has a higher average temperature than some summer day (*Ognjenović, 2008*). Geographical and climatic conditions make this region rich in pastures suitable for breeding small ruminants. They are usually kept in small herds by rural households and spend most of the year grazing.

During our research 937 fecal samples originated from 62 sheep flocks were collected individual at monthly intervals. Examination were performed using standard coprological technique with saturated NaCl solution and sedimentation (*Euzeby, 1981; Pavlović and Rogožarski, 2017*). Total of 57 animals we were analyzed by post-mortem examination. Determination of parasites eggs we performed by keys given by *Euzeby (1981).*

Results and Discussion

The faecal samples were obtained from a different source all together as they were collected from flocks in the field, and the results support the other findings. These counts were also of value in providing some information on the egg rise. Post mortal examination gave us insight into the types of parasites that were present in the infections.

During our examination parasites infection we occured in 65.31% (612/937). Polyparasitsm we established at all examined animals. With coprological examination we established the following genera of gastrointestinal helminths: *Ostertagia sp.* (72.22%), *Trichostrongylus sp.* (68.92%), *Nematodirus sp.* (66.45%), *Haemonchus sp.* (61.44%), *Chabertia ovina* (67.11%), *Oesophagostomum sp.*(39.77%), *Cooperia sp.* (27.66%), *Marshallagia sp.* (22.88%), *Skrjabinema sp.* (19.33%) and *Bunostomum sp.* (11,66%). The intensity of infection and polyparasitsm was monitored in relation to the age of sheep. It was found that in younger animals intensity of infection was lower than that of older animals.

Species in the genus *Ostertagia, Trichostrongylus* and *Nematodirus* were present after the first appearance of those present during the entire study period. Haemonchus contortus is ordered in animals during the warmer and *Marshallagia marshali* during the colder period of the year. Species in the genus *Cooperia*, and

Oesophagostomum. Bunostomum were often present in lambs sacrificed during all the monitoring period. Species in the genus *Cooperia*, and *Oesophagostomum. Bunostomum* were often present in lambs sacrificed during the monitoring period At the beginning of our research, conducted in March, the real extent of gastrointestinal infections strongilidae was 72.22%, after which he soon reached a level of 100% in the same way and moved to the end of follow-up period. Extensity of infection established genera gastrointestinal strongilidae was different. The distribution of the most prevalence genera species - *Ostertagia, Trichostrongylus* and *Nematodirus* was reached during the monitoring period almost the maximum level.

With post-mortem examination we found next parasite species: *Teladorsagia* (*Ostertagia*) *circumcincta*, *Ostertagia trifurcata*, *Ostertagia ostertagi*, *Ostertagia occidentalis*, *Trichostrongylus axei*, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Nematodirus filicoliis*, *Nematodirus spathiger*, *Nematodirus abnormalis*, *Haemonchus contortus*, *Chabertia ovina*, *Oesophagostomum venulosum, Cooperia curticei*, *Cooperia oncophora*, *Cooperia punctata*, *Marshallagia marshalli*, *Skrjabinema ovis* and *Bunostomum trigonocephalum*.

Their localization was different. *Haemonchus contortus, Ostertagia trifurcata, O. ostertagi*, *Marshallagia marshalli* and *Trichostrongylus axei* were found only in abomasus. In the small intestine we occured only species of the genus *Cooperia*, and in the large intestine *Oesophagostomum venulosum* and *Chabertia ovina*. Other species of the genus *Ostertagia* were predominantly localized in the abomasum, rarely in the small intestine, and *Trichostrongylus colubriformis, Trichostrongylus vitrinus*, and species of the genus *Nematodirus predominantly* in the small intestine. *Bunostomum trigonocephalum* were predominantly localized in the small intestine, and a smaller number of parasites were found in abomasus.

The interrelationship of the total number of males and females of the established species of gastrointestinal strongylides varied greatly. In all species of the genus *Ostertagia* was found a larger number of specimens of female parasites. *Haemonchus contortus* and *Marshallagia marshalli* were also represented by a larger number of female parasites. The same case was found in species of the genus *Trichostrongylus*. In species from the genera *Nematodirus, Cooperia, Bunostomum, Oesophagostomum, Skrjabinema* and *Chabertia* we no found significant differences between males and females.

When we compared our results to the examiantion at hilly mountainous area of Serbia like Stara Planina (*Pavlović et al., 2015*), Sjenicko-Pesterski Highland, (*Vujić et al., 1991*) and at Prizren District and north Kosovo (*Pavlović et al., 1995; Milanović et al., 2018*), we were concluded that dominant nematode species in such geographical conditions were *Ostertagia*, *Nematodirus* and *Trichostrongylus*. Same parasitic species we obtained during examination of goats and sheep at Vojvodina (*Pavlović et al., 2017b*), Belgrade area (*Pavlović et al., 2012a*) and south-east Serbia (*Pavlović et al., 2013*), Timok District (*Ilić et al., 1991; Jovanović et al., 1991*). In other Western Balkan countries like Montenegro, Romania, Bulgaria, North Macedonia or Greek were also identified identical species of GI helminths, with different intensities of infections and species ratio (*Denev and Kostov, 1984; Karanfilovski, 1991; Theodoropoulos et al., 2000; Ardeleanu et al., 2007; Georgievski, 1991*).

Conclusion

Results of our examination suggest that infections with helminths present significant problem of sheep in central part of Šumadija region. The infective rate of each of these parasites showed that the most of its followed the same general pattern, having a peak in the spring and an other in the autumn, separate by a trough during the hot dry summer period when the infection rate was low. At the same time, parasitic infections, in addition to harmful effects of sheep, affect the reduction of their production results - less milk yield, reduced growth and poor quality of wool. For these reasons, regular parasitological control of sheep before, during and after the grazing season must be taken into account. Sheep should also be regularly treated for parasites.

Gastrointestinalni helminti ovaca gajenih na području Pomoravskog i Rasinskog okruga (Srbija)

Ivan Pavlović, Violeta Caro-Petrović, Dragana Ružić Muslić, Jovan Bojkovski, Nemanja Zdravković, Renata Relić, Vukašin Stefanović

Rezime

Studija o gastrointestinalnim helmintama ovaca u centralnim delovima Šumadijskog regiona - Pomoravskog i Rasinskog okruga započeta je u martu 2016. godine, a završena u oktobru 2018. Tokom našeg istraživanja prikupljeno je 937 uzoraka fecesa iz 62 stada ovaca pojedinačno u mesečnim intervalima. Istovremeno je patoanatomskim pregledanom 57 zaklanih ili uginulih ovaca. Determinaciju parazita izvršili smo morfometrijskim pregledom pomoću ključeva koje je dao *Euzebi (1981).* Utvrdili smo sledeće vrste parazita: *Haemonchus* *contortus, Teladorsagia* (*Ostertagia*) *circumcincta, Ostertagia trifurcata, Ostertagia ostertagi, Ostertagia occidentalis, Marshallagia marshalli, Trichostrongylus axei, Trichostrongylus colubriformis, Trichostrongylus vitrinus, Nematodirus filicoliis, Nematodirus spathiger, Nematodirus abnormalis, Cooperia curticei, Cooperia oncophora, Cooperia punctata, Cooperia zurnabada, Skrjabinema ovis, Bunostomum trigonocephalum, Oesophagostomum venulosum* i *Chabertia ovina*. Najzastupljenije vrste nematoda su bile vrste iz rodova *Ostertagia, Trichostrongilus* i *Nematodirus*. Iako je većina gastrointestinalnih vrsta imala uobičajeni obrazac sezonske distribucije, pojavile su se neke varijacije u intenzitetu i trajanju infekcija kod nekoliko vrsta parazita. Tako je kod infekcije vrstama Trichostrongilus i Ostertagia kod odraslih ovaca prolećni vrhunac bio izraženiji od jesenje infekcije. Poliparasitizam je utvrđen kod svih pregledanih životinja. Intenzitet infekcije i poliparazizma praćen je u odnosu na starost ovaca. Utvrđeno je da je kod mlađih životinja intenzitet infekcije bio niži od intenziteta kod starijih životinja.

Ključne reči: ovce, gastrointestinalni helminti, Srbija

Acknowledgments

This work was funded by Serbian Ministry of Education, Science and Technological Development (contract number 451-03-9/2021-14/200030 and no. 451-03-9/2021-14/200022).

References

ARDELEANU D., PIVODĂ C., NEACŞU M., IDA A. (2007): Bio-ecolgical phenomenon of poly-parasitism–actual major problem in breeding of sheep and goats. Lucrrari Stiintifica .Zootehnie şi Biotehnologii, 40(2), 309-317.

CABARET J., MAGE C., BOUILHOL M. (2002): Helminth intensity and diversity in organic meat sheep farms in centre of France Veterinary Parasitology, 105(1), 33-47.

DENEV I., KOSTOV R. (1984): Seasonal variations in the principal helminth infections on a sheep-grazing complex.Veterinarno-meditsinski Nauki, 21, 81-88.

EUZEBY J. (1981): Diagnostic experimental de helminthoses animals, Vol 1. ITVC, Paris.

GEORGIEVSKI B. (1998): Rasprostranjenost i dinamika nematoda digestivnog trakta ovaca u Republici Makedonija, Doktorska disertacija, Fakultet veterinarske medicine Skopje, Universiti Kiril i Metodij Skopje, Makedonia.

ILIĆ G., JOVANOVIĆ D., NEŠIĆ D., PAVLOVIĆ I. (1991): Parasitose in goat and its role in pathology of goat at Timok District in period 1986-1989. Proceeding of 1th Internationaln Summer Conference for Advancement of Sheep and Goat Production, Ohrid, SFRJ, 390-395

JOVANOVIĆ D., ILIĆ G., NEŠIĆ D., PAVLOVIĆ I., VALTER D. (1991): Parasitoses of sheep in Timok district during 1986-1989. Proceeding of 1th Internationaln Summer Conference for Advancement of Sheep and Goat Production, Ohrid, SFRJ 383-385.

KARANFILOVSKI G. (1991): Štete od parazita u ovčarstvu, Veterinarska stanica Pljevlja, Pljevlja, SFRJ.

KENYON F., SARGISONN D., SKUCEP J., JACKSON F. (2009): Sheep helminth parasitic disease in south eastern Scotland arising as a possible consequence of climate change. Veterinary Parasitology, 163 (4), 293-297.

MILANOVIC V., PAVLOVIC I., RADOVIC B., MILOŠEVIC B., KRAGOVC Đ., IVANOVIC S., BOJKOVSKI J. (2018): Helminth fauna of small ruminants in north Kosovo Serbia. - The 17th International Symposium Prospects for 3rd Millennium Agriculture, Cluj-Napoca, Romania, Book of Abstracts No5/2018, 404.

OGNJENOVIĆ S. (2008): Ilustrovani atlas Srbije. Evro- Giunti, Beograd.

PAVLOVIĆ I., KULIŠIĆ Z., NEŠIĆ D., ROMANIĆ S. (1995): Endoparasites of sheep and goats in Prizren district. 3rd International Conference of Sheep and Goats Production, Ohrid, Macedonia, 106-110.

PAVLOVIĆ I., SAVIĆ B., IVETIĆ V., RADANOVIĆ O., ŽUTIĆ M., JAKIĆ-DIMIĆ D., BOJKOVSKI J. (2009): The effect of parasitic infections to production results of sheep. IV Balkan Conference of Animal Science BALNIMALCON 2009, Challanges of the Balkan Animal industry and the Role of science and Cooperation, Stara Zagora, Bulgaria, 389-391.

PAVLOVIĆ I., IVANOVIĆ S., ŽUGIĆ G., JOVČIĆ D., BOJKOVSKI J., PAJIĆ M. (2012a): Season distribution of gastrointestinal helminths of small ruminants in spread Belgrade area. Lucrări Ştiinţifice Medicină Veterinară Timişoara XLV(3), 155-160.

PAVLOVIĆ I., IVANOVIC S., ŽUJOVIĆ M., TOMIĆ Z., MEMIŠI N. (2012b): Studies on the endoparasites of goats in spread Belgrade area in period 2009-2010. Archiva Zootechnica, 15(4), 27-31.

PAVLOVIĆ I., IVANOVIĆ S., STOKIĆ-NIKOLIĆ S., BOJKOVSKI J., ŠEKLER M., SAVIĆ B., ŽUTIĆ M. (2013): Season distribution of gastroitestinal helminths of goats in south-east Serbia. - Lucrări Ştiinţifice Medicină Veterinară Timişoara, XLVI (5), 138-143.

PAVLOVIĆ I., IVANOVIĆ S., SAVIĆ M., ĆIRKOVIĆ D., JOVČEVSKI S.R., JOVCEVSKI S.T., SAVIĆ B., BEČKEI Ž., MARČIĆ D. (2015): Gastointestinal helmints of goats breeding at Stara plana area (Serbia). - Lucrări Ştiinţifice Medicină Veterinară Timisoara, XLVIII (3), 159-166.

PAVLOVIĆ I., IVANOVIĆ S., ĆIRKOVIĆ D., PETROVIĆ P.M., CARO PETROVIĆ V., MAKSIMOVIĆ N., IVANOVIC D. (2017a): Gastrointestinal helmints of sheep breeding at south west Serbia. Bulgarian Journal of Veterinary Medicine, 20, Suppl. 1, 402-406.

PAVLOVIĆ I., BECSKEI Z., IVANOVIĆ S., PETROVIĆ P.M., SAVIĆ M., CARO PETROVIĆ V., BOJKOVSKI J. (2017b): Biodiversity of helminths of sheep breed in Vojvojvodina (Northern Serbia). Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca 74 (2), 162-166.

PAVLOVIĆ I., ROGOŽARSKI D. (2017): Parazitske bolesti domaćih životinja sa osnovima parazitologije i dijagnostike parazitskih bolesti. Naučna KMD, Beograd.

PAVLOVIĆ I., IVANOVIĆ S. (2019): Gastrointestinal helminths of goats breed in central part of Serbia. - Proceedings of International symposium on animal science (ISAS) 2019, Herceg Novi, Montenegro, 143-148.

PETROVIĆ P.M., ILIĆ Z., CARO PETROVIĆ V., PAVLOVIĆ I. (2021): Uspešno i isplativo ovčarstvo. Balkanski naučni centar ruske akademije prirodnih nauka, Beograd.

ROSE C.H., JACOBS D.E. (1990): Epidemiology of sheep nematodes in sub-arctic Greenland: sources of infection on rangeland grazing. Acta Veterinaria Scandinavica, 31(3), 339-345.

THEODOROPOULOS G., ZERVAS G., KOUNELI A., MARTINEZ B., GONZALES G., PETRAKOS J., KOSTOPOULOS S. (2000): Seasonal patterns of strongyle infections in grazing sheep under the traditional production system in the region of Trikala, Greece, Veterinary Parasitology, 89(4), 327-335.

VLASSOFF A. (1982): Biology and population dynamics of the free-living stages of gastrointestinal nematodes of sheep. In: Internal parasites of sheep. Ed. A.D.Ross. Animal Industries Workshop, Lincoln College: 11-20.

VUJIĆ B., BOŠKOVIĆ V., SAVIN Ž. (1991): Most important parasites species of sheep and goat and its eradication. Proceeding of I International Summer Conference for Advancement of Sheep and Goat Production, Ohrid, SFRJ, 375-381.

PRODUCTION RELATED DISEASES (TECHNOPATHIES) IN SWINE COMMERCIAL FARM (SERBIA)

Jovan Bojkovski[1], Marina Spinu[2], Mihaela Niculae[2], JasnaProdanov-Radulović[3], Aleksandar Stanojković[4], Ivan Pavlović[5], Nemanja Zdravković[5], Radiša Prodanović[1]

[1]University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, Belgrade, Serbia
[2]University for Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine, Manastur street 3-5, Cluj- Napoca, Romania
[3]Scientific Veterinary Institute Novi Sad, Rumenički put 20, Novi Sad, Serbia
[4]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[5]Scientific Veterinary Institute Serbia, Janisa Janulisa, 14, Belgrade, Serbia
Corresponding author: Jovan Bojkovski, bojkovski@vet.bg.ac.rs

Abstract: The aim of this review aricle was to present our previous research related to production diseases (technopathies) on commercial pig farms. The presence of production diseases is the result of unfavorable conditions in the production itself. Growing and exploitation of boars on commercial farms aims to produce seeds for the needs of the farm. The lifespan of boars, and therefore the length of their exploitation, largely depends on their health condition. The health of the sow is also of paramount importance. We followed two parameters of the assessment of the body score condition of sows and the thin sow syndrome. The assessment of the body condition of sows was exceeded within the optimal values (3 at the time of farrowing and 2.5 during insemination). Thin sow syndrome is a disease of complex etiology. Sows have been observed to lose weight during lactation. Loss of body weight was diagnosed in most cases in sows in the second lactation. We proof aflatoxin in sow milk. Breeding diseases of bacterial and viral etiology were monitored in all categories. On the end of this article we give biochemical parameters by sows. The results of biochemical analysis showed economical validity and represent a significant contribution to monitoring of the health status and production of pigs on commercial farms. The promotion of good pig health includes the implementation of a system of prophylactic measures. Important factors in production are good choice of breed, size of the herd on the farm, ways of applying biotechnical measures in the production process and constant education of employees on the farm.

Key words technopaties, piglets, sows, boar, commercial farm

Introduction

Health care in organized intensive pig production is structured as a complex epidemiological unit given the transmissibility patterns and the elevated risk of infections spread at farm level. Certain pathogenic microorganisms lead to production disease due to intensive rearing and breeding. Development programs in pig breeding are aimed to creating an agglomeration in which the minimal occurrence of certain less important breeding disease is tolerated. Such as cultivations can be formed with help of health monitoring, which becomes an integral part of the management and is based on continuous laboratory analysis. The system of preventive antimicrobial use was banned for several years. The pig health care program contains three connections: diagnostics, immunoprophylaxis and metaphylaxis). An approach targeting production diseases is important. Detection and control measures for this type of pathology are not a legal obligation but an economic need. An organized health care program in an organized manner should include regular parasitic control. Parasitic infections significantly affect the health status of pigs on the farm. Parasitic diagnostics has been included in the regular monitoring of the health status of pigs (*Pavlović et al., 2019)*. Parasitic infections are constant companions of pig production. In intensive pig breeding, there are several valid parameters that can express the success or profitability of production, such as: the number of live or decided piglets, daily gain, length of fattening, and the number of unproductive days of sows (*Petrujkić et al., 2011)*. Only a healthy sow is able to consume a sufficient amount of food necessary for high milk production, on which the favorable number of reared piglets in the litter depends (*Wittemore and Elsley, 1975; Djordjević et al., 2009*). Today, it is common to present pig production on commercial farms by the number of piglets raised, i.e. delivered fatteners per sow during the calendar year (*Bojkovski et al., 2018*). In order to be able to work on improving the production of pigs on the farm, it is important that they provide. This review paper summarizes our many years of research on production diseases (technopathies) of pigs on commercial farms.

Pigs on commercial farm

Production of pigs on commercial farms is largely burdened by diseases of piglets. Pathology of piglets is a very dynamic discipline within the entire herd epidemiology in which the large agglomerations of animals housed in confined spaces can easily allow the horizontal and vertical transmission of infection (*Lončarevič et al., 1997*; *Ivetić et al., 2000*). Great importance is consider to the variations of swine pathogens, not only regarding the elevated antimicrobial resistance, but also the occurrence of genetic recombination, which affect the clinical picture and course of the disease, all of which makes it difficult to diagnose and apply therapy and prophylaxis (*Blackburn, 1995; Bojkovski et al., 1997*). In our pig farms the most frequently encountered pathologies are as follows: Neonatal colibacillosis, endemic disease, necrotic enteritis, circoviral infections, colitis caused by spirochetes, enterohaemorrhagic syndrome, dysentery and respiratory disease complex *(Bojkovski et al., 2008b*; *Šamanc, 2009; Bojkovski et al., 2018*). In recent years, a mass occurrence of porcine respiratory disease complex (PRDC), which is becoming a serious health problem in all technological stages of production, was reported at global level and on our pig farms. This pathology involves respiratory signs in pigs and it is characterized by simultaneous infection of lung tissue with several respiratory pathogens. The isolated pathogens vary between and within production herds *(Honnold, 1999; Golinar et al., 2006; Bojkovski et al., 2018)***.**Control of respiratory disease complex is difficult and complicated. The significance of respiratory diseases complex is based on the interaction of respiratory pathogens. Knowledge of the interaction of respiratory pathogens should be considered in order to implement effective control measures. Respiratory disease of pigs occur if the causative agents are present in the same habitat or if due to an unknown cause for us, the immune response mechanisms of respiratory system weakens *(Ivetić et al., 2006*). Unlike classical spreading control of infectious diseases of pigs that persist in our country whose fighting is a legal obligation, a detection and suppression of technopathies are more an economic need of the producers themselves. Porcine circovirus type2 (PCV_2) and hepatitis E virus (HEV) are the most recently recognized causes of infectious hepatitis of pigs and may or may not act independently in the development of the disease **(***Savić et al., 2012).* Recently it has been suggested that swine torque teno viruses (TTVs) in co-infections with some viral pathogens, may potentiate the severity of disease. In order to search for virus cofactors associated with infectious hepatitis in pigs, we investigated the liver tissues, to determine the presence of TTVs, PCV2 and HEV of naturally infected pigs and analyzed the prevalence of both genogroups of the TTVs in the hepatitis lesions. Histopathological techniques, nested polymerase chain reactions (nPCRs), polymerase chain reaction (PCR) and one-step reverse transcriptase polymerase chain reaction (RT-PCR) were applied to detect hepatitis lesions, TTVs genogroups 1 and 2, PCV2 and HEV infection. Of the livers examined 58% (29/50) had mild to moderate hepatitis and 74% (37/50), 56% (28/50) and 26% (13/50) samples were nPCR, PCR and RT-PCR positive for TTVs PCV2 and HEV respectively. TTVs were detected in 84% (16/19) of the samples which determined to be of mild severity while in almost all (90% or 9 of 10) samples identified as having moderate hepatitis lesions. Additionally, the livers of 12 out 21(57%) pigs without the hepatitis lesions were positive for TTVs. These results demonstrate an association between TTVs and infections hepatitis of pigs in concomitant infections with PCV_2 and/or HEV and indicated that TTVs may play a role as a cofactor in the pathogenesis of disease (*Savić et al., 2012)*. Increased antimicrobial resistance among pathogenic bacteria of animal origin is a growing concern in both veterinary and human medicine. In our country, antimicrobials have a wide application at pig farms as therapeutic and prophylactic treatment. However, their uncontrolled application may lead to the development of resistant microbial strains in both domestic animals and humans. In this study we investigated the antimicrobial resistance of *Escherichia coli* isolated from clinically diseases pigs, derived from swine farms located in Vojvodina region. In the three years period (2013-2015), a total of 53 organ samples were collected from diseased piglets, with clinical signs of severe enteric infection. Applying bacteriological examination (aerobic cultivation and the disk diffusion method) it was detected that the isolates of *E. coli* are sensitive to the restricted number of chemotherapeutics (Ceftriaxone, Enrofloxacin, Neomycin, Gentamicin, Flumequine). The high level of resistance on Amoxicillin (88.37%) and Colistin (67.44%) was detected, which are the most frequently used antibiotics in the therapeutic treatment of suckling and weaned piglets on the swine farms. Analyzing the antimicrobial sensitivity results obtained in previous period, it can be concluded that *E. coli* strains developed resistance to most of widely used chemotherapeutics. The occurrence of strains resistant to most frequently used antibiotics, as well as greater incidence and severity of diarrhea in piglets, demands the use of alternative measures for diseases control (*Prodanov-Radulović et al., 2018).*

Sow on commercial farm

Esophagogastric ulcer

One of the most present technopathies which is described as individual disease in literature is esophago-gastric ulcer which is characterized by erosions and ulcerations in esophagogastric and rarely glandular part of stomach. Esophagogastric ulcer is the disease of multifactorial origin where as the major causes stand: genetic predisposition, nutrition, presence of some pathogenic microorganisms (*Helicobacter pylori)(Bojkovski et al., 2008; 2010a,b,c; 2014).* In commercial farms (under monitoring) can see pigs with chronic ulcers. Those animals are anemic because of occasional bleeding from ulcer(s), have reduced food intake and they lose weight. In these animals when exposed to additional stress in slaughterhouse depot, manipulation, insufficient dizziness before slaughter, the occurrence of pale, soft and exudative (PSE) meat can be seen due to fast postmortal glycolisis. The PSE meat is too dry and more lose weight because of water release during termical processes. It has unwanted sensory characteristics and poor maintenance so it can not be sold as fresh meat but it is only used for processing (*Bojkovski et al., 2010a,b,c; 2013a*). We present the results of frequence of esophagogastric ulcer in pigs during several years of research. In slaughterhouse „A“(under monitoring) the average age of pigs ranged from 6 to 7 months and they weight from 100 to 110 kg. Total of 103 animals were examinated (53 castrated males, 50 gilts mixbreed of landrace and yorkshirerase, pietrain and durroc). All pigs originated from commercial farm“A“. In slaughterhouse „B“ (undr monitoring) the average age of pigs ranged from 7 to 9 months and they weight from 100 to 120 kg. Total of 107 animals were examinated (72 castrated males, 35 gilts mix breed of Landrace and Yorkshire, Pietrain and Duroc). Pigs originated from different commercial farms and were held in different production management. In slaughterhouse „A“ we established following results: enlargement of esophageal surface in 37 samples, erosions of esophageal part in 29 samples, ulcerations in 4 samples. In slaughterhouse „B“ we established following results: enlargement of esophageal surface in 49 samples, erosions of esophageal part in 27 samples, ulcerations in 21 samples (*Bojkovski et al., 2013c*).

Body score condition of sows and the “thin sow” syndrome and Afla toxin M1levels in sow milk

Nowadays, increasing attention is paid to assessment of body condition score of pigs. Body condition score is the most reliably performed by measurement of the thickness of adipose tissue in the back of sows using ultrasound machines, but nevertheless it is often done only visually (*Hutu and Onan, 2008; Petrujkić et al., 2011; Bojkovski et al., 2014*).Visual grading system of body condition can be subjective and in large precentage measures may depend on the competence of assessors. For example, in one Canadian study, sows with body condition score of 3 had adipose tissue in the back from 9 to 28 mm *(Petrujkić et al., 2011)*. Additional information on the mesurment of body fat on back and body condition of sows on three farms in the state Minnesota (USA) indicate that between 18 and 40 percent of sows have back fat smaller than 13 mm. Also, it is measured that sows with body condition score of 3 have from 9 to 24 mm of adipose tissue on the back. American and Canadian authors recommend that less than 20% of sows on the farm should have less than 15 mm of adipose tissue in the back. Based on this data it is tend to develop nutritional method in sows during pregnancy which is going to be based on the thickness of adipose tissue on the back (i.e. body condition score). The goal is to minimize the variations in quantity of adipose tissue on the back between pregnant sows so that at farrowing it is approximately

19 mm (at last rib). American researchers measured adipose tissue on the back and estimated body condition in total of 731 sows with goal to determinate „accuracy of nutrition" of pregnant sows based on the body condition score. The body condition score was in weak correlation ($r^2 = 0.19$) with thickness of adipose tissue on the back. For example, sows with body condition score of 3 had adipose tissue thickness between 7.5 and 23 mm. This proves that it is necessary to find more objective procedure for measurement of body condition (as ultrasound for example) in order to adjust nutrition level and minimize variation in thickness of adipose tissue of sows (*Coffey and Parker*, *1999; Petrujkić et al., 2011; Šamanc, 2009; Lipej, 2015; Simeunović et al., 2016)*. The "thin sow syndrome" is a condition characterized by anorexia and weight loss. Usually, it appears as a consequence of errors in sows' nutrition during parturition and lactating period, as well as different failures in production technology on the farm. The condition may results as a combination of parasitism (helminths or mange), low environmental temperatures and inadequate feed intake, particularly during lactation. In West Balkan (Serbia) climatic conditions, a more frequent occurrence of this syndrome is observed during the winter months as a result of inadequate environmental conditions (temperature less than 21°C and inappropriate ventilation level) in pig barns. The syndrome may also occur during or after recovery period from some infective diseases, such as influenza **(***Lipej, 2015)***.** Some authors suggested that some parasitic infections may also play role in pathogenesis of the thin sow syndrome (*Šamanc***,** *2009; Lipej, 2015).* From the group of endoparasites, the most frequent causes are gastric parasites *Hyostrongylusrubidus*, and from the group of ectoparasites - causative agents may be itch mites (*Šamanc, 2009***)**. However, the parasitism is less important when adequate prophylactic measures and therapy are routinely carried out in the commercial swine production. "The thin sow syndrome" is most often clinically observed after first and second farrowing or lactation. This phenomenon is one of the main reasons for the exclusion of a large number of sows from reproduction, after first and second farrowing. Clinical signs of suboptimal condition include increased weaning to service intervals, small litters and low weaning weights. Piglets of sows in suboptimal condition may be restless and demand milk more frequently *(Petrujkić et al., 2011*). During lactation, the nutritional needs are high, and inappropriate diet is one of the most frequent reasons of significant decrease of sow's body condition. If this phenomenon lasts longer, and if the deficit of nutrients is more pronounced, a " thin sow syndrome" occurs. Thin sows may be identified by observation and systematic condition scoring of the herd. Pressure sores in sows at weaning also indicate poor condition. Some production parameters can also be used to detect thin sows. The particular susceptibility of young gilts is due, among other things, their unequivocal use of large quantities of food during first lactation and small body reserves, which should

be considered that they body is still developing. In extreme cases, the weight loss can involve 30-90% of sows in one herd (*Šamanc, 2009; Lipej, 2015; Bojkovski et al., 2015*). The "thin sow syndrome" is a significant welfare problem and some data can be found in paper by *Lončarević et al. (1997).* In this paper, situation concerning body and health condition of lactating sows at one commercial Serbian farm is discussed. It is not easy to evaluate the body condition in an objective way under practical circumstances. In many herds, body condition is evaluated by the pig producer by visual scoring, on a scale ranging from 1 to 5. Although visual scoring systems may work well in some herds, e.g. in outdoor systems, they have several disadvantages. First, a sow that appears to be thin can still have a fairly high amount of back fat (*Cutler, 2013*). Second, it is a subjective and inaccurate method that largely depends upon the scoring skills of the person. Finally, when visual scoring is performed by the pig producer in the same herd over time, it is likely that less attention will be paid to deviations from the optimal condition due to herd blindness. Determining the optimal body condition by visual scoring is particularly difficult in herds with sows of 1 type of breed because of the inherent variation in conformation existing between breeds (*Whittemore and Schofield, 2000)*. In the experiment we had total of 47 sows in which parity ranged between 2 and 6. Applying ANOVA method there were no significant differences between parities. The number of live born piglets ranged from 9 to 22 piglets and the number of dead born piglets ranged from 0 to 6. It was found that the highest percentage of piglets born alive existed in sows of parity 5. Body condition score at the 90th day of gestation was 5 in 7 sows, estimated body condition 4 had 16 sows and 24 sows had body condition score 3. In ourexperiment, we found that7 sows had abody score condition 5. Sows farrowing to go with body score condition 5h are health- reproduction disorders. For these reasons, we have to try to not go to the farrowing sowswith the assessment of body score condition 5. With a score of body condition 3 had 24 sows. Body score condition 3 is optimal. In our experiment, 16 sows had a body score condition 4. Body score condition 4 gives a chance to the correction of the meal to farrowing, sows that such lack of health-reproduction problems. Data from the farm records and current situation on the farm indicated that zoohygenic, prophylactic and biosecurity measures were not carried out in an adequate regime. Also, the regime of preparing the sows for farrowing and the feeding is not regulated. Decreases of feed intake and weight loss in the lactation period were noticed in animals after the 1st, 3rd, 4th and 5th farrowing. In the cases when gilt condition and nutrition in the lactation period was inadequate, the second litter was smaller than the first. Certainly that low number of newborn may reflect overall sow condition at service. The extended weaning to service intervals and low weaning weights were connected to the poor body condition. The litters of the lean sows were smaller, and in the case of the pig's rejection, it attained less body

weight. In some cases, problems with conception or abortion in pregnant sows were also noticed. On the sows' body, some skin changes (wrinkles and different types of lesions) and swellings were noticed, as well as clogged and dirty hair. The lesions were formed dominantly at the point of bone compression. Most often, thin sows were found in a position lying on the sternum and do not showed any interest for the environment. In some cases, it was very difficult for these animals to take a standing position in the box i.e. The detected post mortal lesions were grossly classified as poor body condition, low fat thickness and as an increased incidence and extent of skin lesions, especially over shoulders and hips where pressure sores can develop (*Bojkovski et al., 2014*). *Anorexia* (loss of appetite) in sows develops after farrowing as part of a "thin sow syndrome", and as a result there is an intense loss of body weight. The clinical signs of this syndrome, observed in the examined cases, show an unusual similarity to the clinical signs of anorexic nerve (*Anorexia Nervosa*). In addition to loosing appetite and body weight, sows limit the intake of normal foods and consume large quantities of straw. Animals spend more time on non-intrusive hyperactive behavior, constantly moving inside the box. The sows affected by anorexic nervousness are easily recognized by the prominent backbone of the spine and their rough and long hair (*Treasure and Owen, 1997*), which is also in line with the observed changes in our experiment.
Many factors affect the appetite of sows in lactation, and the most important are: consumption of the food during pregnancy, air temperature and ventilation in the pig barn, energy level in the meal and the number of feeding per day *(Whittemore and Elsley, 1975; Djordjević et al., 2009; Bojkovski et al., 2018*). The most powerful effect on the level of consumption has the level of energy in the meal, so if lactating sows are not allowed to eat „a*d libitum"* or close to it, than production of milk, body weight and level of body reserves decreases. On the other hand, the needs in nutrients during lactation vary and depend on the concentration of energy in the meal and the previous feeding of the sow. In practical nutrition, the highest efficiency of energy consumption from meals is achieved by controlled diet during gravidity in order to minimize the mobilization of body depots of fat during lactation *(Djordjević, 2009)*. Cases of severe constipation can be avoided by increasing the amount of dietary fiber during the last phase of suppression (*Tabeling et al., 2003*). Ensuring optimum levels of dietary fiber improves the functioning of the bowel and reduces the degree of constipation. It seems that the use of high-fiber meals in the form of coarse humpy 128 nutrients is a useful strategy for improving the health of pigs (*Whittemore and Elsley, 1975*). According to our results, sows with parity 5 gave the best results. Our recommendation for commercial farms is to introduce body condition score in daily routine. "Thin sow syndrome" on commercial farms can be prevented by correction in the feeding technology and feeding sows during the lactation period. It is recommended to

carry out energy and protein balanced diet during gravidity and lactation, and restrictive diet, the first few days after partitution. In the critical period, at the beginning of lactation, the health control of sows should be performed regularly on a daily basis in order to spot and detect the earliest symptoms of the disease (long sleeping periods, reduced appetite and constipation). Certainly that improved sow nutrition at key stages in the breeding cycle will help improve the number, birth weights and piglet vitality. This break through in sow nutrition can help the sow in supporting larger litters, from birth to weaning. Our findings stress the need for further studies in the etiology and prevention of this condition. Aflatoxins (AFs) are one of the most known and investigated group of mycotoxins, which can be found as contaminants in different types of food and feed. Animals are exposed to AFs mainly through the consumption of contaminated feed, particularly products of plant origin. Among AFs, aflatoxin M1 (AFM1) is the monohydroxylated derivative of AFB1 formed in the liver and excreted into the milk of lactating animals. This study encompassed the Vojvodina region of Serbia and was aimed at determining the levels of AFM1 excretion in sows' milk in the first 3–5 days of lactation, after consumption of naturally contaminated with AFB1 maize. A total of 110 sows' milk samples from 11 swine farms in the specific region were analyzed by Enzyme Linked Immunosorbent Assay (ELISA). Different levels of AFM1 were detected in the majority (97%) of the examined milk samples. The obtained results showed AFM1 levels ranging from 5 to 165.4 ng/L. The results of this study pose special health concern associated with aflatoxin contamination of swine feed raw materials in this particular part of Serbia. Moreover, such high incidence of AFM1 detection in sows' milk may suggest the occurrence of long-term low level aflatoxicosis clinical case (*Prodanov-Radulović et al., 2017*).

Boar on commercial farm

Boars use on commercial farms aims for dwindle costs through producing sperm doses for internal use, and in hand mating if needed. Health control measures for pigs are partly regulated by legislative acts, which are mandatory, and other measures are recommended and depend on farm health policy (*Bojkovski et al., 2017*). Diseases that can be transmitted by sperm to inseminated sows and gilts jeopardize their health and cause economic damage to the farm. In order to minimize secondary expanses in pig production there are several worldwide recommendations for disease prevention and control (where particular infective agent threatens). Those are: vaccination against classical swine Fever, Erysipelas, and Parvovirus (PPV) twice a year, serological surveillance tests annually for Brucellosis, Leptspirsis, PRRS, Aujeszky's disease, Swine, Listeriosis, Atrophic

Rhinitis, Inclusion Rhinitis and Parvoviroses. Introduced boars should be annually tested for tuberculosis and Jhone‟s disease. On the other hand, the possible presence of certain bacteria in seeds boars can not be ignored, the case of: *Staphylococcus* spp, *Corynebacterium* spp, *Pseudomonas* spp, *Streptococcus* spp, *Escherichia* spp, *Proteus* spp, *Klebsiella* spp, *Serratia* spp, *Citrobacter* spp, *Bacillus* spp, *Micrococcus* spp, *Enterobacter* spp, *Eubacterium* spp, *Aerobacter* spp, *Bordetella* spp, *Mycoplasma* spp, *Brucellasuis*, *Actinobacillus*, *Pasteurella*, *Salmonella* spp, *Campylobacter* spp and the possible presence of the following highly infective viruses and contaminating viruses that are transmitted by seed: Pseudorabies, PRRS, Porcine Parvo Virus, African Swine Fever, Adenovirus, Cytomegalovirus, Enterovirus, Foot and Mouth Disease, Hog Cholera, Japanese Encephalitis, Reovirus, Swine Influenza, Swine Vesicular Disease, Transmissible Genital Papilloma. *(Petrujkić et al., 2011)*. The possibility of the spread of infectious agents was significantly reduced by introducing technology of artificial insemination, bacteriological examination of native sperm, prepuce swabs and smears and environment in which boar resides (under the pen walls, feeders, drinkers). Health control of breeding boars and contribute to the improvement of reproductive parameters in commercial farms. Porcine circovirus type 2 (PCV-2) is the major causative agent of post weaning multisystemic wasting syndrome (PMWS) and is associated with different syndromes that affect the swine. The term PCVAD (Porcine circovirus associated diseases) was introduced in 2006 to gather many diseases. Porcine circovirus (PCV) is a DNA, non-enveloped, virus that contains a singlestranded, circular genome and belongs to the family Circoviridae, genus Circovirus. PCV was first identified in 1974 as a contaminant of the continuous porcine kidney cell line PK-15 (*Cavanagh, 1997*) and later found to be non-pathogenic based on experimental inoculation of pigs (*Tischer et al., 1986*). A wasting disease in pigs known as post-weaning multisystemic wasting syndrome (PMWS) was first observed in 1991 in western Canada and later associated with a PCV found to be distinct from the nonpathogenic PCV according to nucleotide sequencing (*Allan et al., 1999a,b*). To differentiate between the two PCV variants, it was decided to use the terminology PCV_1 to indicate non-pathogenic isolates and PCV_2 to indicate isolates associated with disease and PMWS (*Ellis et al.,1998*). Later, PCV2 was also found to be associated with respiratory disease, enteritis, reproductive failure, and porcine dermatitis and nephropathy syndrome (PDNS), which are now collectively known as porcine circovirus associated disease (PCVAD). Retrospectively, PCV_2 has been detected in archived tissues collected as early as 1962 in Germany *(Jacobsen et al., 2009)* and is now found globally. PCV_2 has proven to be ubiquitous in nature, causing disease in varying percentages of animals within a group while the rest of the herd typically does not display clinical signs **(***Balasch et al., 1999*). PCV_2 can be further subdivided into subtypes

2a through 2e) **(***Jantafong et al., 2011*). The hallmark lesion associated with PCV_2 infection is lymphadenopathy, or enlargement of most lymph nodes, which correlates with lymphoid depletion at the cellular level**.**

The exact mechanism of PCV2-associated lymphoid depletion is still unknown and there have been contradicting results. In a conventional pig model, the authors demonstrated the presence of PCV2 antigen within apoptotic lymphocytes using PCV_2 specific immunohistochemistry (IHC) and terminal transferased UTP nick end labeling. The hallmark lesion associated with PCV_2 infection is lymphadenopathy, or enlargement of most lymph nodes, which correlates with lymphoid depletion at the cellular level (*Shibahara et al., 2000*). Apoptosis is a programmed mechanism which keeps a balance between cell proliferation and cell death. Presence of PCV_2 DNA was demonstrated in hepatocytes of clinically affected pigs (*Rosell, 1999***)** and in a follow-up study, 88% of the livers from pigs suffering of naturally occurring PMWS contained apoptotic hepatocytes with apoptotic bodies **(***Rosell et al., 2000***)**. However, contradicting results were obtained by another research group in 2004 who investigated 21 pigs categorized in three lesional severity stages and five healthy control pigs (*Resendes et al., 2004a,b*)**.** Apoptotic rates in lymphoid tissues were correlated inversely with viral load in serum and with severity of lesions (*Resendes et al., 2004***)**. A study conducted in gnotobiotic pigs was unable to associate PCV_2 with apoptosis in hepatocytes and concluded that apoptosis was not the chief pathway for hepatocytic cell loss (*Krakowka et al., 2004*). PCV2b was associated with enhancement of PCVAD in field conditions.

There are two main methods to demonstrate apoptosis in tissues, the TUNEL method and the cleaved caspase-3 (CCasp-3) IHC staining, when investigating the role of apoptosis in lymphoid tissues (*Resendes et al., 2004a,b*) PCVAD is multi-factorial and the presence of co-infections can increase and intensify clinical PCVAD *(Ellis et al., 2004*). Porcine reproductive and respiratory syndrome virus (PRRSV), just like PCV2, has also been associated with apoptosis in infected host cells **(***Choi et al., 2002*). Increased disease severity is commonly observed when pigs are co-infected PCV2 and PRRSV (*Wensvoort, 1992).* PRRSV is an enveloped RNA virus with single-stranded, positive-sense genome that belongs to the family Arteriviridae, genus Arterivirus *(Cavanagh, 1997*). Two PRRSV types, 1 (European) and 2 (North American) exist based on genome sequence analysis (*Wensvoort et al.,1992*). PRRSV infection renders the host unable to clear concurrent infections resulting in increased severity of disease and it is associated with enhanced PCV2 replication which results in increased amounts of PCV2 DNA in coinfected pigs *(Rovira et al., 2002***)**. Both PCV2 virus have a tropism towards pulmonary alveolar macrophages (PAMs) and capable of infecting PAMs (*Deen et al., 2010***)**. The ability of PRRSV to replicate in PAMs *(Bautista et al., 1993*) and the inability of PAMs and dendritic cells to degrade PCV2 (27) might lead to modification of macrophage function that could contribute to immune deregulation **(***Sinha, 2011*).

PCV2 subtypes a and b differ from each other genetically in a signature motif located between nucleotides 1472 and 1476 of open reading frame 2 (ORF2) *(Cheung et al., 2006).* Chimeric DNA clones have been constructed with swapped signature motif regions that were used in *in-vitro* and *in-vivo* studies to determine the significance of the signature motif in virulence and it was concluded that clones containing the reciprocal signature motif had decreased virulence (*Allemandou et al., 2011***)**. Hence, an additional goal of the second study was to determine the importance of ORF2 and the PCV_2 signature motif located in ORF2 in PCV2-PRRSV co-infection *in vitro*. As PRRSV enhances PCV_2 replication, there is a potential of increased PCV_2 shedding of and increased PCV_2 viral load in PRRSV-PCV_2 co-infected pigs that might lead to increased transmission rates of PCV2a or PCV2b in certain pig populations. No differences in shedding of PCV2a versus PCV2b were found in experimentally infected boars (*Madson et al., 2008***)**. PCV_2 vaccination is one of the most widely used vaccination strategies in growing pigs to date and existing PRRSV infections at the time of PCV_2 vaccination is of concern to swine producers and veterinarians. Informations on the efficacy of PCV_2 vaccination in PRRSV viremic pigs are scares (*Allan et al., 1999a,b; Sinha et al., 2011).* The aim of our research was to investigate the prevalence of antibodies to PCV2 with boars from commercial farms in Serbia (*Bojkovski et al., 2017*). There is no PCV type 2 seroprevalence investigated in boars on commercial farms so far in Serbia so far which have intended use for in-house reproduction. On industrial farms, we conducted blood sampling in a total of 28 boars of Landrace, Domestic Yorkshire and Duroc breed. All boars were in exploitation and following reproduction parameters: the number of inseminated gilts, the number of inseminated sows, repeated heats, and the number of fertilized gilts, the number of fertilized sows, number of abortions, number of piglets farrowed, and the number of piglets born dead. Blood samples were taken from the jugular vein. After spontaneous blood clotting at room temperature serum was separated by centrifugation and the aliquots were stored at -20°C till analysis. Serum anti-PCV_2IgM and IgG antibodies were detected by commercially available ELISA "INGEZIM circovirusIgG/IgM®" (Inmulogia y geneticaaplicada, s.a., Madrid, Spain) according to the manufacturer instruction Statistical significance of 95% ($p>0.05$) was observed. Statistical data processing was done using number of boars for observations.
Circovirus infection in our commercial farms caused significant losses due to reduced performance in growth and reproduction, treatment costs and culling. Such a situation makes production unprofitable. The term PCVAD (Porcine circovirus

associated diseases) was introduced in 2006 to gather many diseases. *Stankov et al. (2013)* states that on our commercial farms since 2002 losses in the pig production on a monthly basis have reached to 9% and in fattening operation is increasing up to three times, mostly reaching the value to 15%. The most vulnerable were pigs weighing 40 to 60 kg. The clinical symptoms of the disease in weaned piglets was demonstrated by the second or third week after weaning in the form of weight loss and depression, skin pallor, increase in body temperature up to 41^{o}C, rapid and shallow breathing and pale yellow to golden-yellow aqueous diarrhea and jaundice which was a constant finding. The clinical signs in fattening pigs is related to clinical picture in piglets, with hightlight on the respiratory syndrome, bearing in mind that these animals were the affected with other pathogens (mostly *Mycoplasma hyopneumoniae*, and very rarely with *Pasteurella multocida*). The growth rate of fattening pigs decreased by 6 to 12 kg per pig. Health check of breeding boars should be a complex process within the implementation of health care measures on commercial farms.We have established the prevalence of antibodies to porcine circovirus type 2 PCV2 with boars from commercial farms in operation

Use of metabolic profile on commercial farm

Physiological ranges of blood biochemistry parameters differ substantially for each animal species. Assessment of biochemical parameters included in metabolic profiling have multiple significances in swine health care and production. Parameters of metabolic profile may be an indicator of shortage of food, and sometimes can point out to a variety of subclinical and clinical diseases. In clinically healthy animals (sows, boars), disorders in metabolic profile parameters can also occur, despite the fact that there is no visible symptoms. The material for the research included boars deriving from four commercial swine farms (farms under monitoring A, B, C and D). In order to determine whether there has been a deviation in the physiological ranges of certain biochemical parameters, we analyzed biochemical parameters of 23 boars used in exploitation from a commercial farm "A". The following parameters were examined: Phosphorus (mmol/L), Calcium (mmol/L), Ca/P ratio, Bilirubin (µmol/L), Total proteins (g/L), Glucose (mmol/L), Triglycerides (mmol/L), Albumins (g/L) and Urea (mmol/L). In the examined group of boars, the most variable results were observed in urea and calcium levels. In the blood of 28 boars from a commercial farm "B", 27 boars from commercial farm “C” and 3 boars from commercial farm “D”, we analyzed levels of Phosphorus, Total protein, creatinine and AST. The results of biochemical analysis showed economical validity and represent a significant contribution to monitoring of the health status and production of pigs on commercial farms (*Bojkovski et al., 2013a)***.**

Conclusion

The aim of intensive pig production on commercial farms is to produce an optimal number of piglets or fatteners per sow per year. To achieve this aim, adequate health control program should be designed and implemented. The health status of the herd depends on a relatively large number of factors, such as farm tailored technology of breeding, care, nutrition, farm employee regular base training, as well as the systematic implementation of health care measures

Proizvodne bolesti (tehnopatije) svinja na komercijalnim farmama u Srbiji

Jovan Bojkovski, Marina Spinu, Mihaela Niculae, Jasna Prodanov-Radulović, Aleksandar Stanojković, Ivan Pavlović, Nemanja Zdravković, Radiša Prodanović

Rezime

Cilj ovog preglednog rada je bio da se predstave naša dosadašnja istraživanja vezana za proizvodne bolesti (tehnopatije) na komercijalnim farmama svinja. Prisustvo proizvodnih bolesti je rezultat nepovoljnih uslova u samoj proizvodnji. Uzgoj i eksploatacija nerastova na komercijalnim farmama ima za cilj proizvodnju semena za potrebe farme. Životni vek nerastova, pa samim tim i dužina njihove eksploatacije u velikoj meri zavise od njihovog zdravstvenog stanja. Zdravstveno stanje krmača je takodje od izuzetne važnosti. Pratili smo dva parametra: ocenu telesne kondicije krmača i sindrom mršavih krmača. Ocena telesne kondicije krmača praćena je unutar optimalnih vrednosti (3 u vreme prašenja i 2,5 tokom osemenjavanja). Sindrom mršavih krmača je bolest složene etiologije. Uočeno je da krmače gube telesnu masu tokom peroida laktacije. Gubitak telesne mase dijagnostikovan je u većini slučajeva kod krmača u drugoj laktaciji. Ustanovili smo vrednosti afla toksina u mleku krmače. Praćene su uzgojne bolesti bakterijske i virusne etilogije kod svih kategorija. Na kraju smo dali prikaz biohemijskih parametara kod krmača. Analiza biohemisjkih parametara može da se koristi u slučajevima gde je potrebno da se pruže detaljnije analize u cilju postavljanja što tačnije dijagnostike. Promocija dobrog zdravlja svinja uključuje sprovođenje sistema profilaktičkih mera. Važni faktori u proizvodnji su dobar izbor rase,

veličina stada na farmi, načini primene biotehničkih mera u proizvodnom procesu i stalno edukovanje zaposlenih radnika na farmi.

Ključne reči: tehnopatije, prasad, nerastovi, krmače, komercijana farma

Acknowledgment

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-9/2021-14/200143).

References

ALLAN G.M., KENNEDY S., MCNEILLY F., FOSTER J.C., ELLIS J.A., KRAKOWKA S.J., MEEHAN B.M., ADAIR B.M.(1999a): Experimental reproduction of severe wasting disease by 141 co-infection of pigs with porcine circovirus and porcine parvovirus. J. Comp. Pathol. 121, 1-11.

ALLAN G.M., MCNEILLY F., MEEHAN, B.M., KENNEDY, S., MACKIE, D.P., ELLIS, J.A., CLARK, E.G., ESPUNA E., SAUBI N., RIERA P., BØTNER A., CHARREYRE C.E (1999b): Isolation and characterisation of circoviruses from pigs with wasting syndromes in Spain, Denmark and Northern Ireland, Vet. Microbiol. 66, 115-123.

ALLEMANDOU A., GRASLAND B., HERNANDEZ-NIGNOL A.C., KERANFLEC'H A., CARIOLET R., JESTIN A. (2011): Modification of PCV-2 virulence by substitution of the genogroup motif of the capsid protein, Vet. Res., 42, 54.

BAUTISTA E.M., GOYAL S.M., YOON I.J., JOO H.S., COLLINS J.E (1993): Comparison of porcine alveolar macrophages and CL 2621 for the detection of porcine reproductive and respiratory syndrome (PRRS) virus and anti-PRRS antibody. J. Vet. Diagn. Invest., 5, 163-165.

BALASCH M., SEGALÉS J., ROSELL C., DOMINGO M., MANKERTZ A., URNIZA A., PLANA-DURÁN J. (1999): Experimental inoculation of conventional pigs with tissue homogenates from pigs with post-weaning multisystemic wasting syndrome, J. Comp. Pathol., 121, 139-148.

BOJKOVSKI J., DOBRIĆ Đ., ERSKI-BILJIĆ M., ZAKARIJA (1997): Rezistencija domaćih životinja na antibiotike i njena genetska osnova. I Simpozijum mutageneze, genotoksikologije, Zlatibor, 15-18 septembar. Zbornik kratkih sadržaja radova, C37.

BOJKOVSKI J., RELIĆ R., HRISTOV S., STANKOVIĆ B., SAVIĆ B., PETRUJKIĆ T. (2010a): Contribution to knowledge of health, reproduction,

biosecurity and ecological problems in intensive pig production. Bulletin UASVM, Veterinary Medicine, 67 (2), 37-39.
BOJKOVSKI J., RADOJIČIĆ B.PETRUJKIĆ T., BOROZAN S. (2008): A contribution to insight of the most important etiological factors with influence of farm animal health in Serbia. Biotehnology, Bucharest, Romania series F, Special volume, 101-114.
BOJKOVSKI J., STANKOVIĆ B., PETRUJKIĆ T., PETRUJKIĆ B., SAVIĆ B., ĐOKOVIĆ R., PANTIĆ I., TURAJAČANIN D. (2010b): Review of investigations on influence of environmental chemical contaminants on hereditary base and reproductive capacities of landras breed boars from pig farm, center for reproduction and artificial insemination and biosecurity measures in Serbia. Lucrãri stiinifice medicinã veterinarã Timisoara, vol. XLIII (2), 25-33.
BOJKOVSKI J., PETRUJKIĆ T., STANKOVIĆ B., PETRUJKIĆ B. (2010c): Menadžment zdravlja svinja. Zbornik referata i kratkih sadržaja 21. Savetovanje veterinara Srbije, sa međunarodnim učešćem, Zlatibor, Zbornik kratkih sadržaja, 83.
BOJKOVSKI J., RADOJIČIĆ B., PETRUJKIĆ T., BOROZAN S. (2008b): Prilog poznavanju etioloških činiliaca koji utiču na zdravlje farmskih životinja. 20. Savetovanje veterinara Republike Srbije sa međunarodnim učešćem, Zlatibor, Zbornik referata i kratkih sadržaja, 405-412. BOJKOVSKI J., SAVIĆ B., ROGOŽARSKI D., STOJANOVIĆ D., VASILJEVIĆ T., APIĆ I., PAVLOVIĆ I. (2013a): An outline of clinical cases of disease in pigs at commercial farms. Proceedings of 23th International symposium "New Technologies in Conteproary Animal Production", Novi Sad 19-21 jun, 163-166.
BOJKOVSKI J., ROGOŽARSKI D., VASILJEVIĆ T., STOJANOVIĆ D., SAVIĆ B., PAVLOVIĆ I., RELIĆ R., JANJUŠEVIĆ J. (2013b): Morphological changes in the kidneys of pigs caused by ochratoxin-feeding on the slaughter house (case report). Bulltein USAVM, Veterinarinary Medicine, 70(2) 195-197.
BOJKOVSKI J., JANJUŠEVIĆ J., ROGOŽARSKI D., PETRUJKIĆ T., SAVIĆ B., STOJANOVIĆ D., PAVLOVIČ I., DJEDOVIČ S. (2013c): Contribution to knowledge of espophagogastric ulcer in swine in Serbia (case report). Lucrãri stiinifice medicinã veterinarã Timisoara, 67, 4, 17-23. BOJKOVSKI J., PRODANOV-RADULOVIČ J., PRODANOVIČ R., VUJANAC I., NEDIČ S., ZDRAVKOVIČ N., PAVLOVIČ I., RELIČ R., BECKEI Z. (2018): Common pig disease on comercial farm-a review. Lucrãri stiinifice medicinã veterinarã Timisoara, vol. LI (3), 14-27. BOJKOVSKI J. VASIČ A., ZDRAVKOVIĆ N., ROGOŽARSKI D., PETRUJKIĆ T., MILANOV D., KORICA S., DJEDOVIĆ S. (2014): Control of body score condition of sows on comercial farms. Lucrãri stiinifice medicinã veterinarã Timisoara, 62, 1, 15-19.

BOJKOVSKI J., ILIĆ V., VASIĆ A., MALTETIĆ J., VAKANJAC S., SAVIĆ S., ŠTUKELJ M., ZDRAVKOVIĆ N., TZIKA E., NICULAE M., MILANOV D. (2017): Invcestigation of porcine cireko virus type 2(PCV2) antibodies in clinicaly healthy boars from Serbia. Journal of the helenic veterinary medical society, 68, 3, 347-354.

BLACKBURN P.W. (1995): Curent problems on a new approaches to pig health. Chapter 7 In: the health of pigs By Jon Hill and David Sainsbury,Logman Veterinary Health Sourcies, 221-222.

CAVANAGH D. (1997): Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch. Virol., 142, 629-633.

CHANG H.W., JENG C.R., LIN T.L., LIU J.J., CHIOU M.T., TSAI Y.C., CHIA M.Y., JAN T.R., PANG V.F. (2006): Immunopathological effects of porcine circovirus type 2 (PCV2) on swine alveolar macrophages by in vitro inoculation. Vet. Immunol. Immunopathol, 110, 207-219.

CHOI C., CHAE C. (2002): Expression of tumour necrosis factor-alpha is associated with apoptosis in lungs of pigs experimentally infected with porcine reproductive and respiratory syndrome virus. Res. Vet. Sci., 72, 45-49.

CHEUNG A.K., LAGER K.M., KOHUTYUK O.I., VINCENT A.L., HENRY S.C., BAKER R.B., ROWLAND R.R., DUNHAM A.G. (2007): Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. Arch. Virol., 152, 1035-1044.

COFFEY R., PARKER R.G. (1999): Assessing sow body condition. Cooperative extensioin service, University of Kentucky, College of Agricultural.

DEEN J. (2010): Pigs: Behaviour and welfare assessment. Encyclopedia of Animal Behaviour, 731-722.

CUTLER R. (2013): Mananing pig health. 2nd edtion, M.Murihead and T. Alexandar, pp 654.

DJORDJEVIC N., MAKEVIC M., GRUBIC G., JOKIC Ž. (2009): Ishrana domaćih i gajenih zivotinja, Poljoprivredni fakultet, Univerziteta u Beogradu, (on serbian language) pp 749-812.

ELLIS J., HASSARD L., CLARK E., HARDING J., ALLAN G., WILLSON P., STROKAPPE J., MARTIN K., MCNEILLY F., MEEHAN B., TODD D., HAINES D. (1998): Isolation of circo virus from lesions of pigs with post weaning multisystemic wasting syndrome. Can. Vet. J., 39, 1, 44-51.

ELLIS J., CLARK E., HAINES D., WEST K., KRAKOWKA S., KENNEDY S., ALLAN G.M. (2004): Porcine circovirus-2 and concurrent infections in the field. Vet. Microbiol., 98, 159-163.

GOLINAR O.I., VALENČAK Z. (2006): Porcine respiratory disease complex (PRDC) in Slovenia. Proceedings of the 19th Ineternational Pig Veterinary Society Congres, Copenhagen, Denmark, 291.

HONNOLD C.(1999): Porcine respiratory

disease complex. http//www. ces.purdue. edu/ pork health/caryhonnold.html (8 july 2008 date last accessed)

IVETIČ V., ŽUTIĆ M., VALTER D., ŠAMANC H. (2000): Mikoplazmatska pneumonija (MP) i aktinobacilusna pleuropneumonija (APP) komponente kompleksa respiratorne bolesti (PRDC) svinja. Zbornik radova 3 simpozijuma "Uzgoj i zaštita zdravlja svinja", Pilotska akademija Vršac, 69-75.

IVETIĆ V., ŽUTIĆ M., SAVIĆ B., MILOŠEVIĆ B. (2005): Kompleks respiratornih bolesti kod svinja dijagnostika i mere kontrole. Zbornik radova i kratkih sadržaja 17. Savetovanja veterinara Srbije sa medjunarodnim učešćem, 7-10 septembar, 190-198 .

JACOBSEN B., KRUEGER L., SEELIGER F., BRUEGMANN M., SEGALES J., BAUMGAERTNER W. (2009): Retrospective study on the occurrence of porcine circovirus 2 infection and associated entities in Northern Germany. Vet. Microbiol., 138, 27-33.

JANTAFONG T., BOONSOONGNERN A., POOLPERM P., URAIRONG K., LEKCHAROENSUK C., LEKCHAROENSUK P.(2011**)**: Genetic characterization of porcine circovirus type 2 in piglets from PMWS-affected and -negative farms in Thailand. Virol J., 8, 88.

KRAKOWKA S., ELLIS J., MCNEILLY F., MEEHAN B., OGLESBEE M., ALLDINGER S., ALLAN G. (2004): Features of cell degeneration and death in hepatic failure and systemic lymphoid depletion characteristic of porcine circovirus-2-associated postweaningmultisystemic wasting disease. Veterinary Pathology, 41, 471-481.

LONČAREVIĆ A., MARIČIĆ Z., TOSEVSKI J., PAVLOVIĆ I. (1997): Osnove sistematskog zdravstvenog nadzora i programiranje zdravstvene zaštite svinja u intenzivnom odgoju. U monografiji: A. Lončarević: Zdravstvena zaštita svinja u intenzivnom odgoju. Naučni institut za veterinarstvo Srbije, Beograd, 517-523.

LIPEJ Z. (2015): Bolesti svinja. Medicinska naklada, Zagreb, Hrvatska.

MADSON D.M., RAMAMOORTHY S., KUSTER C., PAL N., MENG X.J., HALBUR P.G., OPRIESSNIG T. (2008): Characterization of shedding patterns of porcine circovirus types 2a and 2b experimentally inoculated mature boars. J. Vet. Diagn. Invest., 20, 725-734.

HUTU I., ONAN W.G. (2008): Alternative swine management system. TVT publishing, 25-35. PAVLOVIĆ I., SAVIĆ B., BOJKOVSKI J., DOBROSAVLJAVIĆ I., STOJANOV I., STOKIĆ- NIKOLIĆ S., KURELKUŠIĆ B., JOVČEVSKI S.T., JOVČEVSKI S.R. (2019): Bolesti svinja uzrokovani cestodama. Zbornik radova sedamnaestog savetovanja sa medjunarodnim učešćem, Zdravstvena zaštita, selekcija i reprodukcija svinja, 78-82.

PETRUJKIĆ T., BOJKOVSKI J., PETRUJKIČ B. (2011): Reprodukcija svinja, pp.98-145.

PRODANOV-RADULOVIĆ J., PUŠIĆ I., ŽIVKOV-BALOŠ M., BOJKOVSKI J. (2018): Faktori koji mogu da utiču na učestalost i intenzitet oboljenja digestivnog trakta prasadi. Zbornik radova, XVI Simpozijum 'Zdravstvena zaštita, selekcija i reprodukcija svinja' sa međunarodnim učešćem, 31.5-2.6., Srebrno jezero, Veliko Gradište.

PRODANOV–RADULOVIĆ J., ŽIVKOV–BALOŠ M., JAKŠIĆ S., GRGIĆ Ž., STOJANOV I., BOJKOVSKI J., TASIS P.D. (2017): Afla toxin M1 levels in sow milk. Journal of the Hellenic Veterinary Medical Society, 68, 3, 341-346.

ROSELL C., SEGALÉS J., PLANA-DURÁN J., BALASCH M., RODRÍGUEZ-ARRIOJA G.M., KENNEDY S., ALLAN G.M., MCNEILLY F., LATIMER K.S., DOMINGO M. (1999): Pathological, immunohistochemical, and in-situ hybridization studies of natural cases of post weaning multisystemic wasting syndrome (PMWS) in pigs. J. Comp. Pathol., 120, 59-78**.**

ROSELL C., SEGALÉS J., RAMOS-VARA J.A., FOLCH J.M., RODRÍGUEZ-ARRIOJA G.M., DURAN C.O., BALASCH M., PLANA-DURÁN J., DOMINGO M. (2000): Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. Vet. Rec., 146, 40-43.

RESENDES A.R., MAJO N., SEGALÉS J., MATEU E., CALSAMIGLIA M., DOMINGO M., (2004a): Apoptosis in lymphoid organs of pigs naturally infected by porcine circovirus type 2. J. Gen. Virol., 85, 2837-2844.

RESENDES A.R., MAJO N., SEGALES J., ESPADAMALA J., MATEU E., CHIANINI F., NOFRARIAS M., DOMINGO M. (2004b): Apoptosis in normal lymphoid organs from healthy normal, conventional pigs at different ages detected by TUNEL and cleaved caspase-3 immunohistochemistry in paraffin-embedded tissues. Vet. Immunol. Immunopathol., 99, 203-213.

ROVIRA A., BALASCH M., SEGALÉS J., GARCIA L., PLANA-DÚRAN J., ROSELL C., ELLERBROK H., MANKERTZ A., DOMINGO M.(2002): Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. Virol., 76, 3232-3239.

SAVIĆ B., MILIČEVIĆ V., JAKIĆ-DIMIĆ D., BOJKOVSKI J., PRODANOVIĆ R., KURELJUŠIĆ B., POTKONJAK A. (2012): Genetic caracterization and phylogenetic analysis of porcine cirkovirus type 2 (PCV-2) in Serbia. Archives of Virology, 157(1), 21-28.

STANKOV P., STANIMIROV D., MIRILOVIĆ M. (2013): The effect of vaccination of sows against PCV2 viruses on brood size and farrowing rate. Proc. Symposium "Health care, selection and reproduction pigs", Srebrno jezero, Serbia, 31 May-2 June 2013, pp 49-54.

SIMEUNOVIĆ P., PRODANOVIČ R., VUJANAC I., ŠTUKELJ M., BOJKOVSKI J. (2016): Bolesti svinja. Praktikum, Naučna, Beograd, pp 100.

SHIBAHARA T., SATO K., ISHIKAWA Y., KADOTA K. (2000): Porcine circovirus induces B lymphocyte depletion in pigs with wasting disease syndrome. Journal of Veterinary Medical Science 62, 1125-1131.

SINHA A. (2011): Interactions of porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus. PhD thesis, Iowa State University, USA.

TABELONG R., KAMPHUES J., SHIWIER S. (2003): Effects of different feeding and housing conditions on dry matter content and consistency of faeces in sow. J. Anim. Psyilogy and Animal Nutrition, 87, 3-4, 116-121.

TISCHER I., MIELDS W., WOLFF D., VAGT M., GRIEM W. (1986): Studies on epidemiology and pathogenicity of porcine circovirus. Arch. Virol., 91, 271-276.

TREASURE J.L., OWEN J. (1997): Intrigfuing links between animal behavior and anorexia nervosa. Intenational journal of eating disoders, 2, 4, 1-5.

WENSVOORT G., DE KLUYVER E.P., LUIJTZE E.A., DEN B.A., HARRIS L., COLLINS J.E., CHRISTIANSON W.T., CHLADEK D. (1992): Antigenic comparison of Lelystad virus and swine infertility and respiratory syndrome virus. J. Vet. Diagn. Invest., 4, 134-138.

WHITTEMORE C.T., ELSLEY F.W.H. (1975): Savremena ishrana svinja. Zadrugar, Sarajevo, (prevod sa Engleskog jezika), pp 84-116.

ŠAMANC H. (2009): Bolesti svinja, Naučna KMD, Beograd, pp 248- 329.

CHEMICAL COMPOSITION AND FATTY ACID PROFILE OF TWO TRADITIONAL BULGARIAN DRY-CURED MEAT PRODUCTS MADE OF EAST BALKAN PIG

Teodora Popova[1], Nadezhda Palova[2], Jivko Nakev[3], Maya Ignatova[1]

[1]Institute of Animal Science- Kostinbrod, Pochivka str., 2232 Kostinbrod, Bulgaria
[2]Scientific Center of Agriculture- Sredets, "Bulgaria"square, 8300, Sredets, Bulgaria
[3]Agricultural Institute- Shumen,3"Simeon Veliki" blvd, 9700 Shumen, Bulgaria
Corresponding author: Teodora Popova, tlpopova@yahoo.com

Abstract: The aim of this work was to present two traditional Bulgarian dry-cured products (dry-cured loin and dry-cured sausage called "Strandzhansko dyado") made of meat from East Balkan Pig. This is a Bulgarian indigenous breed raised in the Eastern part of the country with unique meat, suitable for producing of high-quality meat products. The chemical composition, fatty acid profile and lipid nutritional indices in both dry- cured products were analysed. Cured loin had substantially higher protein content than „Strandhansko dyado"(36.71% vs. 18.01%, P<0.0001) but lower fat content (7.97% vs. 29.16%, P<0.0001). Both products showed similar content of saturated fatty acids (38.28%-38.54%). The percentage of monounsaturated fatty acids (MUFA) was higher (P=0.0016) and that of polyunsaturated fatty acids (PUFA) was lower (P<0.0001) in the cured loin. The products had high n-6/n-3 ratio but relatively low atherogenic index.

Keywords: dry-cured products, East Balkan Pig, chemical composition, fatty acids

Introduction

The growing demands of the consumers for healthy diet call for searching of ways to improve food quality. More meat producers are oriented towards the application of alternative ways of raising farm animals, which will allow the production of high quality meat with good nutritional composition and healthy value. More often, the animals are reared under extensive systems, allowing free access to pasture and these systems are applied not only for the ruminant animals but also for pigs *(Blumetto Velazco et al., 2013; Park et al., 2017).* Rearing under extensive production systems has beneficial effect on the fatty acid composition of meat and also allows accumulation of natural antioxidants that are important for healthy nutrition *(Nilzén et al., 2001; Parunovic et al., 2012).* The East Balkan Pig is an indigenous Bulgarian breed similar to the wild boar (Figure 1). According to *Marchev et al. (2018)* until the middle of the last century the East Balkan pigs have been widely spread all over Bulgaria. However, nowadays the breed is reared only in Varna, Shumen and Burgas districts, in the Eastern part of Bulgaria. The animals have excellent adaptability to extensive rearing conditions. Usually they are reared in herds, outdoors with pasture access and additional feed. The meat of the East Balkan pigs has dark red colour and unique flavour. It is suitable for manufacturing of high quality meat products. So far, to our knowledge, traditional meat products made of East Balkan pigs have not been investigated. Hence, our study aims to present the chemical composition and fatty acid profile of two meat products, dry-cured loin and dry-cured sausage "Strandzhansko dyado", made of East Balkan pigs.

Figure 1. East Balkan Pigs (*Photo: T. Popova*)

Material and Methods

Animals and meat

The meat for manufacturing of the products was derived from castrated male and female East Balkan pigs. The animals were reared in the farm of the Scientific Center of Agriculture-Sredets, Bulgaria. They were reared under traditional technology with daily access to pasture in Strandzha mountain. The pasture had high content of cereal components: wiregrass (*Cynodon dactylon*), bluestem (*Dichanthium ischaemum*), bulbous bluegrass (*Poa bulbosa*), perennial ryegrass (*Lolium perenne*), cock's foot (*Dactylis glomerata*), lower participation of legumes: clover (*Trifolium*), alfalfa (*Medicago sativa*), bird's foot trefoil (*Lotus corniculatus*), bush vetch (*Vicia sepium*) and other various species: plantains (*Plantago*), denseflower mullein (*Verbascum densiflorum Bertol*.), dandelion (*Taraxacum officinale*). During autumn, acorns were naturally present in the diet of the pigs. According to their category, the pigs received additionally organic feed (50 % barley and 50% wheat) prepared in the feed unit of the Center. Water was provided *ad libitum*. The animals were slaughter in a certified abattoir at an average live weight 102.9±6.19 kg for the castrated males and 103.25±5.37 kg for the females.

Manufacturing of the products

Two traditional Bulgarian dry-cured products were manufactured according to the traditional technology in a meat processing plant as presented below:

"**Strandzhansko Dyado"** (Figure 2). The product was made of lean meat and meat from the belly of the carcass. The meat was previously cooled and subjected to deboning and sorting. The de boned and sorted meat with removed tendons was cut into pieces (200 g) and placed into refrigerator at -5°C for 24h and further cut into bowel cutter until the meat particles reach 18-20 mm. The meat was seasoned with a commercial mixture containing salt, dextrose and nitrate/nitrite, additionally black pepper, paprika and savoury were used as spices. The stuffing was dense into casings. No air was allowed into the stuffed casings. The sausages were hung and placed into a room for draining and drying at 10-12°C, relative humidity 80-85 % for 24h. The drying was done in drying rooms at 10-12°C, relative humidity 75%. During the drying, the product was pressed 3-4 times. Drying continued 28 -30 days.

Figure 2. Strandzhansko dyado (*Photo: T. Popova*)

Dry-cured loin (Figure 3)**.** The product was made from *m. longissimus dorsi***.** The muscles were removed from the carcasses, the fat was partially removed and then cut into pieces approximately 25-30 cm long. The pieces were covered with sea salt for 5 days. Then the pieces were washed under cold running water and left to drain for 4 h. They were further hung to dry in drying rooms at 10-12°C, relative humidity 75% or 28-30 days.

Two batches of the products were made and three replicates of each batch were taken for analysis.

Figure 3. Dry cured loin (*Photo: T. Popova*)

Chemical composition analysis

Samples of both dry-cured products were ground in a meat grinder until homogenous. Moisture, intramuscular fat, protein and ashes contents were determined according to ISO 1442 (1997); AOCS (2005); ISO 937 (1978) and ISO 936 (1998), respectively. Determinations were made in triplicate per meat sample.

Analysis of the fatty acid profile

The fatty acid composition was determined according the method of *Bligh and Dyer (1959)* with slight modifications as described by *Vargas-Ramella (2020).* For fat extraction, 10 g of the muscle sample were homogenized with 10 mL of chloroform and 20 mL of methanol for 30 s. After, 10 mL of chloroform and 10 mL of NaCl (1% in distilled water) were added to the mixture and homogenized for 30 s. The chloroform layer (with the fatty acids) was then separated from the residues and aqueous layer by centrifugation (4000 rpm for 10 min) and finally the chloroform was evaporated. The fatty acids were transesterified according to the procedure previously described by *Domínguez et al. (2005)*, with some modifications: for the fatty acids transesterification, 20 mg of extracted fat dissolved in 1 mL of toluene were mixed with 2 mL of a sodium methoxide (0.5 N) solution, vortexed for 10 s and allowed to stand for 15 min at room temperature. Then, 4 mL of a H_2SO_4 solution (10% of H_2SO_4 in methanol) was added, vortexed for 10 s and left for 5 min before adding 2 mL of saturated sodium bicarbonate solution. For the extraction of fatty acid methyl esters, 1 mL of hexane was added to the samples, vortexed for 10 s and the organic phase was then transferred to an appropriate GC vials. Separation and quantification of FAMEs were carried out using gas chromatograph CSi 200 (Cambridge Scientific Instruments Ltd., UK) equipped with a capillary column (DM-2330:30 m×0.25 mm×0.20 µm, Dikma Technologies Inc., China) and hydrogen as a carrier gas. The oven temperature was first set to 160 °C for 0.2 min, then raised until 220 °C at a rate of 5 °C/min and then held or 5 min. The temperatures of the detector and injector were 230 °C. Methyl esters were identified through comparison to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters (FAME) identified *(Christie, 1973).*

The amount of each fatty acid was used to calculate the atherogenic (AI) and thrombogenic (TI) indices as proposed by Ulbricht and Southgate (1991):
AI = (4×C14:0+C16:0)/[MUFA+Σ(n-6)+Σ(n-3)]
TI = (C14:0+C16:0+C18:0)/[0.5×MUFA+0.5×(n-6)+3×(n-3)+(n-3)/(n-6)].

Statistical analysis

Data was statistically evaluated through t-test using JMP .7 statistical package. The results were presented as Mean ± SD.

Results and Discussion

Chemical composition of the dry cured products

Table 1. Chemical composition of dry cured loin and "Strandzhansko dyado"

Product	Protein, %	Fat, %	Moisture, %	Ash, %
Cured loin	36.71±1.49	7.97±0.90	44.26±1.64	10.15±0.07
Strandzansko dyado	18.01±0.21	20.16±0.56	35.65±0.86	4.96±0.07
P	<0.0001	<0.0001	0.0006	<0.0001

Data presented as Mean ± SD (n=3)

The chemical composition analysis showed significant difference between both products. As can be seen from Table 1, the cured loin had high protein content (36.7%) and a fat content of 7.97%. Similar values of these components were reported for dry cured loin from Chato Murciano native breed (respectively 37% protein and 8.53 % fat) *(Salazar et al., 2013)*. In their study the authors compared cured loins of native and modern pig breeds and found that the fat content of the cured loin of the modern breed was considerably lower (1.68 %) than in the native breed. Lower fat content in dry cured fillet available in the market (1.94% - 3.60%) was reported by *Nikolov (2019)*. In Celta pigs, fed with chestnuts, *Gomez et al. (2018)* reported 13.97 % fat content in dry cured loin. Fat content is an important quality trait of meat and meat products since it is closely associated with sensory traits such as tenderness, juiciness and flavor *(Thompson, 2004)*. Also, despite the health concerns, many consumers prefer fattier meat *(Frank et al., 2016)*. In this regard, the fat content of "Strandzansko dyado" was much higher (29.16%, P<0.0001) than in the cured loin while the protein content is considerably lower (18.01%, P<0.0001). This is not surprising, since both products are completely different and meat from very different anatomical locations is used for each type of the product. The fat content of "Strandzansko dyado" might be comparable to that of other dry-fermented sausages made of minced pork *(Kovacevic et al., 2010; Parunovic et al., 2017; 2019)*. *Alves et al. (2015)*, however reported higher content of fat in Portuguese and Serbian dry-fermented sausages ranging from 35.3%-40.7%. The higher fat content could be attributed to the specifics of the technology. In our product we used only meat and no additional solid fat or backfat. As seen from Table 1. the dry-cured loin had significantly higher moisture (P=0.0006) and ash content (P<0.0001) in comparison to ”Strandzhansko dyado”. The moisture and ash content of both products are within the range of the values reported by other scientist for traditional dry cured sausages *(Parunovic et al., 2013)* and dry cured loin *(Pateiro et al., 2014)*.

Fatty acid composition of the dry-cured products

A total of 17 fatty acids were identified in the two products. The major fatty acids were palmitic (C16:0) and oleic acid (C18:1n-9) as presented in Table 2. The content of C16:0 was slightly lower in “Strandzhansko dyado” compared to the dry cured loin (23.39 % vs. 26.06%, P=0.0030), while the percentage of C18:1n-9 was very similar (50.06%-50.37%), and did not differ between both products. The other saturated fatty acids with significant content were stearic (C18:0) followed by myristic fatty acid (C14:0). It could be noticed that while the cured loin had significantly lower content of C18:0 than “Strandzhansko dyado” (9.89% vs. 13.20%, P=0.0002), the latter showed lower levels of C14:0 (1.53% vs. 2.07%, P=0.0133). Furthermore, both products displayed high content of C18:1n-9, and this is beneficial for the human health. Numerous studies have shown that C18:1n-9 presents modulatory effects in a wide physiological functions, and also suggest a beneficial effect on cancer, autoimmune and inflammatory diseases *(Sales-Campos et al., 2013)*. This compensates for the significant content of C16:0 and C14:0 that are considered hypercholesterolemic *(Mensink et al., 2005)*. The content of C18:1n-9 in the two dry cured products was very similar to the content determined in a previous study in *m. LD* in East Balkan pigs *(Popova et al., 2015)*. The cured loin had lower proportion of the essential fatty acids C18:2n-6 (3.90% vs. 6.05%, P<0.0001) and C18:3n-3 (0.29% vs. 0.68%, P=0.0116), however it had higher content of C20:4n-6 (0.52% vs. 0.37%,) than the dry cued sausage. The content of these three fatty acids in the dry cured products was much lower than their proportion reported in meat *(Popova et al., 2015)* which indicates well the changes that have become during the process and drying of the products. The same could be observed in regard to the long chain polyunsaturated fatty acids (LC-PUFA).

While C20:5n-3 was not detected in the cured loin, tendencies towards lower percentage of C22:5n-3 and higher of C22:6 n-3 were observed in this product, when compared to the Strandzhansko dyado. *Parunovic et al. (2017)* reported similar fatty acid proportions in regard to

C14:0; C16:0; C18:0 in dry cured pork sausages made of indigenous breeds Mangalitsa and Moravka. However, the authors found lower C18:1n-9 and considerably higher content of C18:2n-6 when compared to “Strandzhansko dyado”. In dry cured loins made of meat derived from Large White and Great York crossed pigs, *Hoz et al. (2007)* reported lower content of C14:0 and C18:1n-9, but higher proportion of C18:2n-6, C18:3n-3 and the LC-PUFA. Overall, the fatty acid profile may vary considerably and is difficult to compare with other studies since it can be very strongly affected by factors such as the animal nutrition, but also breed. Usually the meet of the modern pig breeds have thinner backfat and low intramuscular fat content which is associated with lower content of C18:1n-9 and higher of C18:2n-6 *(Wood et al., 2008)*. In regard to the total contents of the fatty acids dry cured loin and “Strandzhansko dyado” had the same percentage of saturated fatty acids (38.28%-38.54%), however, the content of monounsaturated fatty acids (MUFA) was higher (56.43% vs. 53.66%, P=0.0016) and that of PUFA was lower (5.29%-7.80%, P<0.0001) in the dry cured loin.

Table 2. Fatty acid composition of dry cured loin and “Strandzhansko dyado” (% FAME)

Fatty acids	Dry cured loin	Strandzansko dyado	P
C14:0	2.07±0.31	1.53±0.02	0.0133
C15:0	0.08±0.01	0.08±0.00	0.9561
C16:0	26.06±0.91	23.39±0.32	0.0030
C16:1	6.04±0.16	2.86±0.04	<0.0001
C17:0	0.18±0.01	0.34±0.02	<0.0001
C17:1	0.33±0.03	0.43±0.03	0.0062
C18:0	9.89±0.51	13.20±0.25	0.0002
C18:1n-9	50.06±0.79	50.37±0.39	0.4849
C18:2n-6	3.90±0.01	6.05±0.12	<0.0001
C18:3n-6	0.18±0.02	0.03±0.02	0.0049
C18:3n-3	0.29±0.16	0.68±0.09	0.0116
C20:2n-6	0.16±0.02	0.34±0.02	0.0008
C20:3n-6	0.06+0.03	0.09±0.01	0.0596
C20:4n-6	0.52±0.17	0.37±0.05	0.1059
C20:5n-3	ND	0.08±0.00	-
C22:5n-3	0.09±0.01	0.12±0.02	0.1254
C22:6n-3	0.09±0.01	0.05±0.01	0.1495
SFA	38.28±0.71	38.54±0.49	0.5691
MUFA	56.43±0.66	53.66±0.42	0.0016
PUFA	5.29±0.05	7.80±0.08	<0.0001

SFA –saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA, polyunsaturated fatty acids, Data presented as Mean ± SD (n=3); ND- not detected

The lipid nutritional indices (Table 3) showed that the ratio n-6/n-3 is higher than the recommended valued of 4.0 *(Enser et al., 2001)*,10.25 vs. 7.39, respectively for the dry-cured loin and "Strandzhansko dyado". On the other hand in both products the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) was lower that 0.4 that was recommended as the minimum value *(Wood et al. 2003)*. In dry fermented sausages, *Parunovic et al. (2013)* reported n-6/n-3 ratio within the range of 17.6-23, while the PUFA/SFA ratio varied within 0.32-0.37. In dry cured loin from Korean native pigs, *Seong et al. (2015)* observed ratio PUFA/SFA decreasing from 0.25 to 0.17 during the ripening time of 90 days. Atherogenic (AI) and thrombogenic (TI) indices are lipid quality indicators depending on the changes of particular groups of fatty acids. They show the potential effects of the lipids on the risk of development of cardiovascular diseases. The AI and TI for both products in this study showed lower values than the observed by *Parunovic et al. (2017).* The athrogenic and thromobogenic indices were within the range of the reported from *Kasprizyk et al. (2015)* in local Pulawska breed and Polish Landrace.The low values of AI and TI in both dry-cured products in our study are due to the high content of MUFA and PUFA and may indicate certain advantage in regard to the healthy value of this kind of products.

Table 3. Lipid nutritional indices

Product	n-6/n-3	PUFA/SFA	AI	TI
Dry-cured loin	10.25±0.49	0.13±0.004	0.55±0.04	1.18±0.02
Strandzansko dyado	7.39±0.39	0.20±0.004	0.48±0.008	1.15±0.02
P	0.1302	<0.0001	0.0119	0.0704

PUFA/SFA- ratio between polyunsaturated and saturated fatty acids, AI-atherogenic index, TI-thrombogenic index, Data presented as Mean ± SD (n=3)

Conclusions

The study characterized the chemical composition and the fatty acid profile of dry-cured loin and sausage "Strandzhansko dyado", showing considerable differences in the analysed traits between them. Both dry-cured products displayed high protein and fat content, however the dry-cured loin showed some advantage in regard to the significantly higher protein but lower fat in comparison to the dry-cured sausage. Fatty acid profile showed higher percentage of MUFA in the dry-cured loin, however, "Strandzhansko dyado" had favourably higher PUFA proportion Both products had high n-6/n-3 ratio and relatively low atherogenic indices.

Hemijski sastav i profil masnih kiselina dva tradicionalna bugarska suhomesnata proizvoda od istočnobalkanske svinje

Teodora Popova, Nadezhda Palova, Jivko Nakev, Maya Ignatova

Rezime

Cilj ovog rada bio je da se predstave dva tradicionalna bugarska suhomesnata proizvoda (suhomesnata pečenica i suvo sušena kobasica pod nazivom „Strandzhansko dyado“) od mesa istočnobalkanske svinje. Ovo je bugarska autohtona rasa uzgajana u istočnom delu zemlje sa jedinstvenim mesom, pogodna za proizvodnju visokokvalitetnih mesnih prerađevina. Analiziran je hemijski sastav, profil masnih kiselina i nutritivni indeksi lipida u oba sušena proizvoda. Suva pečenica ima znatno veći sadržaj proteina od proizvoda „Strandhansko dyado“ (36,71% vs. 18,01%, P <0,0001), ali niži sadržaj masti (7,97% vs. 29,16%, P <0,0001). Oba proizvoda su pokazala sličan sadržaj zasićenih masnih kiselina (38,28%-38,54%). Procenat mononezasićenih masnih kiselina (MUFA) bio je veći (P = 0,0016), a procenat polinezasićenih masnih kiselina (PUFA) je bio niži (P <0,0001) u suvoj pečenici. Proizvodi su imali visok odnos n-6/n-3, ali relativno nizak aterogeni indeks.

Ključne reči: suhomesnati proizvodi, istočnobalkanska svinja, hemijski sastav, masne kiseline

References

ALVES, S. P., ALFAIA, C. M., ŠKRBIĆ, B., ĐURIŠIĆ-MLADENOVIĆ, N., FERNANDES, M. J., BESSA, R. J. B., FRAQUEZA, M. J. (2015): Tracing nutritional composition of dry fermented sausages from distinct origins. Journal of Food Processing and Preservation, 39(6), 2969–2978.
AOCS (2005) AOCS Official Procedure Am 5 -04. Rapid determination of oil/fat utilizing high temperature solvent extraction (Urbana, IL, USA, American Oil Chemists Society).
BLIGH, E. G., DYER, W. Y. (1959): A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911-917.
BLUMETTO VELAZCO, O. R., CALVET SANZ, S., ESTELLÉS BARBER, F., VILLAGRÁ GARCÍA, A. (2013): Comparison of extensive

and intensive pig production systems in Uruguay in terms of ethologic, physiologic and meat quality parameters. Revista Brasileira de Zootecnia, 42(7), 521-529.

CHRISTIE, W.W. (1973): Lipid analysis. Pergamon Press. Oxford. 352 p.

DOMÍNGUEZ, R., CRECENTE, S., BORRAJO, P., AGREGÁN, R., LORENZO, J.M. (2015): Effect of slaughter age on foal carcass traits and meat quality. Animal, 9, 1713–1720.

FRANK, D., JOO, S. T., WARNER, R. (2016): Consumer Acceptability of Intramuscular Fat. Korean Journal of Food Science and Animal Resources, 36(6), 699-708.

GÓMEZ, M., CACHALDORA, A., FONSECA, S., DOMÍNGUEZ, R., CARBALLO, J., FRANCO, I. (2018): Biochemical, oxidative, and lipolytic changes during vacuum-packed storage of dry-cured loin: effect of chestnuts intake by Celta pigs. Journal of Food Quality, 2018, Article ID 7690501.

HOZ, L., CAMBERO, I., SANTOS, C., HERRANZ, B., ORDÓÑEZ, J. A. (2007): Fatty acids and sensory characteristics of Spanish dry-cured loin enriched in acid α-linolenic and α-tocopherol. Food Chemistry, 101(4), 1701–1706.

ISO 1442 (1997): Determination of moisture content, in: International standards meat and meat products (Geneva, Switzerland, International Organization for Standardization).

ISO 936 (1998): Determination of ash content, in: International standards meat and meat products (Geneva, Switzerland, International Organization for Standardization).

ISO 937 (1978): Determination of nitrogen content, in: International standards meat and meat products (Geneva, Switzerland, International Organization for Standardization).

KOVAČEVIĆ, D., MASTANJEVIĆ, K., ŠUBARIĆ, D., JERKOVIĆ, I., MARIJANOVIĆ, Z. (2010): Physico-chemical, colour and textural properties of Croatian traditional dry sausage (Slavonian Kulen). MESO: Prvi hrvatski časopis o mesu 12 (5), 270-275.

MARCHEV, Y., DONEVA, R. K., DIMITROVA, D. (2018): East Balkan Swine-autochthonous Bulgarian breed. Archivos de Zootecnia, Proceedings IX Simposio Internacional sobre el Cerdo Mediterráneo: 61-65.

MENSINK, R. P. (2005): Effects of stearic acid on plasma lipid and lipoproteins in humans. Lipids, 40(12), 1201-1205.

NIKOLOV, V. (2020). Hygiene and qualitative studies in the production of raw-dried meat products. PhD thesis, University of forestry, Sofia.

NILZÉN, V., BABOL, J., DUTTA, P. C., LUNDEHEIM, N., ENFÄLT, A. C., LUNDSTRÖM, K. (2001): Free range rearing of pigs with access to pasture grazing - effect on fatty acid composition and lipid oxidation products. Meat Science, 58(3), 267-275.

PARK, H.S., MIN, B., OH, S. H. (2017): Research trends in outdoor pig production - A review. Asian-Australasian Journal of Animal Science, 30 (9), 1207-1214.

PARUNOVIĆ, N., RADOVIĆ, Č., SAVIĆ, R. (2017): Sensory properties and fatty acids profiles of fermented dry sausages made of pork meat from various breeds. IOP Conference Series: Earth and Environmental Science, 85, 012014.

PARUNOVIĆ, N., PETROVIĆ, M., MATEKALO-SVERAK, V., RADOJKOVIĆ, D., RADOVIĆ, Č. (2013): Fatty acid profiles, chemical content and sensory properties of traditional fermented dry Kulen sausages. Journal of Food Processing and Preservation, 38(5), 2061–2068.

PARUNOVIC, N., PETROVIC, M., MATEKALO-SVERAK, V., TRBOVIC, D., MIJATOVIC, M., RADOVIC, C. (2012): Fatty acid profile and cholesterol content of m: longissimus of free-range and conventionally reared mangalitsa pigs. South African Journal of Animal Science, 42(2), 101-113.

PARUNOVIC, N., SAVIC, R., RADOVIC, C (2019): Qualitative properties of traditionally produced dry fermented sausages from meat of the autochthonous Mangalitsa pig breed.IOP Conference Series: Earth and Environmental Science, Volume 333, The 60th International Meat Industry Conference MEATCON2019 22–25 September 2019, Kopaonik-Serbia.

PATEIRO, M., FRANCO, D., CARRIL, J.A., LORENZO, J. M. (2014): Changes on physico-chemical properties, lipid oxidation and volatile compounds during the manufacture of Celta dry-cured loin. Journal of Food Science and Technology, 52(8), 4808–4818.

SALAZAR, E., CAYUELA, J. M., ABELLÁN, A., POTO, A., PEINADO, B., TEJADA, L. (2013): A comparison of the quality of dry-cured loins obtained from the native pig breed (Chato Murciano) and from a modern crossbreed pig. Animal Production Science, 53(4), 352-359.

SALES-CAMPOS, H., SOUZA, P. R., PEGHINI, B. C., DA SILVA, J. S., CARDOSO, C. R. (2013): An overview of the modulatory effects of oleic acid in health and disease. Mini Review in Medicinal Chemistry, 13(2), 201-210.

SEONG, P. N., PARK, K.M., KANG, G. H., CHOO, S. H., PARK, B. Y., BA, H. V. (2015): The impact of ripening time on technological quality traits, chemical change and sensory characteristics of dry-cured loin. Asian-Australasian Journal of Animal Sciences, 28(5), 677-685.

THOMPSON, J. M. (2004): The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. Australian Journal of Experimental Agriculture, 44(7), 645-652.

ULBRICHT, T. L., SOUTHGATE, D. A. T. (1991): Coronary heart disease: seven dietary factors. Lancet, 338, 985–992.

VARGAS-RAMELLA, M., PATEIRO, M., BARBA, F. J., FRANCO, D., CAMPAGNOL, P. C. B., MUNEKATA, P. E. S., TOMASEVIC, I., DOMÍNGUEZ, R., LORENZO, J. M. (2020): Microencapsulation of healthier oils to enhance the physicochemical and nutritional properties of deer pâté. LWT – Food Science and Technology, 125, 109223.

WOOD, J. D., ENSER, M., FISHER, A.V., NUTE, G. R., SHEARD, P. R., RICHARDSON, R. I., HUGHES, S. I., WHITTINGTON, F. M. (2008): Fat deposition, fatty acid composition and meat quality: A review, Meat Science, 78(4), 343–358.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

PRECISION FARMING IN IMPROVEMENT OF DAIRY CATTLE WELFARE

Branislav Stanković[1], Slavča Hristov[1], Dušica Ostojić Andrić[2], Ivana Milošević-Stanković[1], Dimitar Nakov[3], Mirjana Rašović Bojanić[4]

[1]University of Belgrade, Faculty of Agriculture, Department of Animal Science, Nemanjina 6, 11080 Zemun, Belgrade, Republic of Serbia
[2]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia
[3]Ss. Cyril and Methodius University, Faculty of Agricultural Sciences and Food, 16-ta Makedonska brigada 3, 1000 Skopje, Republic of North Macedonia
[4]University of Montenegro, Biotechnical Faculty, Mihaila Lalića 1, 81000 Podgorica, Republic of Montenegro
Corresponding author: Branislav Stanković, baxton@agrif.bg.ac.rs

Abstract: The welfare of dairy cattle is a complex phenomenon, which requires multilevel, multidimensional and planned approach. Precision livestock farming (PLF) enables farm animal welfare focusing from the group level to monitoring and managing individual animals of different categories, which is enabled by use of new advanced technologies.

The basic principle of precision agriculture is the use of sensor technologies in order to improve the efficiency of given narrow thresholds resource use. A range of precision livestock monitoring and control technologies have been developed, primarily to improve livestock production efficiency, but more precise and delicate use may be very applicable in early detection of certain conditions, for example initial lameness in dairy cows, real-time surveillance when calving, or distant body temperature variations measuring of individual animals, when early and more efficient therapeutic measures could be undertaken. Environmental monitoring and control in barns can improve animal comfort, and automatic milking systems facilitate animal choice and improve human-animal interactions.

According literature data, previous and future investigations are encouraging possibility of PLF mechanisms use into automated barn surveillance systems in order to assess, control and improve dairy cattle welfare in entire production process through prompt reaction.

Key words: cattle, improvement, precision livestock farming, sensors, surveillance, welfare

Introduction

The welfare of dairy cattle is a complex phenomenon, which requires multilevel, multidimensional and planned approach. For instance, Five Domains describe a highly structured approach to assessing animal welfare and is centred on the four internal domains of nutrition, environment, health, and behaviour, with the aggregation of these affects into the fifth domain, mental state (*Mellor, 2017*). These Five Domains are defined by the set of 118 contributing factors, approximately 10 times more than the Welfare Quality framework (*Van Erp-van der Kooij and Rutter, 2020*).

The measures which determinate the animal welfare quality when animal exhausted short–term or long term problems may be physiological, behavioural, or concerned with individual production or disease. Individuals vary in the coping methods which they use, so any one measure may indicate poor welfare and absence of evidence using one measure does not mean that there is no welfare problem (*Broom, 1988*).

During the time, animal welfare not only started to use, but developed multiple methodologies, where empiricism, engagement and ethics join to know what 'matters' for the animal and to ensure, as far as is possible, that what matters is met. In both, animal welfare science has been remarkably successful in providing the evidence and the procedures for defining, identifying, and precise assessing animal welfare, leading to significant legislative and regulatory change (*Amon et al., 2001; Milman et al., 2004; Blokhuis et al., 2008; Melfi, 2009*).

To be more accurate, some precision livestock farming funds (PLF) originate many years before term itself became in use, described in domestic literature, such as certain aspects of animal rearing and health protection (*Hristov et al., 1996; Đuričić et al., 1997; Stanković, 1998; Ostojić Andrić et al., 2011*), genetic and reproduction (*Šahinović et al., 1997; Stanković et al., 2005*), nutrition (*Grubić et al., 2009*) and welfare (*Hristov et al., 2014*). This paper aims to present certain aspects of PLF services in contemporary cattle welfare protection.

Precision livestock farming sensors and different aspects of animal welfare

Most reviews of welfare now start with listing the needs of the animal, including needs to show certain behaviours. This approach has used sophisticated studies of what is important to animals and has replaced the earlier general guidelines described as freedoms. Many measures of welfare are now used and indicate how good or how poor the welfare is (*Broom, 2011*). Therefore, PLF enables farm animal welfare focusing from the group level to monitoring and managing individual animals of different categories, which is enabled by use of new advanced technologies (*Van Erp-van der Kooij and Rutter, 2020*). PLF uses advanced technologies for automatic, real-time monitoring of animal behaviour and health, and their influence on environment and production (*Beckermans, 2017*), in order to detect variableness at an early stage and improve current state of animal health, welfare and efficiency, expecting to improve production sustainability (*Beckermans, 2014*). PLF relies on four elements (*Wathes, 2010*):

1. The continuous sensing of the process responses at an appropriate frequency and scale with a continuous exchange of information with the process controller;
2. A compact, mathematical model, which predicts the dynamic responses of each process output to variation of the inputs and can be and is best estimated online in real time;
3. A target value and/or trajectory for each process output, e.g. a behavioural pattern, pollutant emission or growth rate;
4. Actuators and a model-based predictive controller for the process inputs.

According to *Buller et al. (2018)*, protecting the welfare of farmed animals has entered the public policy mainstream in many countries. The development of animal welfare policy and science faces new challenges, particularly in the context of the increasingly in food security, climate change, and human nutrition, opening numerous practical and ethical questions. These issues have potential impact on the welfare science and policy development and therefore need to be discussed, especially two of them; the first is the growing incorporation of animal welfare into contemporary understandings of sustainability (IFC, 2014), now formally endorsed in the United Nations Committee on World Food Security Draft Recommendation, and the second is intertwining of animal and human health, increasingly represented by the ‘One Health’ and ‘One Welfare’ agendas (*Gibbs, 2014; Pinillos et al., 2016*).

Hotzel (2014) considers that animal welfare science should be revolutionizing production systems since no system can be considered as ‘sustainable’ if it does not ensure high quality animal welfare (*Wathes et al., 2013; IFC, 2014*). This is already happening through a growing attention being paid to ‘reflexive interactive design’ and its application, notably to the development of sustainable dairy systems (*Bos, 2008; Bos et al., 2009*) and the technological innovations and improved welfare possibilities associated with precision farming (*Berckmans, 2006; Dawkins, 2014*). It is happening with the expanding incorporation of welfare criteria in environmental certification and assurance schemes, but these are often limited to relatively high-end food supply chains, as well as legislative changes reactions (*Buller and Roe, 2008; Buller, 2018*).

In the very informing study of *Van Erp-van der Kooij and Rutter (2020)*, different types of PLF sensors in use or expected in future to be in use were presented (Table 1).

Table 1. Examples of PLF sensors commercially for use on-farm

Sensor	Location	Measure	Reason
Accelerometer	Leg-mounted	Activity	Oestrus, health
	Neck-mounted	Activity	Oestrus, health, rumination
	Rumen bolus	Activity	Oestrus, health
	Ear tag	Activity	Oestrus, health
	Tail-mounted	Tail posture	Onset of calving
Temperature sensor	Ear tag	Body temperature	Health
	Rumen bolus	Body temperature	Health
pH sensor	Rumen bolus	Body temperature	Health
Milk characteristic	Milking machine, online or inline	Progesterone, BHB, urea, LDH	Pregnancy, ketosis, digestion, mastitis
Milk characteristic	Milking machine	Milk flow, colour, conductivity	Mastitis
Sound analysis	Neck tag	Rumination	Health, stress
Vision	Camera	Body Condition Score	Health, nutrition
Vision	Camera	Face recognition	Identification
Positioning	Beacons and neck tags	Locomotion, behaviour	Health, stress, reproduction
	Wireless sensor network	Locomotion, behaviour	Health, stress, reproduction
Weighing device	Dairy farm, feeder	Weight and feed intake	Growth
Pressure sensor	Floor sensor	Leg pressure	Lameness
Ultrasonic sensor	Foot bath sensor	Claw shape	Lameness, claw health
Vision	Camera	Posture	Lameness
Heart rate sensor	Chest band	Heart rate	Health, stress

(from: *Van Erp-van der Kooij and Rutter, 2020*)

There are several levels of PLF, varying from collecting and analysing data at the group level down to monitoring individual animals, utilising sensors that can be static, moving or animal-mounted (*Rutter, 2012*). The automatic monitoring systems may be based on sound, images and collection of environmental data (*Tullo et al., 2013*), and the technology ranges from monitoring production and fertility to health and behaviour; some systems monitor environmental factors to control climate conditions and there are robotic systems that automate human handling such as milking, feeding and cleaning (*Van Erp-van der Kooij and Rutter, 2020*). Many PLF systems are already commercially available, with further systems in development and likely to be improved and commercialised in the future.

Feeding and drinking sensors - data concerning feeding and water consumption could be collected directly from automatic feeders (*Rushen et al., 2012*) or waterholes (*Meiszberg et al., 2009)*, or indirectly from sensors that monitor behaviour, location or the animals. In dairy cows, feeding behaviour and grazing can be monitored automatically using activity meters, location sensors or sound sensors *(Rutten et al., 2013; Vanrell et al., 2018; Werner et al., 2018)*. Rumination can be monitored by sensors on a neck collar, based on accelerometer data or sound *(Ambriz-Vilchis et al., 2015; Bar and Soloman, 2010)*, and sound may be also analysed to measure behaviour of lying and ruminating time in dairy cows *(Meen et al., 2015)*.

Animal health sensors - Several sensor systems can be used to detect disturbed health condition in farm animals, using animal-mounted sensors or sensors as a part of farm infrastructure. Body temperature can be measured directly - with animal-mounted sensors, or indirectly - with thermographic cameras *(Sellier et al., 2014; Arican et al., 2018)*. Thermographic cameras can be used for mastitis detection in dairy cows *(Hovinen et al., 2008; Bortolami et al., 2015)*. Body temperature can be monitored with rumen boluses in dairy cows, which can also monitor rumen motility and pH, as an indicator for metabolic disease *(Mottram, 2015; Nogami et al., 2017; Arai et al., 2019)*. Accelerometers measuring activity in dairy cows not only detect oestrus but also behavioural changes signalling disease *(Rutter, 2012; Chanvallon et al., 2014; Roelofs and Van Erp-Van Der Kooij, 2015)*, such as lameness *(Sadiq et al., 2017; Vázquez Diosdado et al., 2018; Barker et al., 2018)*. Symptoms of disease can be detected with sound analysis, for example coughing calves *(Vandermeulen et al., 2016)*. Lameness in cows can be detected with force plates or pressure mats *(Maertens et al., 2011)*. In milk samples, beta-hydroxybutyrate (BHB) and lactate dehydrogenase (LDH) can be measured automatically as an indicator for metabolic disease or mastitis in dairy cows *(Asmussen and Foss, 2010)*.

Housing conditions and animal comfort sensors - This type of sensors are used mostly for pig and poultry farms, since these farms are under no influence of the outside environmental conditions *(Van Erp-van der Kooij and Rutter, 2020)*. These farms require automatic control systems, regulating the indoor climate, temperature, relative humidity (RH), air speed and carbon dioxide (CO_2). In dairy farms, commercial climate condition monitoring is in development *(Antanaitis et al., 2016)*.

Animal behaviour sensors - Behaviours of animals can be monitored using location or activity data from animal-mounted sensors in dairy cows *(Meunier et al., 2018; Pastel et al., 2018)*. In dairy farms, activity sensors developed for heat detection are also used for behaviour monitoring and for reporting deviations in behaviour *(Van Erp-Van der Kooij et al., 2016;)* [25, 76–79]. Lying, walking, eating and standing behaviour of dairy cows can be measured quite accurately with activity meters on a leg, neck or ear tag *(Van Erp-Van der Kooij et al., 2016)* [25, 76, 80]. It is also possible to detect some abnormal and damaging behaviour, which is mostly used in pig and poultry production *(Van Erp-van der Kooij and Rutter, 2020)*. For instance, it has been reported that lame cows reveal changes to both feeding and lying behaviour. They are slower to respond to food being made available *(Yunta et al., 2012)* and feed faster, although for a reduced overall duration per day *(Norring et al., 2014)*. Changes in lying behaviour are also described, although there are discrepancies between studies *(Barker et al., 2018)*; increased lying was described by *Singh et al. (1993)* and *Blackie et al. (2011)*, no difference by *Ito et al. (2010)* and *Yunta et al. (2012)*, and decreased lying by *Cook et al. (2008)*. Therefore, automated individual cow behaviours monitoring could be useful in the early detection of lameness.

Distress detecting sensors – According to Van Erp-*van der Kooij and Rutter (2020)*, different stress calls in cows can be recognised. The rise in heat production during stress can be measured using a thermographic camera in cows *(Stewart et al., 2005; Stewart et al., 2017)*. Finally, automatic heart rate measurements, corrected for activity, can be used to measure stress *(Behmann et al., 2016)*.

Measures used in on-farm welfare assessment systems are often classified into resource-based measures: housing systems, space allowances, animal management practises, and animal-based measures: low incidence of disease or injury, normal behaviour *(Main et al., 2003)*. Animal-based measures provide more direct assessment of the state of the animals *(Barnett and Hemsworth, 2009)* and nowadays PLF has an important role in the welfare assessment.

PLF implications on animal welfare

The creation of an automated assessment of animal welfare was attempted through integration and combining of certain measures *(Van Erp-van der Kooij and Rutter, 2020)*, but still no system offers everything that could be achieved by using a full combination of all systems operating together, and almost without exception, the different technologies operate ‘stand-alone’ and will not communicate with each other *(Caja et al., 2016)*.

PLF has the potential to monitor, manage and control many aspects of livestock production, simultaneously and automatically *(Wathes et al., 2008)*. Regarding calving prediction, recognition and early detection enable timely assistance, which is necessary to ensure the survival of cows and their offspring *(Lopes et al., 2016)*. There are some calving detection sensors on the market, which resort to the detection of different changes that occur around or during calving.

According to *Saint-Dizier and Chastand-Maillard (2015)*, these sensors should point out variations of rectal and vaginal temperatures (0.4 to 0.6 ºC and 0.6-0.7 ºC respectively lower on the day of calving than 48 hours before), for both beef and dairy cattle. On the calving day, their behaviour changes, the animals tend to isolate themselves from the rest of the herd, have increased activity and lie down and stand up more often, increased movements by the tail close to parturition and rising of the tail head as early as 5 days before parturition. There are decreases of feeding, drinking and rumination activity which also should be noticed.

Bioacoustics has been used to evaluate conditions such as stress and welfare through screams, calls and vocalizations, and to assess health by monitoring coughs and sneezes *(Ferrari et al., 2010)*. Furthermore it is a simple, cheap and non-invasive technology. Respiratory diseases, such as bovine respiratory disease (BRD), are one of the most prevailing pathologies in young categories and early recognition of cough sounds is being used as a method of diagnosing respiratory diseases. Cough sounds can only be assessed during a visit to the farm and an automatic monitoring tool for animals' coughs can contribute to improved farm management through opportune treatments *(Vandermeulen et al., 2016)*.

Use of different types of imaging, such as infrared thermographic imaging (IRT), magnetic resonance (MR), computer tomography (CT) are accurate and saving time but expensive and in logistic aspect demanding methodology for diagnostic of certain primarily health disorders. According to *Arican et al. (2018)*, thermographic examination may have potential as a detection tool for laminitis. MR transversal images provided excellent depiction of anatomical structures and many biometric researches in the bovine hoof can be easily investigated, especially during the initial active phase of laminitis. However, the usefulness of IRT, MRI, CT in evaluating laminitis in different situations remains still open. Diagnostic imaging technique such as radiography and ultrasonography provide limited information for evaluation of the bovine digits and claw. Radiography has limited value to evaluation of soft tissue.

In order to to evaluate the potential application of thermographic imaging compared to SCC and bacteriological culture for infection detection in cow affected by subclinical mastitis, *Bortolami et al. (2015)* took thermographic images from each functional udder quarter and nipple. Authors found that infrared thermography was correlated to SCS ($p<0.05$) but was not able to discriminate between positive and negative cows. The association found between SCS and temperatures suggests the use of thermographic imaging as a screening tool helpful in the evaluation of an inflammation status of the udder, but seems to have a poor diagnostic value. Similar results regarding thermal imaging in assessing body temperature of calves were published by *Bell et al. (2019)*, suggesting that accurate measures of core body temperature using thermal imaging cannot be achieved under commercial farm conditions and that further research is needed.

Another approach to animal status assessment traditionally includes manual and visual scoring, but the large number of man-hours required for these methods involves high costs, and use of a sensor attached to the animals can be invasive and may alter the outcome *(Cangar et al., 2008)*. For this reason, the use of automatically collected images to analyse farming systems is becoming more and more common *(Tullo et al., 2013).* Early detection of certain symptoms of health disorders might be useful when immediate therapy is required, such as real-time rumen temperature monitoring by utilizing an ingestible biosensor *(Kim et al., 2019).* It proved to be right and that mastitis is accompanied with a high rise in body temperature.

PLF can combine audio and video information into on-line automated tools that can be used to control, monitor and model the behaviour of animals and their biological response *(Tullo et al., 2013)*. The PLF approach can easily be applied to different aspects of management, with a focus on the animals and/or the environment, and at different scales, from the individual to the entire flock/herd *(Wathes, 2010).*

The human-animal relationship is important for animal welfare and could be measured automatically. An integrated approach to animal welfare assessment should be possible, but this approach needs to be further defined and validated *(Van Erp-van der Kooij and Rutter, 2020).*

The greatest uptake of PLF technologies to date has been in intensive animal production systems, but *Van Erp-van der Kooij and Rutter (2020)* deem that there is a risk for the technological intensification of production may be neglected animal welfare enhancement by promoting positive experiences *(Stevenson, 2017)* and decreased contact between farmer and animals, therefore disturbing the human-animal relationship and decrease the opportunities to directly observe the health and well-being of the animals *(Hostiou et al., 2017)*. Over-relining on PLF might cause missing of other diseases signs *(Wathes et al., 2008)*. Farmers often do not understand PLF systems and need to be maintained and calibrated *(Hartung et al., 2017)*. Therefore, automated detection system must work on any farm in any conditions, and data standardisation is strongly dependent on manual labelling, which is necessary for data analysis and model development. Key indicators and standards must be clear and precise *(Tullo et al., 2013).*

PLF systems should improve welfare by optimising feeding and systematically monitoring growth and/or weight measurements *(Wathes et al., 2008)*, by early detection of disease, such as lameness or mastitis, as well as by improving housing conditions with devices such as robot scrapers and automated climate control systems *(Blokhuis, 2010)*. *Webster (2016)* is right claiming that giving animals the ability to make choices that promote their own quality of life could help improve welfare, which could be managed through individual feeding, robotic milking or voluntary showering facilities. PLF systems may increase welfare if the farmer responds adequately to the PLF system alerts; however, good tools do not automatically guarantee good utilisation by a stockperson *(Van Erp-van der Kooij and Rutter, 2020).*

Conclusion

The welfare of dairy cattle is a complex phenomenon, which requires multilevel, multidimensional and planned approach. Precision livestock farming (PLF) enables farm animal welfare focusing from the group level to monitoring and managing individual animals of different categories, which is enabled by use of new advanced technologies. A number of developed PLF sensors increase as investigations advance, although welfare assessment systems are not efficient enough yet and further research is needed. PLF is a useful tool for the farmer to monitor and improve animal welfare, upgrading living conditions for the cows and to detect early symptoms of health disorders. According literature data, previous and future investigations are encouraging possibility of PLF mechanisms use into automated barn surveillance systems in order to assess, control and improve dairy cattle welfare in entire production process through early reaction.

Precizna poljoprivredna proizvodnja za poboljšanje dobrobiti mlečnih goveda

Branislav Stanković, Slavča Hristov, Dušica Ostojić Andrić, Ivana Milošević-Stanković, Dimitar Nakov, Mirjana Rašović Bojanić

Rezime

Dobrobit mlečnih goveda je složen fenomen koji zahteva višestepeni, višedimenzionalni i planski pristup. Precizno uzgajanje stoke (PLF) omogućava fokusiranje dobrobiti domaćih životinja sa grupnog nivoa na praćenje i upravljanje pojedinim životinjama različitih kategorija, što je moguće upotrebom novih naprednih tehnologija.
Osnovni princip precizne poljoprivrede je upotreba senzorskih tehnologija u cilju poboljšanja efikasnosti korišćenja resursa u okviru zadatih uskih graničnih vrednosti parametara. Razvijen je niz preciznih tehnologija za nadzor i kontrolu životinja, prvenstveno radi poboljšanja efikasnosti stočarske proizvodnje, ali se one

mogu upotrebiti preciznije i delikatnije u ranom otkrivanju određenih stanja, na primer početne hromosti kod muznih krava, nadzor u realnom vremenu u vreme teljenja ili za daljinsko merenje telesne temperature za pojedinačna grla, u cilju ranog i efikasnijeg preduzimanja terapijskih mera. Monitoring i kontrola parametara životne sredine u štalama mogu poboljšati udobnost životinja, a sistemi za automatsku mužu olakšavaju odabir grla i poboljšavaju interakcije ljudi i životinja.
Prema literaturnim podacima, sprovedena i buduća istraživanja ohrabruju mogućnost upotrebe PLF mehanizama u automatizovanim sistemima za nadzor štala u cilju procene, kontrole i poboljšanja dobrobiti mlečnih goveda u celom proizvodnom procesu mogućnošću brzog reagovanja.

Ključne reči: goveda, poboljšanje, precizno stočarstvo, senzori, nadzor, dobrobit

Acknowledgment

This paper was created as a result of research within the "Agreement on the implementation and financing of scientific research in 2021 between the Faculty of Agriculture in Belgrade and the Ministry of Education, Science and Technological Development of the Republic of Serbia", contract record number: 451-03-9 / 2021-14 / 200116.

References

AMBRIZ-VILCHIS V., JESSOP N.S., FAWCETT R.H., SHAW D.J., MACRAE A.I. (2015): Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments. Journal of Dairy Science, 98, 3, 1750–1758.
AMON T., AMON B., OFNER E., BOXBERGER J. (2001): Precision of assessment of animal welfare by the'TGI 35 L'Austrian needs index. Acta Agriculturae Scandinavica, Section A-Animal Science, 51, S30, 114-117.
ANTANAITIS R., ZILAITIS V., JUOZAITIENE V., STOSKUS R., TELEVICIUS M. (2016): Changes in reticulorumen content temperature and pH according to time of day and yearly seasons. Polish Journal of Veterinary Sciences, 19, 4, 771–776.
ARAI S., OKADA H., SAWADA H., TAKAHASHI Y., KIMURA K., ITOH T. (2019): Evaluation of ruminal motility in cattle by a bolus-type wireless sensor. Journal of Veterinary Medical Science 81, 12, 1835–41.
ARICAN M., HATIPLOGU F., EROL H., KANAT O., YAVUZ O., PARLAK K., COK O. (2018): Comparison of Thermographic Imaging and Other Diagnostic

Techniques in Diagnosis of Cattle with Laminitis. Acta Scientiae Veterinariae, 46, 1, 7.

ASMUSSEN T., FOSS A.S. (2010): Herd navigator or “how to benefit from frequent measurements”. In: ICAR 37th Annual Meeting. Riga: ICAR; 291–293.

BAR D., SOLOMAN R. (2010): Rumination collars: what can they tell us. In: The First North American Conference on Precision Dairy Management, Toronto, Canada, 2.

BARKER Z.E., VÁZQUEZ DIOSDADO J.A., CODLING E.A., BELL N.J., HODGES H.R., CROFT D.P., AMORY J.R. (2018): Use of novel sensors combining local positioning and acceleration to measure feeding behavior differences associated with lameness in dairy cattle. Journal of Dairy Science, 101, 7, 6310–21.

BARNETT J.L., HEMSWORTH P.H. (2009): Welfare monitoring schemes: using research to safeguard welfare of animals on the farm. Journal of Applied Animal Welfare Science, 12, 114-131.

BEHMANN J., HENDRIKSEN K., MÜLLER U., BÜSCHER W., PLÜMER L. (2016): Support Vector machine and duration-aware conditional random field for identification of spatio-temporal activity patterns by combined indoor positioning and heart rate sensors. GeoInformatica, 20, 4, 693–714.

BELL D.J., MACRAE A.I., MITCHELL M.A., MASON C.S., JENNINGS A., HASKELL M.J. (2020): Comparison of thermal imaging and rectal temperature in the diagnosis of pyrexia in pre-weaned calves using on farm conditions. Research in Veterinary Science, 131, 259-265.

BERCKMANS D. (2006): Automatic on-line monitoring of animals by precision livestock farming. In Livestock Production and Society; Wageningen Academic Publishers: Wageningen, The Netherlands, 287–295.

BERCKMANS D. (2008): Automatic real-time monitoring of locomotion and posture behaviour of pregnant cows prior to calving using online image analysis. Computers and Electronics in Agriculture 64, 1, 53-60.

BERCKMANS D. (2014): Precision livestock farming technologies for welfare management in intensive livestock systems. OIE Revue Scientifique et Technique, 33, 1, 189–96.

BERCKMANS D. (2017): General introduction to precision livestock farming. Animal Frontiers. 7, 1, 6–11.

BLOKHUIS H.J. (2010): The Welfare Quality® project and beyond: safeguarding farm animal well-being. Acta Agriculturae Scandinavica Section A Animal Science 60, 3, 129–40.

BLOKHUIS H.J., KEELING L.J., GAVINELLI A., SERRATOSA J. (2008): Animal welfare’s impact on the food chain. Trends in Food Science & Technology, 19, S79–S87.

BOJKOVSKI J., BOROZAN S., HRISTOV S., STANKOVIĆ B., JOKSIMOVIĆ-TODOROVIĆ M., DAVIDOVIĆ V. (2007): Uticaj kontaminacije sredine na zdravstveno stanje farmskih životinja. „Dobrobit životinja i biosigurnost na farmama”, Poljoprivredni fakultet, Zemun, 251-258.

BORTOLAMI A., FIORE E., GIANESELLA M., CORRO M., CATANIA S., MORGANTE M. (2015): Evaluation of the udder health status in subclinical mastitis affected dairy cows through bacteriological culture, somatic cell count and thermographic imaging. Polish Journal of Veterinary Sciences, 18, 4, 799–805.

BOS A.P. (2008): Instrumentalization theory and reflexive design in animal husbandry. Social Epistemology, 22, 29–50.

BOS A.P., GROOT KOERKAMP P.W.G., GOSSELINK J.M.J., BOKMA S. (2009): Reflexive interactive design and its application in a project on sustainable dairy husbandry systems. Outlook on Agriculture, 38, 137–145.

BROOM D.M. (1988): The scientific assessment of animal welfare. Applied Animal Behaviour Science, 20, 1-2, 5-19.

BROOM D.M. (2011): A history of animal welfare science. Acta biotheoretica, 59, 2, 121-137.

BULLER H. (2018): Public Opinion and the Retailer. In Animal Welfare in a Changing World; BUTTERWORTH A., Ed.; CABI: Wallingford, UK, 100–109.

BULLER H., BLOKHUIS H. JENSEN P., KEELING L. (2018):Towards farm animal welfare and sustainability. Animals, 8(6), 81.

BULLER H., BLOKHUIS H., JENSEN P., KEELING L. (2018):Towards farm animal welfare and sustainability. Animals, 8, 6, 81.

BULLER H., ROE E. (2008): Food and Animal Welfare; Bloomsbury Press: London, UK.

CAJA G., CASTRO-COSTA A., KNIGHT C.H. (2016): Engineering to support wellbeing of dairy animals. Journal of Dairy Research, 83, 2, 136–47.

CANGAR Ö., LEROY T., GUARINO M., VRANKEN E., FALLON R., LENEHAN J., MEE J., BLACKIE N., AMORY J.R., BLEACH E., SCAIFE J. (2011): The effect of lameness on lying behaviour of zero grazed Holstein dairy cat-tle. Applied Animal Behaviour Science, 134, 85–91.

CHANVALLON A., COYRAL-CASTEL S., GATIEN J., LAMY J.M., RIBAUD D., ALLAIN C., CLÉMENT' P., SALVETTI P. (2014): Comparison of three devices for the automated detection ofestrus in dairy cows. Theriogenology, 82, 5, 734–41.

COOK N.B., MARIN M.J., MENTINK R.L., BENNETT T.B., SCHAEFER M.J. (2008): Comfort zone-design free stalls: Do they influence the stall use behavior of lame cows? Journal of Dairy Science, 91, 4673–4678.

DAWKINS M.S. (2014): Precision Farming and Animal Welfare. Sci. Parliam. 71, 14–15.

ĐURIČIĆ B., MILOŠEVIĆ B., RADOJIČIĆ S., OBRENOVIĆ S., ARANĐELOVIĆ S., KNEŽEVIĆ D., VUKŠA P., STANKOVIĆ B., MIĆOVIĆ M. (1997): Aerosol vaccination: drop size influence on postvaccinal immunity in chickens vaccinated against Newcastle disease. Proceedings of XIth International Congress of the World Veterinary Poultry Association, 203, Budapest, Hungary, 18-22nd August 1997.
FERRARI S., PICCININI R., SILVA M., EXADAKTYLOS V., BERCKMANS D., GUARINO M. (2010): Cough sound description in relation to respiratory diseases in dairy calves. Preventive Veterinary Medicine 96, 3-4, 276-280.
GIBBS E.P.J. (2014): The evolution of One Health. Veterinary Record, 174, 85–91.
GRUBIĆ, G., NOVAKOVIĆ, Ž., ALEKSIĆ, S., SRETENOVIĆ, L., PANTELIĆ, V., OSTOJIĆ-ANDRIĆ, D. (2009): Evaluation of the body condition of high yielding cows. Biotechnology in Animal Husbandry, 25, 1-2, 81-91.
HARTUNG J., BANHAZI T., VRANKEN E., GUARINO M. (2017): European farmers' experiences with precision livestock farming systems. Animal Frontiers, 7, 1, 38–44.
HOSTIOU N, FAGON J, CHAUVAT S, TURLOT A, KLING-EVEILLARD F, BOIVIN X, ALLAIN C. (2017): Impact of precision livestock farming on work and human-animal interactions on dairy farms. A review. Biotechnology, Agronomy, Society and Environment 21, 4, 268–75.
HÖTZEL M.J. (2014): Improving farm animal welfare: Is evolution or revolution needed in production systems. In Dilemmas in Animal Welfare; Appleby, M., Sandoe, P., Weary, D., Eds.; CABI: Wallingford, UK, 67–84.
HOVINEN M., SIIVONEN J., TAPONEN S., HÄNNINEN L., PASTELL M., AISLA A.M., PYÖRÄLÄ S. (2008): Detection of clinical mastitis with the help of a thermal camera. Journal of Dairy Science, 91, 12, 4592–4598.
HRISTOV S, STANKOVIĆ B, OSTOJIĆ-ANDRIĆ D. (2014): Different approaches to assess the welfare of dairy cows with some results in Serbia. Proceedings of the International Symposium on Animal Science 2014, September 2014, Belgrade – Zemun, 320-328.
HRISTOV S., ANOJČIĆ B., VUČINIĆ M., STANKOVIĆ B. (1996): Uticaj prašine vazduha na organizam životinja. VII Savetovanje DDDDD, Zbornik radova, 268-275.
INTERNATIONAL FINANCE CORPORATION. Improving Animal Welfare in Livestock Operations; IFC: Washington, WA, USA, 2014.51.
ITO K., VON KEYSERLINGK M.A.G., LEBLANC S.J., WEARY D.M. (2010): Lying behavior as an indicator of lameness in dairy cows. Journal of Dairy Science, 93, 3553–3560.

KIM H., MIN Y., CHOI B. (2019): Real-time temperature monitoring for the early detection of mastitis in dairy cattle: Methods and case researches. Computers and Electronics in Agriculture, 162, 119-125.
LOPES S.I., BEXIGA R., ARAÚJO J.P., CERQUEIRA J.L., ABREU C., PAREDES C., ALONSO J.M. (2016): Precision livestock farming for reproductive performance optimization: a survey. In Food futures: ethics, science and culture, Wageningen Academic Publishers, 3197-3211.
MAERTENS W., VANGEYTE J., BAERT J., JANTUAN A., MERTENS K.C., DE CAMPENEERE S., PLUK A, OPSOMER G, VAN WEYENBERG S, VAN NUFFEL A. (2011): Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: The GAITWISE system. Biosystems Engineering, 110, 1, 29–39.
MAIN D.C.J., KENT J.P., WEMELSFELDER F., OFNER E., TUITTENS F.A.M. (2003): Applications for methods of on-farm assessment. Animal Welfare Journal, 12, 445-456.
MEEN G.H., SCHELLEKENS M.A., SLEGERS M.H.M., LEENDERS N.L.G., VAN ERP-VAN DER KOOIJ E., NOLDUS L.P.J.J. (2015): Sound analysis in dairy cattle vocalisation as a potential welfare monitor. Computers and Electronics in Agriculture, 118, 111–115.
MEISZBERG A.M., JOHNSON A.K., SADLER L.J., CARROLL J.A., DAILEY J.W., KREBS N. (2009): Drinking behavior in nursery pigs: determining the accuracy between an automatic water meter versus human observers. Journal of Animal Science, 87, 12, 4173–80.
MELFI V.A. (2009): There are big gaps in our knowledge, and thus approach, to zoo animal welfare: A case for evidence-based zoo animal management. Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association, 28, 574–588.
MELLOR D.J. (2017): Operational details of the five domains model and its key applications to the assessment and management of animal welfare. Animals, 7(8):1–20.
MEUNIER B., PRADEL P., SLOTH K.H., CIRIÉ C., DELVAL E., MIALON M.M., VEISSIER I. (2018): Image analysis to refine measurements of dairy cow behaviour from a real-time location system. Biosystems Engineering, 173, 32–44.
MILLMAN S., DUNCAN I., STAUFFACHER M., STOOKEY J.M. (2004): The impact of applied ethologists and the International Society for Applied Ethology in improving animal welfare. Applied Animal Behaviour Science, 86, 299–311.
MOTTRAM T. (2015): Precision livestock farming in rumen sensing, feed intake and precise feeding. In: HALACHMI I, ed. Precision livestock farming applications. Wageningen: Wageningen Academic Publishers, 291-298.

NOGAMI H., ARAI S., OKADA H., ZHAN L., ITOH T. (2017): Minimized bolus-type wireless sensor node with a built-in three-axis acceleration meter for monitoring a Cow's Rumen conditions. Sensors (Switzerland), 17, 4, 1–10.
NORRING M., HÄGGMAN J., SIMOJOKI H., TAMMINEN P., WINCKLER C., PASTELL M. (2014): Short communication: Lameness impairs feeding behavior of dairy cows. Journal of Dairy Science, 97, 4317–4321.
OSTOJIĆ ANDRIĆ D., ALEKSIĆ S., HRISTOV S., NOVAKOVIĆ Ž., PETROVIĆ M.M., NIKŠIĆ D., STANIŠIĆ N. (2012): Serbia in the implementation of SEUROP standard for beef carcass classification: Legislation, parameters and evaluation criteria: Part A. Biotechnology in Animal Husbandry, 28, 1, 47-58.
PASTELL M., FRONDELIUS L., JÄRVINEN M., BACKMAN J. (2018): Filtering methods to improve the accuracy of indoor positioning data for dairy cows. Biosystems Engineering, 169, 22–31.
PINILLOS R.C., APPLEBY M.C., MANTECA X., SCOTT-PARK F., SMITH C., VELARDE A. (2016): One Welfare - A platform forimproving human and animal welfare.Veterinary Record, 179, 412–413.
ROELOFS J.B., VAN ERP-VAN DER KOOIJ E. (2015): Estrus detection tools and their applicability in cattle: recent and perspectival situation. Animal Reproduction, 12, 3, 498–504.
RUSHEN J., CHAPINAL N., DE PASSILLÉ A.M. (2012): Automated monitoring of behavioural-based animal welfare indicators. Animal Welfare, 21, 3, 339–50.
RUTTEN C.J., VELTHUIS A.G.J., STEENEVELD W., HOGEVEEN H. (2013): Invited review: sensors to support health management on dairy farms. Journal of Dairy Science 96, 4, 1928–52.
RUTTER S.M. (2012): A "smart" future for ruminant livestock production? Cattle Practice, 20, 3, 186–93.
RUTTER S.M. (2012): A "smart" future for ruminant livestock production? Cattle Practice, 20, 3, 186–93.
SADIQ M.B., RAMANOON S.Z., MOSSADEQ W.M.S., MANSOR R., SYED-HUSSAIN S.S. (2017): Association between lameness and indicators of dairy cow welfare based on locomotion scoring, body and hock condition, leg hygiene and lying behavior. Animals, 7(11):1–17.
ŠAHINOVIĆ R., STANČIĆ B., STANKOVIĆ B. (1997): In vitro maturation of porcine follicular oocytes after 24 and 48 hours. 5th KOK and 3rd Symposium on the Animal Reproduction, 150, 2-6th September, Ohrid, Macedonia, 1997.
SAINT-DIZER M., CHASTAND-MAILLARD S. (2015): Methods and on-farm devices to predict calving time in cattle. Veterinary Journal, 205, 349-356.

SELLIER N., GUETTIER E., STAUB C. (2014): A review of methods to measure animal body temperature in precision farming. American Journal of Agricultural Science and Technology, 2, 2, 74–99.
SINGH S.S., WARD W.R., LAUTENBACH K., MURRAY R.D. (1993): Behaviour of lame and normal dairy cows in cubicles and in a straw yard. Veterinary Record, 133, 204–208.
STANKOVIĆ B. (1998): Uticaj prašine u vazduhu poreklom iz prostirke na uspeh vakcinacije pilića putem aerosola. Magistarska teza, Fakultet veterinarske medicine, Beograd.
STANKOVIĆ B., PETRUJKIĆ T., HRISTOV S., RELIĆ R. (2005): Freezing the boars semen: Some limitations and perspectives. Savremena poljoprivreda, 54, 1-2, 139-144.
STEVENSON P. (2017): Precision livestock farming: could it drive the livestock sector in the wrong direction? In: 8th European Conference on Precision Livestock Farming; Nantes, France, 736–45.
STEWART M., WEBSTER J.R., SCHAEFER A.L., COOK N.J., SCOTT S.L. (2005): Infrared thermography as a non-invasive tool to study animal welfare. Animal Welfare 14, 4, 319–25.
STEWART M., WILSON M.T., SCHAEFER A.L., HUDDART F., SUTHERLAND M.A. (2017): The use of infrared thermography and accelerometers for remote monitoring of dairy cow health and welfare. Journal of Dairy Science, 100, 5, 3893–901.
TULLO E., FONTANA I., GUARINO M. (2013): Precision livestock farming: an overview of image and sound labelling. In European Conference on Precision Livestock Farming 2013: (PLF) EC-PLF, KU Leuven. 30-38.
VAN ERP-VAN DER KOOIJ E., RUTTER S. M. (2020): Using precision farming to improve animal welfare. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 15(051).
VAN ERP-VAN DER KOOIJ E., VAN DE BRUG M, ROELOFS J. (2016): Validation of Nedap Smarttag Leg and neck to assess behavioural activity level in dairy cattle. In: Kamphuis C, Steeneveld W, editors. Precision fairy farming 2016. Leeuwarden: Wageningen Academic Press; 321–326.
VANDERMEULEN J., BAHR C., JOHNSTON D., EARLEY B., TULLO E., FONTANA I., GUARINO M., EXADAKTYLOS V., BERCKMANS D. (2016): Early recognition of bovine respiratory disease in calves using automated continuous monitoring of cough sounds. Computers and Electronics in Agriculture; 129, 15–26.
VANRELL S.R., CHELOTTI J.O., GALLI J.R., UTSUMI S.A., GIOVANINI L.L., RUFINER H.L., MILONE D.H. (2018): A regularity-based algorithm for

identifying grazing and rumination bouts from acoustic signals in grazing cattle. Computers and Electronics in Agriculture, 151, 392–402.
VÁZQUEZ DIOSDADO J.A., BARKER Z.E., HODGES H.R., AMORY J.R., CROFT D.P., BELL N.J., CODLING E.A. (2018): Space-use patterns highlight behavioural differences linked to lameness, parity, and days in milk in barn-housed dairy cows. PLOS One 13, 12, 1–23.
WATHES C.M. (2010): The prospects for precision livestock farming. Journal of the 327 Royal Agricultural Society of England 171, 26-32.
WATHES C.M., BULLER H., MAGGS H., CAMPBELL M.L. (2013): Livestock production in the UK in the 21st century: A perfect storm averted? Animals, 3, 574–583.
WATHES C.M., KRISTENSEN H.H., AERTS J.M., BERCKMANS D. (2008): Is precision livestock farming an engineer's daydream or nightmare, an animal's friend or foe, and a farmer's panacea or pitfall?, Computers and Electronics in Agriculture, 64, 2–10.
WEBSTER J. (2016): Animal welfare: freedoms, dominions and "a life worth living". Animals, 6, 6, 2–7.
WERNER J., LESO L., UMSTATTER C., NIEDERHAUSER J., KENNEDY E., GEOGHEGAN A., SHALLOO L., SCHICK M. O'BRIEN B. (2018): Evaluation of the RumiWatchSystem for measuring grazing behaviour of cows. Journal of Neuroscience Methods, 300, 138–46.
YUNTA C., GUASCH I., BACH A. (2012): Short communication: Lying behavior of lactating dairy cows is influenced by lameness especially around feeding time. Journal of Dairy Science, 95, 6546–6549.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

LAMENESS DETECTION IN CATTLE USING ICT TECHNOLOGY

Tina Bobić, Andrea Bejteš, Pero Mijić, Vesna Gantner, Maja Gregić

Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia
Corresponding author: Tina Bobić, tbobic@fazos.hr

Abstract: Since the problem of lameness is very common on dairy farms, it is necessary to apply newer technologies that can contribute to reducing the frequency of lameness. Information Computer Technologies (ICT) is becoming increasingly widespread to monitor the levels of the production, functional, welfare and health traits. With automated lameness detection methods a large amount of data can be collected in short period of time, which can improve lameness prediction accuracy. The application of ICT technology in the detection and prevention of lameness in cows in milk production is possible and has a future. Currently, ICT are strongly represented at the level of scientific approach with a tendency to be applied in practice on a daily basis. Significant effort and cooperation of several different groups of people (scientists, IT specialists, and farmers) are needed in order for ICT to be successfully implemented in continuous practical application.

Key words: information computer technologies, lameness, cattle, detection

Introduction

Precision Livestock Farming (PLF) and the use of Information Computer Technologies (ICT) is becoming increasingly widespread to monitor the levels of the production, functional, welfare and health traits (*Andonovic et al., 2018*; *Azarpajouh et al., 2020).* Precision Dairy Farming is the use of technologies to improve management strategies and farm performance (*Bewley, 2010)*. ICT with software techniques and engineering techniques have been applied to collects and analyses (with reasonable accuracy) various behaviour and production measurements (rumination, eating, standing, lying, walking rhythm and speed, etc.) which can be the foundation for identifying key animal conditions such as the onset of illness.

Lameness is large and frequent problem on the dairy farms which directly affecting production, economic losses and animal welfare (*Green et al., 2010; Viazzi et al., 2014*). In addition to being extremely painful to the animal, the appearance of lameness leads to large economic losses due to the milk production decrease, treatment and early culling *(Ózsvári, 2017)*. Detection of lameness early in cows is important from the animal welfare point of view and for reducing economic losses (*Lokesh Babu et al., 2018*). With larger numbers of cows per farm, farmers no longer have the same time traditionally had to care for their animals. *Norton and Berckmans (2017)* emphasized that the application of technology is becoming more important for EU dairy farmers than ever before.

Visual scoring is the most common method to evaluate lameness, which requires a lot of time and labor. Therefore, *Azarpajouh et al. (2020)* considered that with automated lameness detection methods (that do not rely on the human eyes and are not subjective) a large amount of data can be collected in short period of time, which can improve lameness prediction accuracy.

The aim of this paper is to present some of the ICT methods for lameness detection in cattle on dairy farms.

Automatic lameness detection

Different scientific approaches have been used in order to develop a fully automated and continuous lameness detection system based on kinematic analysis, behavioral change detection using sensors, and data and image analysis. According to *Alsaaod et al. (2019)* the automatic methods of lameness detection consist in three categories or combinations thereof:

1. Kinematic gait analysis (changes in the position of specific body segments over time using image-processing techniques or accelerometers),
2. Kinetic gait analysis (force applied to the body using 1- or 3-dimensional (D) ground reaction force systems or pressure-sensitive walkways)
3. Indirect methods (analysis of variables not involving any gait characteristics using thermographic imaging, social behaviour production and health parameters).

Flower et al. (2005) state that kinematic analysis measures the movement geometry, without considering the causing forces and calculates different gait aspects such as stride length, and stance and swing duration. Furthermore, according to previous researches (*Chapinal et al., 2009; Kramer et al., 2009; Pastell et al., 2010; Poursaberi et al., 2010, 2011*) parameters such as:

uneven weight distribution among the legs, increased lying times and bouts, reduced walking speed, changes in feeding behavior and activity, back posture, can be used as lameness indicators.

Pressure Sensitive Walkway

Maertens et al. (2011) and *Van Nuffel et al. (2013)* were used pressure sensitive walkway and they indicated that variables of asymmetry and speed seem most promising for further research on cattle lameness detection. Same authors also emphasized that measurements with the pressure sensitive walkway were highly repeatable and suited for gait characterization. *Pastell et al. (2008)* introduced a special pressure mat made of electromechanical film, which automatically detect dynamic forces and cow’s leg problems. This tool can be setup in any corridor along which the cows walk, and it has potentials to separate lame cows from healthy cows by different force-time behavior.

Accelerometer

The accelerometer is an electronic device containing one or several sensors that measures, records and transmits acceleration data (regarding to various locomotor behaviours) in one or all three dimensions. *Alsaaod et al. (2012)* demonstrated that pedometer measurements such as activity and lying behavior in combination with machine learning tools have the potential to detect cattle lameness.

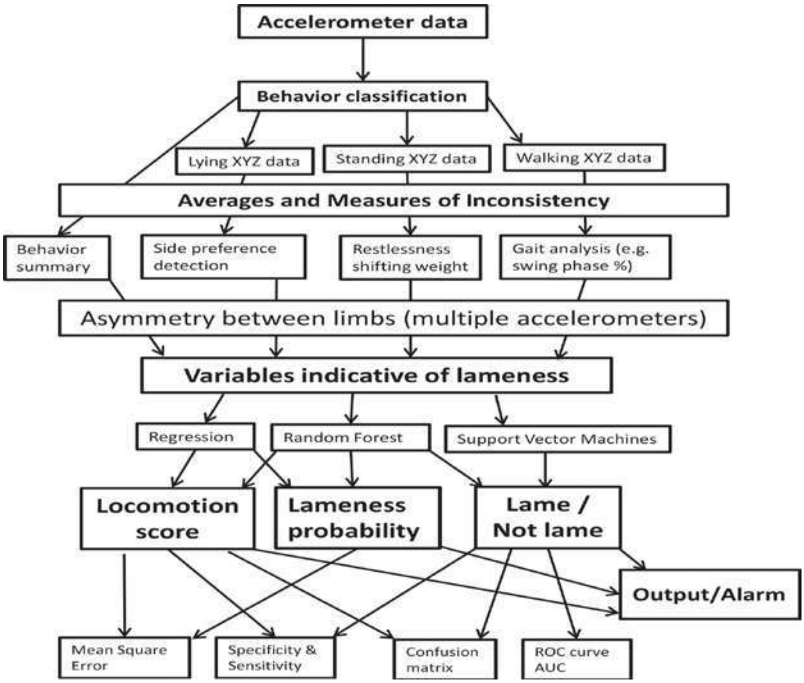


Figure1. A schematic diagram of how lameness classification could be implemented using accelerometers *(O'Leary et al., 2020)*

Pastell et al. (2009) reported that lame cows have a higher asymmetry of variance during acceleration compared to healthy cows. Similar to that, *Chapinal et al. (2011)* reported that lame cows with high gait scores had a greater asymmetry of variance during acceleration in both front and rear legs. According to *O'Leary et al. (2020)* lameness detection systems using accelerometer (one per cow; resolution < 100 Hz) with gait measurement functions are suggested to balance cost and data requirements. The same authors presented a schematic diagram about lameness classification using accelerometers (Figure 1), and they point out that high priority should be given to the development of novel gait measures and testing their ability to differentiate lame from nonlame cows.

Camera and Image Analysis

In resent time more studies was done with camera and picture analyses for lameness detection, for example, *Norton and Berckmans (2017)* used image analysis for collecting real-time field data aiming to monitor lameness of cows. Authors emphasized that there is no need for physical contact with animals, no need to recover sensors from living animals, and costs are reduced since one camera can monitor a very large group of animals, as each of them will pass under the camera every day. *Norton and Berckmans (2017)* used data from real-time image analysis of the camera in combination with data collected from accelerometers as basis for developing a real-time PLF algorithm for lameness detection (Figure 2).

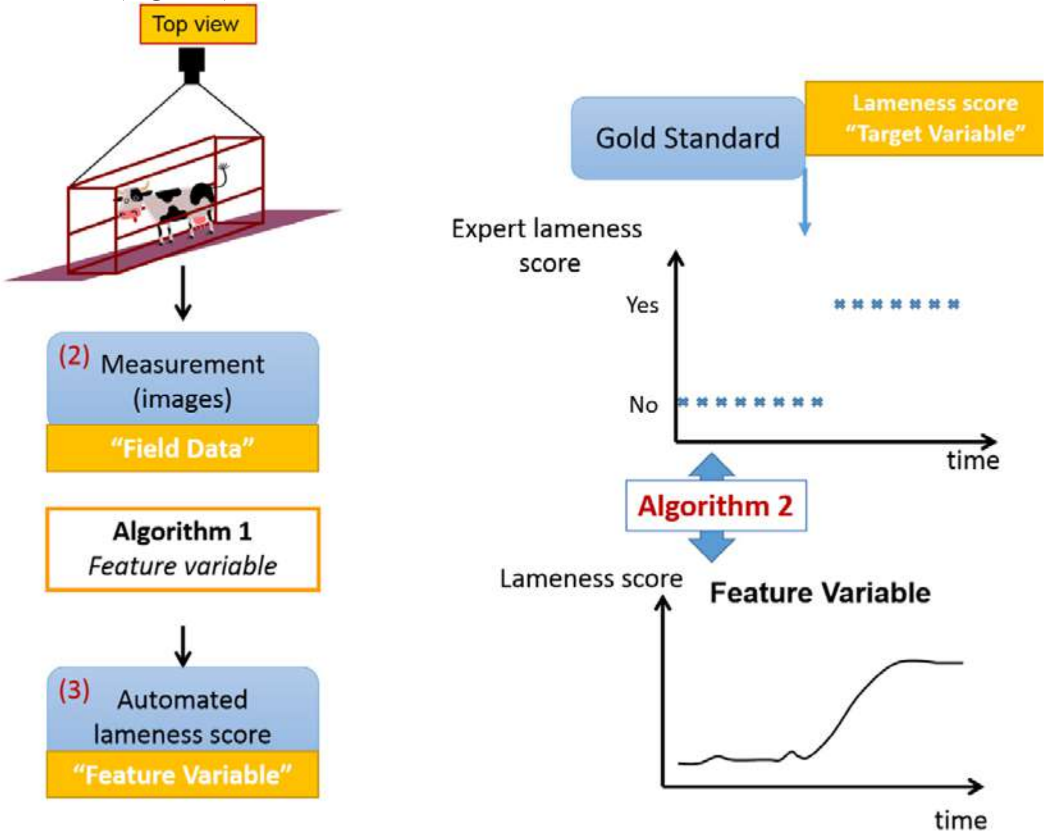


Figure 2. Measurements, labeling, and gold standard to develop algorithms *(Norton and Berckmans, 2017)*

In study of the *Piette et al. (2020)* camera were used for tracking of back posture of the individual Holstein cows, and authors were used algorithm and the quantity of the historical data for lameness detection. *Piette et al. (2020)* were established a high-performing lameness detection system and demonstrates the importance of the historical window length for healthy reference calculation in order to ensure the use of meaningful historical data in deviation detection algorithms.

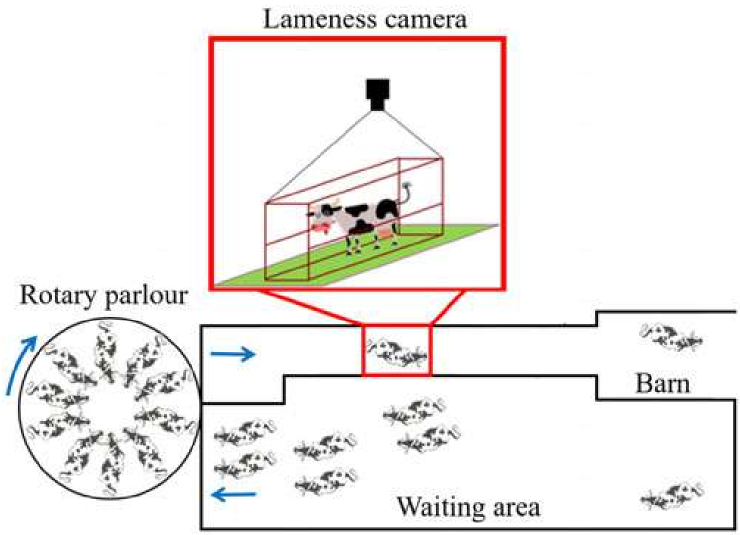


Figure 3. Schematic plan for the dairy cow lameness monitor in the farm *(Piette et al., 2020)*

Infrared Thermography

Infrared thermography (IRT) is non-invasive diagnostic tool able to detect lesions of hooves associated with lameness by measuring the changes in skin surface temperature (*Nikkhah et al., 2005; Alsaood and Büscher, 2012; Poikalainen et al., 2012; Bobić et al., 2018*). In dairy herds, hoof surface temperature could be monitored regularly to assess hoof health status, and with IRT can be detected foot lesions well before the appearance of clinical signs (*Bobić et al., 2017*).

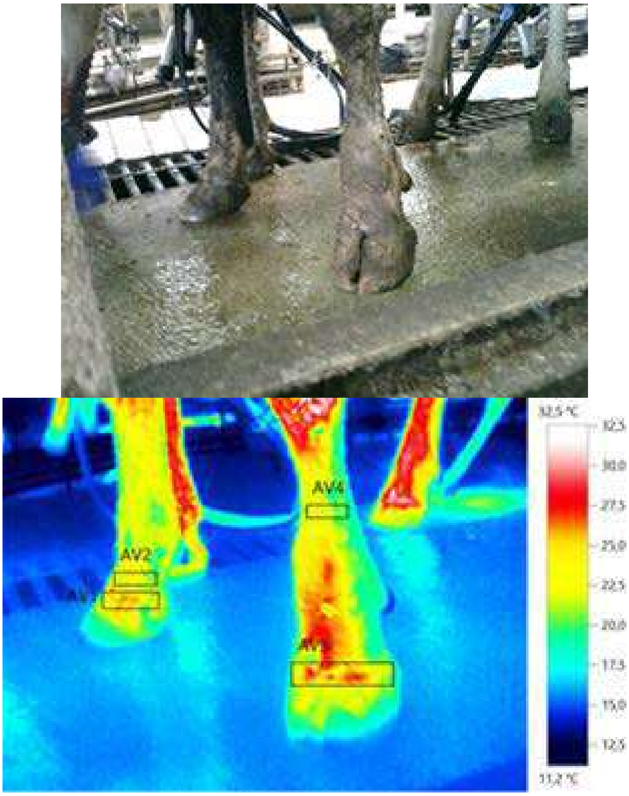


Figure 4. Digital and infrared picture of the cows hoof (*Bobić et al., 2020*)

The application of IRT in practice is possible, useful and desirable, but it is not simple (special conditions and preparation of the animal are required, presented by *Lokesh Babu et al. 2018* in Figure 5). Various factors that affect changes in body temperature and can lead to erroneous conclusions should be taken into account, such as: wind, ambient temperature, sunlight exposure, hoof humidity, physiological condition of the animal, etc.

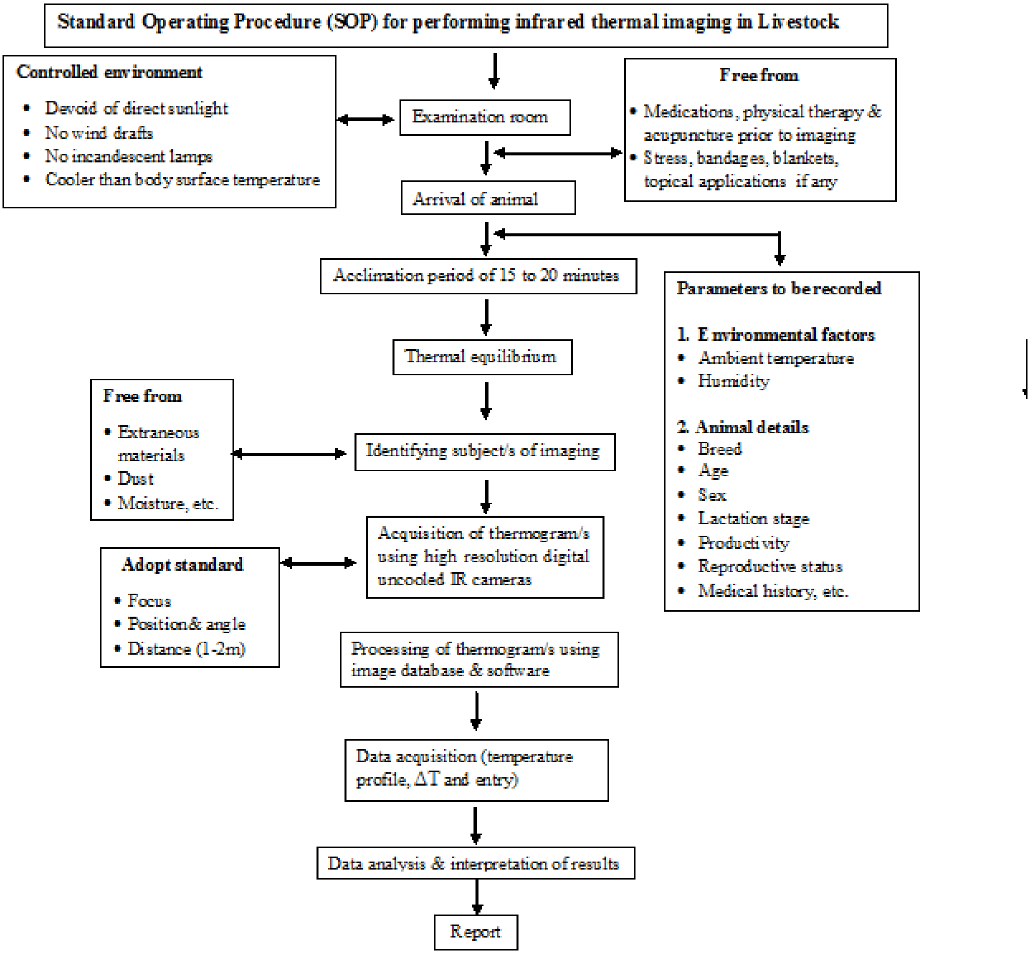


Figure 5. A schematic diagram of standard operating procedure for performing thermography ***(Lokesh Babu et al., 2018)***

Conclusion

The application of ICT technology in the detection and prevention of lameness in cows in milk production is possible and has a future. Currently, ICT is strongly represented at the level of scientific approach with a tendency to be applied in practice on a daily basis. Significant effort and cooperation of several different groups of people (scientists, IT specialists, farmers) is needed in order for ICT to be successfully implemented in continuous practical application.

Otkrivanje hromosti kod goveda korišćenjem ICT tehnologije

Tina Bobić, Andrea Bejteš, Pero Mijić, Vesna Gantner, Maja Gregić

Rezime

Budući da je problem hromosti vrlo čest na farmama za proizvodnju mleka, potrebno je primeniti novije tehnologije koje mogu doprineti smanjenju učestalosti hromosti. Informacione računarske tehnologije (ICT) postaju sve raširenije za praćenje nivoa proizvodnje, funkcionalnosti, dobrobiti i zdravstvenih osobina. Automatskim metodama otkrivanja hromosti velika količina podataka može se prikupiti u kratkom vremenskom periodu, što može poboljšati tačnost predviđanja hromosti. Primena ICT tehnologije u otkrivanju i sprečavanju hromosti kod krava u proizvodnji mleka je moguća i ima budućnost. Trenutno su ICT snažno zastupljene na nivou naučnog pristupa sa tendencijom svakodnevne primene u praksi. Da bi se ICT tehnologija uspešno primenjivala u kontinuiranoj praktičnoj primeni potrebni su značajni napori i saradnja nekoliko različitih grupa ljudi (naučnika, IT stručnjaka i farmera).

Ključne reči: informacione računarske tehnologije, hromost, goveda, detekcija

Acknowledgement

The paper is the result of collaboration with a student Andrea Bejteš in preparing her graduation thesis at the Faculty of Agrobiotechnical Sciences Osijek.

Detekcija hromosti kod krava primenom ICT tehnologije

Tina Bobić, Andrea Bejteš, Pero Mijić, Vesna Gantner, Maja Gregić

Rezime: S obzirom da je problem hromosti veoma čest na mlečnim farmama, neophodno je primeniti novije tehnologije koje mogu doprineti smanjenju učestalosti hromosti. Informacione računarske tehnologije (ICT) postaju sve raširenije za praćenje nivoa proizvodnje, funkcionalnih, socijalnih i zdravstvenih karakteristika. Automatske metode otkrivanja hromosti mogu da prikupe veliku količinu podataka u kratkom vremenskom periodu, što može poboljšati tačnost predviđanja hromosti. Primena ICT tehnologije u otkrivanju i sprečavanju hromosti krava u proizvodnji mleka je moguća i ima budućnost. Trenutno je ICT snažno zastupljena na nivou naučnog pristupa sa tendencijom svakodnevne primene u

praksi. Potrebni su značajni napori i saradnja nekoliko različitih grupa ljudi (naučnika, informatičara, farmera) kako bi se ICT uspešno primenjivala u kontinuiranoj praktičnoj primeni.

Ključne riječi: informacione računarske tehnologije, hromost, goveda, detekcija

References

ALSAAOD M., FADUL M., STEINER A. (2019): Automatic lameness detection in cattle. The Veterinary Journal, 246, 35–44.
ALSAAOD M., BÜSCHER W. (2012): Detection of hoof lesions using digital infrared thermography in dairy cows. Journal of Dairy Science, 95, 735–742.
ALSAAOD M., RÖMER C., KLEINMANNS J., HENDRIKSEN K., ROSE-MEIERHÖFER S., PLÜMER L., BÜSCHERA W. (2012): Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. Applied Animal Behaviour Science, 142, (3–4), 134–41.
ANDONOVIC I., MICHIE C., COUSIN P. JANATI A., PHAM C., DIOP M. (2018): Precision Livestock Farming Technologies. Global Internet of Things Summit, GIoTS 2018. IEEE, ESP.
AZARPAJOUH S., CALDERÓN DÍAZ J. A., TAHERI H. (2020): Precision livestock farming: automatic lameness detection in intensive livestock systems. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources, 15, 31, 1-12.
BEWLEY J. (2010): Precision Dairy Farming: Advanced Analysis Solutions for Future Profitability. The First North American Conference on Precision Dairy Management 2010.
BOBIĆ T., MIJIĆ P., GREGIĆ M., BAGARIĆ A., GANTNER V. (2017): Early detection of the hoof diseases in Holstein cows using thermovision camera. Agriculturae Conspectus Scientificus, 82, 2, 197-200.
BOBIĆ T., MIJIĆ P., GANTNER V., GLAVAŠ H., GREGIĆ M. (2018): The effect of the parity and stage of lactation on detection of the hoof diseases in dairy cows using thermovision camera. Journal of Central European Agriculture, 19, 4, 777-783.
BOBIĆ, T., BANK, F., MIJIĆ, P., BABAN, M., GANTNER, V., GREGIĆ, M. (2020): Prevencija šepavosti kod krava infracrvenom termografijom. Proceedings & abstract of the 13th International Scientific Professional Conference, Agriculture in Nature and Environment Protection, Osijek: Glas Slavonije d.d., Osijek, 2020, 201-206.

CHAPINAL N., de PASSILLE A.M.B., WEARY D.M., von KEYSERLINGK M. A. G., RUSHEN J. (2009): Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. Journal of Dairy Science, 92, 9, 4365-4374.

CHAPINAL N., de PASSILLE A.M., PASTELL M., HANNINEN L., MUNKSGAARD L., RUSHEN J., (2011): Measurement of acceleration while walking as an automated method for gait assessment in dairy cattle. Journal of Dairy Science, 94, 2895–2901.

FLOWER F, SANDERSON D, WEARY D. (2005): Hoof pathologies influence kinematic measures of dairy cow gait. Journal of Dairy Science, 88, 3166–73.

GREEN L. E., BORKERT J., MONTI G., & TADICH N. (2010): Associations between lesion-specific lameness and the milk yield of 1,635 dairy cows from seven herds in the X^{th} region of Chile and implications for management of lame dairy cows worldwide. Animal Welfare, 19, 4, 419-427.

KRAMER E., CAVERO D., STAMER E., KRIETER J. (2009): Mastitis and lameness detection in dairy cows by application of fuzzy logic. Livestock Science, 125, 92–6.

LOKESH BABU D.S., JEYAKUMAR S., VASANT P. J., SATHIYABARATHI M., MANIMARAN A., KUMARESAN A., PUSHPADASS H. A., SIVARAM M., RAMESHA K. P., KATAKTALWARE M. A., SIDDARAMANNA (2018): Monitoring foot surface temperature using infrared thermal imaging for assessment of hoof health status in cattle: a review. Journal of Thermal Biology, 78, 10-21.

MAERTENS W., VANGEYTE J., BAERT J., JANTUAN A., MERTENS K. C., de CAMPENEERE S., PLUK A., OPSOMER G., van WEYENBERG S., van NUFFEL A. (2011): Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: the GAITWISE system. Biosystems Engineering, 110, 1, 29–39.

NIKKAH A., PLAIZIER J. C., EINARSON M.S., BERRY R.J., SCOTT S.L., KENNEDY A.D. (2005): Infrared thermography and visual examination of hooves of dairy cows in two stages of lactation. Journal of Dairy Science, 88, 2479-2753.

NORTON T., BERCKMANS D. (2017): Developing precision livestock farming tools for precision dairy farming. Animal Frontiers, 7, 1, 18-23.

O'LEARY N. W., BYRNE D. T., O'CONNOR A. H., SHALLOO L. (2020): Invited review: Cattle lameness detection with accelerometers. Journal of Dairy Science, 103, 3895–3911.

ÓZSVÁRI L. (2017): Economic Cost of Lameness in Dairy Cattle Herds. Journal of Dairy, Veterinary & Animal Research 6, 2, 00176.

PASTELL M., KUJALA M., AISLA A.M., HAUTALA M., POIKALAINEN V., PRAKS J., VEERMÄE I., AHOKAS J. (2008): Detecting cow's lameness using force sensors. Computers and Electronics in Agriculture, 64, 1, 34–8.

PASTELL M., TIUSANEN J., HAKOJARVI M., HANNINEN L. (2009): A wireless accelerometer
system with wavelet analysis for assessing lameness in cattle. Biosystems Engineering, 104, 545–551.

PIETTE D., NORTON T., EXADAKTYLOS V., BERCKMANS D. (2020): Individualised automated lameness detection in dairy cows and the impact of historical window length on algorithm performance. Animal, 14, 2, 409–417.

POIKALAINEN V., PRAKS J., VEERMÄE I. KOKIN E. (2012): Infrared temperature patterns of cow's body as an indicator for health control at precision cattle farming. Agronomy Research Biosystem Engineering, Special Issue 1, 187-194.

POURSABERI A., BAHR C., PLUK A., van NUFFEL A., BERCKMANS D. (2010): Real-time automatic lameness detection based on back posture extraction in dairy cattle: shape analysis of cow with image processing techniques. Computers and Electronics in Agriculture, 74, 1, 110–9.

POURSABERI A., BAHR C., PLUK A., BERCKMANS D., VEERMAE I., KOKIN E., POKALAINEN, V. (2011): Online lameness detection in dairy cattle using body movement pattern (bmp). In: Proceedings of the 11th International Conference on Intelligent Systems Design and Applications (ISDA). Cordoba, Spain: IEEE.

Van NUFFEL A., VANGEYTE J., MERTENS K.C., PLUYM L., De CAMPENEERE S., SAEYS W., Van WEYENBERG S. (2013): Exploration of measurement variation of gait variables for early lameness detection in cattle using the GAITWISE. Livestock Science, 156, 88–95.

VIAZZI S., BAHR C., van HERTEM T., SCHLAGETER-TELLO A., ROMANINI C.E.B., HALACHMI I., LOKHORST C.L., BERCKMANS D. (2014): Comparison of a threedimensional and two-dimensional camera system for automated measurement of back posture in dairy cows. Computers and Electronics in Agriculture, 100, 139–47.

THE EFFECT OF SEASON ON THE ESTIMATED AMMONIA EMISSION OF HOLSTEIN FIRST PARITY COWS

Mirna Gavran[1], Dragan Solić[2], Danko Šinka[3], Vesna Gantner[1]

[1]Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek, Vladimira Preloga 1, Osijek, Croatia
[2] Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, Osijek, Croatia
[3]Belje Plus Ltd, Industrijska zona 1, Mece, 31326 Darda, Croatia
Corresponding author: Mirna Gavran, mgavran@fazos.hr

Abstract: The objective of this research was to determine the differences in nitrogen related compounds (daily urea content in milk, milk urea nitrogen and ammonia emission) in milk samples of Holstein first parity cows reared in Eastern Croatia depending on season of milk recording. Test-day records of first parity Holstein cows reared in Eastern Croatia were used for the statistical analysis. The results of analyses of variance of daily urea content in milk, milk urea nitrogen and ammonia emission of Holstein first parity cows regarding the season (spring, summer, autumn and winter) are presented in the paper. All analysed traits differed statistically highly significant ($p < 0.001$) regarding the seasons. The highest content of daily milk urea was recorded in summer (27.195 mg/dl), also, milk produced in summer had the highest MUN (mg/dl) as well as the highest ammonia emission (g/cow daily). The lowest content of daily milk urea (20.647 mg/dl) was determined in winter, along with the lowest milk urea nitrogen (9.498 mg/dl) and ammonia emission (72.773 g/cow daily). The research results indicate that the variability of daily milk urea content, milk urea nitrogen and consequently ammonia emission in Holstein first parity cows depends of daily milk production, stage of lactation, age at first calving, and season of milk recording. Therefore, those effects should be taken into account in the statistical model for estimation of the ammonia emission from dairy cattle farms based on milk recording data.

Key words: ammonia emission, first parity Holsteins, milk recording

Introduction

In indoor dairy farming, there is an evident correlation between the concentration of urea nitrogen in milk and the concentration of urea nitrogen in urine (*Jonker et al. 1998; Kauffman & St-Pierre 2001; Spek et al. 2013*). A tool used in present dairy farming to control the effectiveness of protein utilization is milk urea nitrogen (MUN) (*Guliński et al., 2015*). Urea (70%), allantoin (8%), hippuric acid (6%), creatinine (4%), creatine (3%) and ammonia (3%) are the main components of bovine urine. Furthermore, nearly 2.5-3.0% of the entire amount of urea in the body, dairy cows remove with milk (*Spek, 2013; Guliński et al., 2015*). Milk recording data provide an evaluation of the influence of a dairy farm on environmental pollution. Accordingly, *Bijgaart (2003)* pointed out that farms in the Netherlands are regulated based on urea content in milk that allows the ascertainment of possible contamination and fresh information of farmers concerning the important measures. The milk recording process starts with the gathering of animal identification, a calving date of milking cows, the quantity of milk given, and the date with time or time frame of a day (*ICAR, 2017*). During the milk recording, milk production characteristics are recorded which represent meaningful data for control and breeding of dairy herds. This is also an ideal way for farmers to track their best and worst animals. Therefore, farmers can make management decisions and do the selection (*ICBF, 2019*). The objective of this research was to determine the differences in nitrogen related compounds (daily urea content in milk, milk urea nitrogen and ammonia emission) in milk samples of Holstein first parity cows reared in Eastern Croatia depending due to season of milk recording.

Materials and Methods

Test-day records of first parity Holstein cows reared in Eastern Croatia were used for the statistical analysis. The records were collected in period from January/2008 to December/2012 within the regular milk recording that is performed accordingly to the alternative milk recording method (AT4 / BT4). Sampled milk was analysed in the laboratory in accordance with accredited laboratory methods; infrared spectrophotometry using MilkoScan FT 6000.

After logical control dataset consisted of 175,162 test day records from 23,368 animals reared on 1,132 farms in Eastern Croatia. The milk urea nitrogen (MUN) content was calculated using milk urea content (UREA) accordingly to the following equation:

MUN (mg/dl) = UREA * 0.46 (*Spiekers & Obermaier, 2012*)

The ammonia emission (AMMONIA) was calculated using milk urea nitrogen (MUN) accordingly to the following equation:

AMMONIA (g/cow daily) = 25.0 + 5.03 * MUN (*Burgos et al., 2010*)

Basic statistical parameters of analysed traits are shown in Table 1.

Table 1. Basic statistical parameters of analysed traits

Variable	N	Mean	SD	CV	Minimum	Maximum
DMY	170660	22.009	7.874	35.775	3.000	85.200
UREA	169291	23.495	8.801	37.461	0.500	60.000
MUN	169291	10.808	4.049	37.461	0.230	27.600
AMMONIA	169291	79.362	20.364	25.660	26.157	163.828

*DMY – daily milk yield (kg); UREA – daily urea content in milk sample (mg/dl); MUN – milk urea nitrogen (mg/dl); AMMONIA – ammonia emission (g/cow daily)

For the evaluation of the effect of season on the variability of daily milk urea content, milk urea nitrogen and ammonia emission following statistical model was used:

$$y_{ijklmn} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3\ln(305/d_i) + b_4\ln^2(305/d_i) + b_5 m_j + A_k + P + M_{ml} + e_{ijklmn}$$

Where:

y_{ijklm} = estimated trait (daily milk urea content, milk urea nitrogen and ammonia emission);

μ = intercept;

b_1, b_2, b_3, b_4, b_5 = regression coefficients;

d_i = days in milk (i = 5 to 500 day);

m_j = daily milk yield (j = 3.00 to 96.00 kg);

A_k = fixed effect of age at first calving class k (k = 21 to 36 month);

S_l = fixed effect of recording season l (l = Spring, …, Winter);

e_{ijklm} = residual.

The significance of the differences between the season of milk recording was tested by Scheffe's method of multiple comparisons using the PROC GLM procedure in SAS (*SAS Institute Inc., 2019*).

Results and Discussion

The results of analyses of variance of daily milk urea, milk urea nitrogen and ammonia emissions in regard to the season of milk recording (spring, summer, autumn, winter) are shown in continuation. Statistically highly significant effect ($p < 0.001$) of all independent variables (days in milk; daily milk yield; age at first calving class; and season of milk recording) included in used statistical model on analysed traits (daily urea content in milk, milk urea nitrogen and ammonia emission) was determined (Table 2).

Table 2. Significance of the effects included in statistical model on analysed traits

Effects		**UREA**			**MUN**			**AMMONIA**		
Source	**DF**	**MS**	**F Value**	**Pr > F**	**MS**	**F Value**	**Pr > F**	**MS**	**F Value**	**Pr > F**
DMY	1	80144.7429	1130.22	<.0001	16958.6276	1130.22	<.0001	429068.541	1130.22	<.0001
AFC	1	3336.3271	47.05	<.0001	705.9668	47.05	<.0001	17861.596	47.05	<.0001
sch1	1	19314.1322	272.37	<.0001	4086.8704	272.37	<.0001	103401.499	272.37	<.0001
sch2	1	12840.6448	181.08	<.0001	2717.0804	181.08	<.0001	68744.581	181.08	<.0001
sch3	1	22303.5190	314.53	<.0001	4719.4246	314.53	<.0001	119405.690	314.53	<.0001
sch4	1	19074.4864	268.99	<.0001	4036.1613	268.99	<.0001	102118.514	268.99	<.0001
season	3	301200.6482	4247.61	<.0001	63734.0572	4247.61	<.0001	1612529.007	4247.61	<.0001

*DMY – daily milk yield (kg); AFC – age at first calving; sch1 – sch4; regression coefficients of lactation curve (effect of days in milk); UREA – daily urea content in milk sample (mg/dl); MUN – milk urea nitrogen (mg/dl); AMMONIA – ammonia emission (g/cow daily)

The differences in analysed traits (daily urea content in milk, milk urea nitrogen and ammonia emission) regarding the season class are shown in the Table 3.

Table 3. LSMs of analysed traits regarding the season of milk recording

SEASON	**UREA**	**MUN**	**AMMONIA**
Spring	22.994^{A}	10.577^{A}	78.204^{A}
Summer	27.195^{B}	12.5099^{B}	87.924^{B}
Autumn	22.958^{A}	10.561^{A}	78.121^{A}
Winter	20.647^{C}	9.498^{C}	72.773^{C}

*UREA – daily urea content in milk sample (mg/dl); MUN – milk urea nitrogen (mg/dl); AMMONIA – ammonia emission (g/cow daily); LSMeans marked with different letters (A, B, C) differ statistically highly significant ($p < 0.001$)

The results of analyses of variance of daily urea content in milk, milk urea nitrogen and ammonia emission of Holstein first parity cows regarding the season (spring, summer, autumn and winter) are presented in Table 3. All analysed traits differed statistically highly significant ($p < 0.001$) regarding the seasons. The highest content of daily milk urea was recorded in summer (27.195 mg/dl), also, milk produced in summer had the highest MUN (mg/dl) as well as the highest ammonia emission (g/cow daily). The lowest content of daily milk urea (20.647 mg/dl) was determined in winter, along with the lowest milk urea nitrogen (9.498 mg/dl) and ammonia emission (72.773 g/cow daily).

A significantly higher urea content in milk produced during the summer period was also determined by *Ruska et al. (2017).* Urea content in milk is an indicator of suitable or appropriate protein and energy balancing in fodder for cows with various productivity features. In accordance to *Ruska et al. (2017)*, urea content in milk produced in a farm where tie-stall housing system is, cows are not classified and are grazed in summer was significantly higher. In accordance to *Spohr and Wiesner (1991)* and *Spann (1993)*, a complication related to providing highly productive dairy cows with fodder dosage having sufficient amounts of energy and protein was revealed by increased urea in milk. Accordingly, *Kohn et al. (2002)* and *Bucholtz et al. (2007)* pointed out that numerous researches conducted in Europe have used urea content in milk; at the same time, researches carrying out in the USA are normally using diverse parameters – milk urea nitrogen (MUN) content. Acceptable MUN content should have 8.0–12.0 mg/dl and it is used for effectiveness control. In case the MUN threshold is surpassed, subsequently, farms have to keep an eye out for the use of proteins in fodder and their balancing with energy in a particular feed dose. In accordance to *Aguilar et al. (2012)*, data gathered in the USA relating to fodder protein and MUN content point that for going up to the MUN limit of 12 mg/dl, it is required to reduce protein amount in food to 12.8% in dry matter. Accordingly, *Godden et al. (2001)* and *Haig et al. (2002)*, noticed that for estimating and creating the farming model is recommended using the urea content parameter from the view of experts from countries evaluating nitrogen usage and effectiveness. For identifying metabolism processes in the animal body, such as predicting possible diseases (ketosis, acidosis) timely and maintaining farming performance, it is determined that milk content features can be applied for that and not just for assessing animal productivity. Also, there is a significant association between milk urea content and nitrogen content in animal urine and manure (*Burgos et al., 2010; Eckersall & Bell, 2010; Klein et al., 2011; Spek et al., 2013*).

Conclusion

The research results indicate that the variability of daily milk urea content, milk urea nitrogen and consequently ammonia emission in Holstein first parity cows depends of daily milk production, stage of lactation, age at first calving, and season of milk recording. Therefore, those effects should be taken into account in the statistical model for estimation of the ammonia emission from dairy cattle farms based on milk recording data.

Uticaj sezone na procenjenu emisiju amonijaka prvotelki holštajn rase

Mirna Gavran, Dragan Soli, Danko Šinka, Vesna Gantner

Rezime

Cilj ovog istraživanja bio je da se utvrde razlike u jedinjenjima povezanim sa azotom (dnevni sadržaj uree u mleku, azot uree mleka i emisija amonijaka) u uzorcima mleka prvotelki holštajn rase uzgojenih u istočnoj Hrvatskoj, u zavisnosti od sezone kontrole mlečnosti. Za statističku analizu korišćena je evidencija o test danima prvotelki holštajn rase uzgojenih u istočnoj Hrvatskoj. Rezultati analiza varijanse dnevnog sadržaja uree u mleku, emisije azota uree u mleku i amonijaka holštajn prvotelki prema sezoni (proleće, leto, jesen i zima) prikazani su u radu. Sve analizirane osobine razlikovale su se statistički visoko značajno ($p<0,001$) u odnosu na godišnja doba. Najveći sadržaj dnevne mlečne uree zabeležen je leti (27,195 mg/dl), takođe, mleko proizvedeno leti imalo je najveću vrednost MUN (mg/dl), kao i najveću emisiju amonijaka (g/krava dnevno). Najniži sadržaj dnevne mlečne uree (20.647 mg/dl) utvrđen je zimi, zajedno sa najnižim azotom uree u mleku (9.498 mg/dl) i emisijom amonijaka (72.773 g/kravi dnevno). Rezultati istraživanja pokazuju da varijabilnost dnevnog sadržaja uree u mleku, azota uree u mleku i posledično emisije amonijaka kod prvotelki holštajn rase zavisi od dnevne proizvodnje mleka, faze laktacije, starosti pri prvom teljenju i sezone kontrole mlečnosti. Stoga, te efekte treba uzeti u obzir u statističkom modelu za procenu emisije amonijaka sa farmi mlečnih goveda na osnovu podataka o kontroli mlečnosti.

Ključne reči: emisija amonijaka, prvotelke holštajn rase, kontrola mlečnosti

References

AGUILAR M., HANIGAN D., TUCKER B., JONES L., GARBADE S.K., MCGILLIARD M.L., STALLINGS C.C., KNOWLTON K.F., JAMES R.E. (2012): Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. Journal of Dairy Science 95, 7261–7268.

BIJGAART H. van den. (2003): Urea. New applications of mid–infra–red spectrometry. Bulletin of the IDF 383, 5–15.

BUCHOLTZ H., JOHNSON T., EASTRIDGE M.L. (2007): Use of milk urea nitrogen in herd management. In: Tri–State Dairy Nutrition Conference. Proceedings. Ft. Wayne, Indiana, 63–67.

BURGOS S.A., EMBERTSON N.M., ZHAO Y., MITLOEHNER F.M., DEPETERS E.J., FADEL J.G. (2010): Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to ammonia emissions. Journal of Dairy Science 93(6), 2377–2386.

ECKERSALL P.D., BELL R. (2010): Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Veterinary Journal 185, 23–27.

GODDEN S.M., LISSEMORE K.D., KELTON D.F., LESLIE K.E., WALTON J.S., LUMSDEN J.H. (2001): Relationships between milk urea concentrations and nutritional management, production and economic variables in Ontario dairy cows. Journal of Dairy Science 84, 1128–1139.

GULIŃSKI P., SALAMOŃCZYK E., MŁYNEK K. (2015): Improving nitrogen use efficiency of dairy cows in relation to urea in milk – a review. Animal Science Papers and Reports vol. 34 (2016) no. 1, 5-24.

HAIG P.A., MUTSVANGWA T., SPRATT R., MCBRIDE B.W. (2002): Effects of dietary protein solubility on nitrogen losses from lactating dairy cows and comparison with predictions from the cornell net carbohydrate and protein system. Journal of Dairy Science 85, 1208–1217.

INTERNATIONAL COMMITTEE FOR ANIMAL RECORDING, ICAR (2017): Section 2 - Guidelines for Dairy Cattle Milk Recording

IRISH CATTLE BREEDING FEDERATION, ICBF (2019): 10 things you need to know about milk recording.

JONKER J.S., KOHN R.A, ERDMAN R. (1998): Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. Journal of Dairy Science 81: 2681-92.

KAUFFMANN A.J., ST-PIERRE N.R. (2001): The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. Journal of Dairy Science 84: 2284- 2294.

KLEIN M.S., BUTTCHEREIT N., MIEMCZYK S.P., IMMERVOLL A.K., LOUIS C., WIEDEMANN S., JUNGE W., THALLER G., OEFNER P.J., GRONWALD W. (2011): NMR Metabolomic analysis of dairy cows reveals milk glycerophoshocholine to phosphocholine ratio as prognostic biomarker for risk of ketosis. Journal of Proteome Reserch 11, 1373–1381.

KOHN, R.A., KALSHEUR, K.F., RUSSEK-COHEN, E. (2002). Evaluation of models to estimate urinary nitrogen and expexted milk urea nitrogen. Journal of Dairy Science 85, 227–233.

RUSKA D., JONKUS D., CIELAVA L. (2017): Monitoring of ammonium pollution from dairy cows farm according of urea content in milk. Agronomy Research, Vol.15, No.2, pp.553-564.

SPANN B. (1993): Futterungsberater Rind: Kalber, Milchvieh, Mastrinder. Stuttgart: Ulmer Eugen Verlag. 183 S.

SPEK J.W. (2013): Variation of milk urea in dairy. A study on factors that affect the relationship between

urea concentration in milk and urea excretion in urine. PhD thesis, Wageningen University, 1-162.

SPEK J.W., DIJKSTRA J., van DUINKERKEN G., BANNIK A. (2013): A review of factors influencing milk urea concentration and its relationship with urea excretion in lactating dairy cattle. Journal of Agricultural Science 151, 407–42.

SPEK J.W., DIJKSTRA J., van DUINKERKEN G., HENDRIKS W.H., BANNIK A. (2013): Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis. Journal of Dairy Science 96: 4310- 4322.

SPIEKERS H., OBERMAIER A. (2012): Milchharnstoffgehalt und N–Ausscheidung (Milk urea content and N excretion) [tiešsaiste]. Institut für Tierernährung und Futterwirtschaft, Prof.– Dürrwaechter–Platz 3, 85586 Poing–Grub.

SPOHR M., WIESNER H.U. (1991): Kontrolle der Herdengesundheit und Milchproduktion mit Hilfe der erweiterten Milchleistungsprufung. Milchpraxis 29, 231–2

THE VARIABILITY IN THE ESTIMATED PREVALENCE RISK OF METABOLIC DISORDERS (KETOSIS/ACIDOSIS) IN SIMMENTAL FIRST PARITY COWS DUE TO RECORDING SEASON

Franjo Poljak[1], Marija Špehar[1], Zvonimir Steiner[2], Mirna Gavran[2], Vesna Gantner[2]

[1] Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, Osijek, Croatia
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia, Corresponding author: Vesna Gantner, vgantner@fazos.hr

Abstract: The purpose of this research was to determine the prevalence risk of subclinical disorders of Simmental first parity cows regarding the recording season. Test-day records of Simmentals collected during the five years (January/2008 – December/2012) given by the Croatian Agricultural Agency were used for the statistical analysis. During the regular milk recording performed monthly following the alternative milk recording method (AT4 / BT4) on dairy cattle farms in Croatia, test-day records were gathered. The highest daily fat content (4.31%) and the highest daily protein content (3.54%) was ascertained in winter. The lowest values of daily fat (3.83%) and protein content (3.33%) were determined in summer period. Additionally, the lowest value of fat to protein ratio (F/P) was observed in summer in amount of 1.16. Higher value of F/P (1.22) was observed in winter period. Further, the study showed that daily fat and protein content, together with F/P ratio significantly, differ due to recording month with the higher values of F/P ratio in winter period indicating higher ketosis prevalence risk, and lower values of F/P ratio in summer period indicating higher acidosis prevalence risk. Mentioned factors should be taken into consideration when predicting the ketosis/acidosis prevalence in dairy cows based on milk recording data because they influence the variability of daily fat and protein content, and therefore the fat to protein ratio along with the prevalence risk of metabolic disorders.

Key words: metabolic disorders, ketosis, acidosis, test-day records, Simmental cows

Introduction

In terms of physiology, postpartum period is one of the most critical periods for dairy cows. In the first 10 days after calving, postpartum disorders usually occur *(Ingvartsen et al. 2003; LeBlanc, 2010; Antanaitis et al. 2015)*. In accordance to *Mulligan and Doherty (2008)*, the cause of stress might be environmental circumstances, for example, reorganization, while *Broucek et al. (2007)*, pointed out inappropriate (micro)climatic conditions. In lactating dairy cows, the most frequent disorders are ketosis and acidosis. Ketosis is a disorder that can appear both in clinical and subclinical forms. According to *Gillund et al. (2001)*, clinical ketosis frequently occurs in high-producing cows at the start of lactation (2^{nd}-7^{th} weeks) as a consequence of an unbalanced diet and management of the farm. Ketosis preponderance could vary because of breed, parity, season, and herd-connected circumstances *(Dohoo and Martin, 1984; Rajala-Schultz et al., 1999)*. Further, clinical ketosis causes economic losses for farmers for the sake of reduced milk production, decreased reproduction, treatment costs, and ultimately increased animal elimination rates *(Rajala-Schultz and Gröhn, 1998; Suthar et al., 2013)*. A developing problem in high-productive dairy cows is also subacute ruminal acidosis (SARA), because of that, prevention and animal surveillance are extremely significant. The greatest prevalence of SARA was determined in animals at maximum dry matter intake and in peak of lactation *(Dirksen et al., 1985; Bramley et al., 2005; Oetzel, 2005; O'Grady et al., 2008)*. Given that both metabolic disorders (ketosis / acidosis) cause high costs on dairy farms, it is extremely important to detect a possible disorder in a timely manner and prevent the occurrence of the clinical form. For this purpose, the results of milk recording, i.e. the ratio of milk fat and protein, can be used as an excellent indicator of the prevalence risk. Since many factors affect the variability of the daily milk content and consequently the fat to protein ratio (F/P), this study aimed to determine the variability in daily milk fat and protein content, the F/P ratio as well as in prevalence risk of subclinical disorders of Simmental first parity cows, depending on the recording season.

Materials and Methods

Test-day records of first parity Simmental cows reared in Eastern Croatia were used for the statistical analysis. The records were collected in period from January/2008 to December/2012 within the regular milk recording that is performed accordingly to the alternative milk recording method (AT4 / BT4). Sampled milk was analysed in the laboratory in accordance with accredited laboratory methods; infrared spectrophotometry using MilkoScan FT 6000. The test-day records with missing information regarding parity, breed, and missing or nonsense daily milk traits accordingly to standards of ICAR (ICAR standards, 2017) were excluded from the dataset. After logical control dataset consisted of 59,150 test day records from 11,258 first parity Simmental cows reared on 1,591 farms in Eastern Croatia. Further, in relation to the date of recording, the test day records were categorized into four seasons: spring (March, April and May), summer (June, July and August), autumn (September, October and November), and winter (December, January and February). Basic statistical parameters of analysed traits (daily milk yield, daily fat and protein content, as well as fat to protein ratio) are shown in Table 1.

Table 1. Basic statistical parameters of analysed traits

Variable	N	Mean	SD	CV	Minimum	Maximum
DMY	58893	15.773	5.195	32.938	3.000	82.400
FAT	56606	4.093	0.900	21.988	1.500	9.000
PROTEIN	57286	3.457	0.458	13.240	1.440	6.900
F/P	56598	1.193	0.269	22.513	0.347	4.194

*DMY – daily milk yield (kg); FAT – daily fat content (%); PROTEIN – daily protein content (%); F/P – fat to protein ratio

For the evaluation of the effect of recording season on the variability of analysed traits (daily fat and protein content, together with fat to protein ratio) in Simmental first parity cows, following statistical model was used:

$$y_{ijklmn} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + b_5 m_j + A_k + P + M_{ml} + e_{ijklmn}$$

Where:

yi_{jklmn} = estimated trait (daily fat and protein content, as well as fat to protein ration);

μ = intercept;

b_1, b_2, b_3, b_4, b_5 = regression coefficients;

d_i = days in milk (i = 5 to 500 day) as the polynomial regressions by Ali and Schaeffer (1987);

m_j = daily milk yield (j = 3.00 to 96.00 kg);

A_j = fixed effect of age at first calving class j (j = 21 to 36 month);

P_l = fixed effect of parity l (l = I., II., III., and IV.);

M_j = fixed effect of recording month m (m = January to December);

e_{ijklmn} = residual.

The significance of the differences between recording season classes was tested by Scheffe's method of multiple comparisons (using the PROC GLM procedure in SAS (SAS Institute Inc., 2019)).

Results and Discussion

The results of analyses of daily fat content, daily protein content, and fat to protein ratio associated with the season of milk recording (spring, summer, autumn, winter) are presented in continuance. Statistically highly significant effect ($p < 0.001$) of all independent variables (days in milk – lactation stage; daily milk yield; age at first calving class; and season of milk recording) involved in the used statistical model on analysed traits (daily fat content, daily protein content, and fat to protein ratio) was ascertained (Table 2).

Table 2. Significance of the effects included in statistical model on analysed traits

Effects		**FAT**			**PROTEIN**			**F/P**		
Source	**DF**	**MS**	**F Value**	**Pr > F**	**MS**	**F Value**	**Pr > F**	**MS**	**F Value**	**Pr > F**
DMY	1	705.812	969.60	<.0001	52.668	363.14	<.0001	29.641	82.66	<.0001
AFC	1	8.905	12.23	0.0005	5.246	36.17	<.0001	0.013	0.03	0.8708
sch1	1	66.665	91.58	<.0001	1.232	8.50	0.0036	5.410	980.35	<.0001
sch2	1	88.703	121.86	<.0001	11.879	81.91	<.0001	3.031	356.28	<.0001
sch3	1	19.525	26.82	<.0001	14.380	99.15	<.0001	9.252	11.74	0.0006
sch4	1	2.730	3.75	0.0528	33.642	231.96	<.0001	8.660	121.67	<.0001
season	3	1597.157	731.36	<.0001	138.407	954.28	<.0001	11.154	160.47	<.0001

*DMY – daily milk yield (kg); AFC – age at first calving; sch1 – 4; regression coefficients of lactation curve (effect of days in milk); *FAT – daily fat content (%); PROTEIN – daily protein content (%); F/P – fat to protein ratio

The LSmeans of analysed traits (daily fat content, daily protein content and fat to protein ratio) regarding the recording season class (spring, summer, autumn and winter) are shown in the Table 3.

Table 3. LSmeans of daily fat, daily protein content, as well as fat to protein ratio regarding the recording season

SEASON	FAT	PROTEIN	F/P
Spring	4.07^{A}	3.41^{A}	1.20^{A}
Summer	3.83^{B}	3.33^{B}	1.16^{B}
Autumn	4.14^{C}	3.52^{C}	1.18^{C}
Winter	4.31^{D}	3.54^{C}	1.22^{D}

*FAT – daily fat content (%); PROTEIN – daily protein content (%); F/P – fat to protein ratio; LSMeans marked with different letters (A, B, C, D) differ statistically highly significant ($p < 0.001$)

All analysed traits differed statistically highly significant ($p < 0.001$) regarding the seasons. Furthermore, the highest daily fat content was determined in winter (4.31%), while the highest daily protein content (3.54%) was also ascertained in winter. The lowest values of daily fat (3.83%) and protein content (3.33%) were determined in summer period. Additionally, the lowest value of fat to protein ratio (F/P) was observed in summer in amount of 1.16. Higher value of F/P (1.22) was observed in winter period. Determined results prove that daily fat and protein content together with F/P ratio particularly vary as a consequence of recording season. Higher LSMs values of F/P ratio in the winter period imply higher ketosis prevalence risk, on the other hand, lower LSMs values of F/P ratio signify higher acidosis prevalence risk throughout the summer period. Accordingly, *Palmquist et al. (1993), Doreau et al. (1999),* stated that elements that can cause a decrease in the percentage of milk fat are increased milk production, reduced feed particle size, feeding with too much starch (> 28% of the total meal), a diet with the addition of polyunsaturated fatty acids (linoleic and linolenic) as free oils and heat stress. If high levels of milk fat occur in cows after calving, it is a sign of digestive diseases and is correlated with ketosis, fast weight loss, reduced milk yield, lasting liver damage, rennet dislocation, mastitis, and many different infections. On the other hand, at the end of lactation, high values of milk fat are normal given the reduction in milk yield, that signifies they are not a sign of digestive disorders. Numerous factors such as breed, order and stage of lactation, season (of calving, of milk recording), milking frequency, udder health, nutrition (energy supply and the proportion of voluminous feed in the meal), and individual characteristics of the animal are regulating the fat content of milk *(Hargrove and Gilbert, 1984; Arsov, 1986; Keowen et al., 1986; Erdman and Varner, 1995; Klei et al., 1997; Ouweltjes, 1998; Weiß et al., 2002).* In accordance to *Palmquist et al. (1993), Doreau et al. (1999),* the increased proportion of voluminous feeds, more frequent feeding, feeding with acclaimed oilseed levels (< 2.5 kg), feeding with a bigger proportion of saturated fats such as palmitic (c 16:00) and stearin (c 18:00), reduced fitness and weight loss are components that can improve milk fat content. Moreover, factors like nutrition (amount of digestible protein in the meal), season (lower content is typical for summer season), breed, order and stage of lactation, udder health, and specific characteristics of each cow, cause variation in protein content in milk *(Hargrove and Gilbert, 1984; Arsov, 1986; Keowen et al., 1986; Murphy and O'Mara, 1993; Erdman and Varner, 1995; Klei et al., 1997; Ouweltjes, 1998; Eicher et al., 1999; Weiß et al., 2002)*. The values of 3.2% - 3.8% is the most desirable for protein content in milk. Furthermore, low protein content signifies lack of energy and digestible protein, while too high protein content means the general overnutrition of the animal. The values of 1.1 - 1.5 are the ideal values of fat to protein ratio (F/P). The variation in the value of the ratio is slight in healthy animals in good condition. Differences in the F/P ratio can be caused by lacking feed, unsuitable environmental conditions of occurrence of animal disorder/disease *(Duffield, 2004; Eicher, 2004)*. This study shows that daily production level, stage of lactation, parity, age at first calving, and milk recording season statistically highly significant ($p < 0.001$) influenced the variability of daily fat and protein content as well as the F/P ratio. Furthermore, the results of the study show that the prevalence risk of metabolic diseases significantly differ throughout the year with registered higher ketosis prevalence risk in the winter period, along with higher acidosis prevalence risk during the summer period.

Conclusion

The purpose of this research was to determine the prevalence risk of subclinical disorders of Simmental first parity cows regarding the milk recording season. The carried analysis determined an important effect of daily milk production, stage of lactation, parity, age at first calving, milk recording, and recording season on the variability of daily fat and protein content as well as F/P ratio. Further, the study showed that daily fat and protein content, together with F/P ratio significantly, differ due to recording season with the higher values of F/P ratio in winter period indicating higher ketosis prevalence risk, and lower values of F/P ratio in summer period indicating higher acidosis prevalence risk. Mentioned factors should be taken into consideration when predicting the ketosis/acidosis prevalence in dairy cows based on milk recording data because they influence the variability of daily fat and protein content, and therefore the fat to protein ratio along with the prevalence risk of metabolic disorders.

Varijabilnost u procenjenom riziku prevalencije metaboličkih poremećaja (ketoza/acidoza) kod simentalskih prvotelki pod uticajem sezone kontrole mlečnosti

Franjo Poljak, Marija Špehar, Zvonimir Steiner, Mirna Gavran, Vesna Gantner

Rezime

Svrha ovog istraživanja bila je da se utvrdi rizik od prevalencije subkliničkih poremećaja kod simentalskih prvotelki u odnosu na sezonu kontrole mlečnosti. Za statističku analizu korišćeni su podaci kontrole mlečnosti prikupljeni tokom pet godina (januar/2008. - decembar/2012.), Hrvatske poljoprivredne agencije. Tokom redovne kontrole mlečnosti mesečno prema alternativnoj metodi kontrole mlečnosti (AT4 / BT4) na farmama mlečnih goveda u Hrvatskoj, prikupljeni su podaci za test dane. Najveći dnevni sadržaj masti (4,31%) i najveći dnevni sadržaj proteina (3,54%) utvrđeni su zimi. Najniže dnevne vrednosti za mast (3,83%) i protein (3,33%) utvrđene su u letnjem periodu. Pored toga, najniža vrednost odnosa masti i proteina (F/P) zabeležena je leti u iznosu od 1,16. Veća vrednost F/P (1,22) primećena je u zimskom periodu. Dalje, studija je pokazala da se dnevni sadržaj masti i proteina, zajedno sa odnosom F/P značajno razlikuju pod uticajem meseca kontrole mlečnosti, sa većim vrednostima odnosa F/P u zimskom periodu što ukazuje na veći rizik od prevalencije ketoze i niže vrednosti F/P odnosa u letnjem periodu koji ukazuje na veći rizik od prevalencije acidoze. Navedene faktore treba uzeti u obzir pri predviđanju prevalencije ketoze/acidoze kod muznih krava na osnovu podataka iz kontrole mlečnosti, jer oni utiču na varijabilnost dnevnog sadržaja masti i proteina, a samim tim i odnos masti i proteina zajedno sa rizikom prevalencije metaboličkih poremećaja.

Ključne reči: metabolički poremećaji, ketoza, acidoza, podaci iz test dana, simentalske krave

References

ALI T.E., SCHAEFFER L.R. (1987): Accounting for covariances among test day milk yields in dairy cows. Can. J. Anim. Sci. 67, 637-644.

ANTANAITIS R., ŽILAITIS V., KUČINSKAS A., JUOZAITIENĖ V., LEONAUSKAITĖ K. (2015): Changes in cow activity, milk yield, and milk conductivity before clinical diagnosis of ketosis, and acidosis. Veterinarija ir Zootechnika 70(92):3–9.

ARSOV A., GOLC S., KASTELIC D. (1986): Higiensko pridobivanje mleka. Knjižica za pospeševanje kmetijstva. Ljubljana, ČZP Kmečki glas, p.125.

BRAMLEY E., LEAN I.J., COSTA N.D., FULKERSON W.J. (2005): Acidosis in pasture fed dairy cows: risk factors and outcomes. J Dairy Sci, 88, 95.

BROUCEK J., RYBA S., MIHINA S., UHRINCAT M., KISAC P. (2007): Impact of thermal-humidity index on milk yield under conditions of different dairy management. J Anim Feed Sci, 16, 329–344.

DIRKSEN G., LIEBICH H., MAYER H. (1985): Adaptive changes of the ruminal mucosa and functional and clinical significance. Bovine Practice, 20, 116–120.

DOHOO I.R., MARTIN S.W. (1984): Subclinical ketosis: prevalence and associations with production and disease. Can J Comp Med, 48, 1–5.

DOREAU M., CHILLIARD Y., RULQUIN H., DEMEYER D.I. (1999): Manipulation of milk fat in dairy cows. Rec Adv Anim Nutr. 81–109.

DUFFIELD T.F. (2004): Monitoring strategies for metabolic disease in transition dairy cows. 23rd World Buiatrics Congress, Quebec City, Canada.

DUFFIELD T.F., KELTON D.F., LESLIE K.E., LISSEMORE K., LUMSDEN J.H. (1997): Use of test day milk fat and milk protein to predict subclinical ketosis in Ontario dairy cattle. Can Vet J, 38, 713 – 718.

EICHER R., BOUCHARD E., BIGRAS-POULIN M. (1999): Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. Prev. Vet. Med. 39: 53 – 63.

EICHER R. (2004): Evaluation of the metabolic and nutritional situation in dairy herds: Diagnostic use of milk components. Proceedings of World Buiatrics Congress, Québec, Canada, July 11-16, 2004.

ERDMAN R.A., VARNER M. (1995): Fixed Yield Responses Increased Milking Frequency. J. Dairy Sci., 78: 1199 – 1203.

GILLUND P., REKSEN O., GRÖHN Y.T., KARLBERG K. (2001): Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J Dairy Sci, 84, 1390-1396.

HARGROVE G.L., GILBERT G.R. (1984): Differences in Morning and Evening Sample Milkings and Adjustment to Daily Weights and Percents. J Dairy Sci, 67, 194-200.

INGVARTSEN K. L., DEWHURST R. J., FRIGGENS N. C. (2003): On the relationship between lactational performance and health: is it yield or metabolic imbalance that causes production diseases in dairy cattle? A position paper. Livestock Production Science 83, 277–308.

INTERNATIONAL COMMITTEE FOR ANIMAL RECORDING, ICAR (2017): Section 2 - Guidelines for Dairy Cattle Milk Recording.

KEOWN J. F., EVERETT R. W., EMPET N. B., WADELL L. H. (1986): Lactation curves. J. Dairy Sci., 69: 769.

KLEI L.R., LYNCH J.M., BARBANO D.M., OLTENACU P.A., LEDNOR A.J., BANDLER D.K. (1997): Influence of Milking Three Times on Milk Quality. J Dairy Sci, 80, 427-436.

LEBLANC S. (2010): Assessing the association of the level of milk production with reproductive performance in dairy cattle. J Reprod Develop, 56, S1 – S7.

MULLIGAN F.J., DOHERTY M.L. (2008): Production diseases of the transition cow. The Veterinary Journal, 176, 1, 3-9. https://doi.org/10.1016/j.tvjl.2007.12.018.
MURPHY J.J., O'MARA F. (1993): Nutritional manipulation of milk protein concentration and its impact on the dairy industry. Livestock Production Science. 35(1–2): 117 – 134.
O'GRADY L., DOHERTY M.L., MULLIGAN F.J. (2008): Subacute ruminal acidosis (SARA) in grazing dairy cows. Vet J, 176 (1), 44–49.
OETZEL G.R. (2005): Applied aspects of ruminal acidosis induction and prevention, J Dairy Sci, 88, 643.
OUWELTJES W. (1998): The relationship between milk yield and milk interval in dairy cows. Livest Prod Sci, 56(3), 193 – 201.
PALMQUIST D.L., BEAULIEU A.D., BARBANO D.M. (1993): Feed and animal factors influencing milk fat composition. J Dairy Sci, 76, 1753 – 1771.
RAJALA P.J., GROHN Y.T. (1998): Effects of dystocia, retained placenta, and metritis on milk yield in dairy cows. J Dairy Sci, 81, 3172–3181.
RAJALA-SCHULTZ P.J., GROHN Y.T., MCCULLOCH C.E. (1999): Effects of milk fever, ketosis, and lameness on milk yield in dairy cows. J Dairy Sci, 82, 288–294.
SAS USER'S GUIDE (2019): Version 9.4 Edition. SAS Institute Inc. Cary, NC.
SUTHAR V.S., CANELAS-RAPOSO J., DENIZ A., HEUWIESER W. (2013): Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci, 96, 2925–2938.
WEISS D., HILGER M., MEYER H.H.D., BRUCKMAIER R.M. (2002): Variable milking intervals and milk composition. Milchwissenschaft, 57(5), 246 – 249.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

THE EFFECT OF RECORDING SEASON ON THE ESTIMATED PREVALENCE RISK OF MASTITIS IN FIRST PARITY SIMMENTAL COWS

Ivana Jožef[1], Dragan Solić[2], Zvonimir Steiner[3], Vesna Gantner[3]

[1]Faculty of Agriculture, University in Novi Sad, Trg Dositeja Obradovića 8, Novi Sad, Serbia
[2]Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, Osijek, Croatia
[3]Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia
Corresponding author: Ivana Jožef, ivana.jozef@stocarstvo.edu.rs

Abstract: The aim of this study was to determine the variability of daily lactose content and somatic cell count (SCC), as an indicator of mastitis risk, depending on the season and based on milk recording data. The statistical analysis used data on test-day records of the first parity Simmental cows, collected during the five-year period, during regular milk recording. All independent variables (days in milk - DIM; daily milk yield - DMY; age at the first calving - AC and milk recording season - MRS) included in the statistical model statistically highly significant effect ($p < 0.001$) affected the analysed traits (daily lactose content and logarithmic SCC (logSCC)). The highest daily lactose content was recorded during the spring season (4.590%), and the lowest (4.513%) in the autumn months. In contrast, the log values of somatic cell count were the lowest in spring (16,017), and highest in autumn (16,180). The research results indicate that the variability of daily lactose content and SCC, as well as prevalence risk of mastitis in Simmental first parity cows depends of daily milk production, stage of lactation, age at first calving, and season of milk recording. Therefore, those effects should be taken into account in the statistical model for the estimation of the mastitis prevalence risk in dairy cows based on milk recording data.

Key words: mastitis, lactose, milk recording, seasons, indirect estimation

Introduction

Mastitis (inflammation of the udder), which occurs in clinical, subclinical, acute and chronic forms, has been identified as the most common (*Seegers et al., 2003; Petrovski et al., 2006*) and most expensive disease in dairy cattle (*Gráff and*

Mikó, 2015; Ibrahim, 2017). The prevalence of mastitis ranges from 20% (*Hasan et al., 2018*) to 71.9% (*Sayeed et al., 2020*) in herds around the world and causes significant business losses due to reduced milk production, treatment costs, reduced udder functionality and shortens the productive life of dairy cows. Precision dairy cattle technologies maximize the potential of animals at the individual level and minimize the use of drugs by applying preventive health measures (*Bewley, 2010*). Milk record data, already used by dairy producers as part of precision dairy farming, can also be used as an excellent tool for early detection of mastitis in individual animals, enabling cost reduction and adequate optimization of dairy farm management. Mastitis damages the udder tissue and reduces the synthetic capacity of secretory cellular enzyme systems, thus reducing lactose biosynthesis (*Kitchen, 1981*). Damage of the cells of the alveolar epithelium causes a decrease in the lactose content, because there is a leakage of lactose from the milk through the paracellular pathways that multiply during mastitis (*Alhussien and Dang, 2018*). Elevated SCC in milk is an indicator of inflammation of the mammary glands of lactating cows (*Botton et al., 2019*), and in a case of indirect measure of subclinical mastitis, some authors believe that the most promising parameter for monitoring of subclinical mastitis is lactose content (*Pyorala, 2003*). Furthermore, climatic factors have an impact on the composition and quality of milk, primarily as a result of the impact of heat stress on cows (*Gantner et al., 2017*).

The aim of this study was to determine the variability of daily lactose content and SCC, as an indicator of mastitis, depending on the season and based on milk recording data.

Materials and Methods

The data were collected on test days (TD) of the first parity Simmental cows between January 2008 and December 2012. Data were collected during regular milk recording in Eastern Croatia, performed in accordance with an alternative milk recording method (AT4 / BT4), which includes measuring and sampling milk during evening or morning milking every four weeks. The recording was performed by field officers of the Croatian Agency for Agriculture and Food, while milk samples were analysed in the Central Laboratory for Milk Quality Control in accordance with accredited laboratory methods; infrared spectrophotometry for determining lactose content and fluoro-optoelectronic method for somatic cell counting. Milcoscan FT6000 was used to determine milk composition, while Fossomatic FC5000 was used for SCC. The following limits were set for the TD record, during the logical data control: lactation phase for (> 5 days and <500 d), age at first calving (> 21 and <36 mo) and all records of test days with missing information regarding parity, breed and missing or meaningless characteristics of daily milk production in accordance with ICAR standards were deleted from the data set (ICAR, 2017). After logical control dataset consisted of 59,150 TD records from 11,258 first parity Simmental cows reared on 1,591 farms in Eastern Croatia. Furthermore, basic variability of analysed traits is shown in Table 1.

Table 1. Basic statistical parameters of analysed traits

Variable	N	Mean	SD	CV	Minimum	Maximum
DMY	58893	15.773	5.195	32.938	3.000	82.400
LACT	57221	4.553	0.205	4.507	3.000	5.310
SSC	58864	205.929	457.300	222.067	5.000	4999.00
logSSC	58864	16.095	2.017	12.533	12.288	22.253

*DMY – daily milk yield (kg); LACT – daily lactose content in milk sample (%); SCC – somatic cell count; logSCC – logarithmic value of SCC

Following statistical model was used, for the evaluation of the effect of recording season on the variability of daily lactose content, and log value of somatic cell count in Simmental first parity cows:

$$y_{ijklm} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + b_5 m_j + A_k + S_l + e_{ijklm}$$

Where:

y_{ijklm} = estimated trait (daily lactose content, and log value of somatic cell count);

μ = intercept;

b_1, b_2, b_3, b_4, b_5 = regression coefficients;

d_i = days in milk (i = 5 to 500 day);

m_j = daily milk yield (j = 3.00 to 96.00 kg);

A_k = fixed effect of age at first calving class k (k = 21 to 36 month);

S_l = fixed effect of recording season l (l = Spring, …, Winter);

e_{ijklm} = residual.

To obtain the significance of the differences between the seasons of milk recording, Scheffe's method of multiple comparison was used (by the PROC GLM procedure in SAS (*SAS Institute Inc., 2019*)).

Results and Discussion

All independent variables (DIM, DMY, AC and MRS) included in the used statistical model showed a statistically highly significant effect ($p < 0.001$) on the analysed traits (daily lactose content and logSCC). Significance of the effects included in statistical model on analysed traits are presented in the Table 2.

Table 2. Significance of the effects included in statistical model on analysed traits

Effects		**LACTOSE**			**logSCC**		
Source	**DF**	**MS**	**F Value**	**Pr > F**	**MS**	**F Value**	**Pr > F**
DMY	1	33.067	848.43	<.0001	2019.487	509.29	<.0001
AFC	1	7.296	187.19	<.0001	201.961	50.93	<.0001
sch1	1	1.202	30.84	<.0001	0.338	0.09	0.7702
sch2	1	0.611	15.69	<.0001	0.663	0.17	0.6826
sch3	1	2.825	72.49	<.0001	4.018	1.01	0.3141
sch4	1	4.717	121.03	<.0001	16.617	4.19	0.0407
season	3	15.795	405.27	<.0001	87.600	22.09	<.0001

*DMY – daily milk yield (kg); AFC – age at first calving; sch1 – sch4; regression coefficients of lactation curve (effect of days in milk); LACTOSE – daily lactose content in milk sample (%); logSCC –logarithmic value of SCC

The least square means (LSMs) values of analysed traits (daily lactose content in milk and log value of somatic cell count) regarding the recording season class are shown in the Table 3.

Table 3. LSMs of analysed traits regarding the season of milk recording

SEASON	**LACTOSE**	**logSCC**
Spring	4.590^{A}	16.017^{A}
Summer	4.546^{B}	16.048^{B}
Autumn	4.513^{C}	16.180^{B}
Winter	4.566^{D}	16.141^{A}

*LACTOSE – daily lactose content in milk sample (%); logSCC – logarithmic value of SCC; LSMeans marked with different letters (A, B, C) differ statistically highly significant ($p < 0.001$)

The LSmeans values of daily lactose content in milk of Simmental first parity cows differed statistically highly significant ($p < 0.001$) regarding the recording seasons (spring, summer, autumn and winter). The daily lactose content fluctuates during the seasons, so the highest value was recorded during the spring

(4.590%), and the lowest (4.513%) in the autumn months. For the second analysed trait, the log value of the number of somatic cells, the lowest LSmean values were determined in the spring season (16.017), as opposed to the autumn season when the highest values were recorded (16.180).

This results showed the influence of the recording season on mastitis indicators (daily lactose content and SCC), also determined by *Nobrega and Langoni (2011*). They found that the incidence of cows with intramammary infections (IMI) was higher in the rainy season than in the dry season, and the same cows had higher lactose rates in the dry season than in the rainy season (*Nóbrega and Langoni, 2011*). Mastitis reduces the synthetic ability of udder cells, so the lactose content in milk from infected glands is usually low and as such is a good indicator of mastitis. Furthermore, there is an inverse relationship between the severity of mastitis (as shown by the SCC) and the lactose content of milk (*Sharif et al., 2014*). Changes in milk composition, SCC and mastitis frequencies are associated with heat stress mainly expressed during the summer season (*Gantner et al., 2011*). Lactose synthesis largely depends on the energy status of the cows, which in turn is directly related to food intake, and the lactose content is also related to the seasons: the heat stress that usually affects lactating cows during the summer and autumn (*Haygert-Velho et al., 2018*). When clinical or subclinical udder inflammation is present, accompanied by an increase in SCC, the lactose content tends to decrease (*Costa et al., 2019*). Additionally, *Weber et al. (2020)* determined the influence of the season on the quality and composition of milk, because they are of better quality in winter and spring, while in the hotter months of summer and autumn the quality is reduced (*Weber et al., 2020*).

Conclusion

The research results indicate that the variability of daily lactose content and log value of somatic cell count, as well as prevalence risk of mastitis in Simmental first parity cows depends of daily milk production, stage of lactation, age at first calving, and season of milk recording. Therefore, these effects should be taken into account in the statistical model for estimation of the mastitis prevalence risk in dairy cows based on milk recording data.

Uticaj godišnjeg doba kontrole na procenjenu prevalenciju rizika od mastitisa kod prvotelki simentalske rase

Ivana Jožef, Dragan Solić, Zvonimir Steiner, Vesna Gantner

Rezime

Cilj ove studije bio je da se utvrdi varijabilnost dnevnog sadržaja laktoze i broja somatskih ćelija, kao pokazatelja rizika od mastitisa, u zavisnosti od godišnjeg doba i na osnovu podataka o kontroli mlečnosti. Statistička analiza koristila je podatke dobijene u kontroli mlečnosti prvotelki simentalske rase, prikupljene tokom petogodišnjeg perioda, tokom redovne kontrole mlečnosati. Sve nezavisne varijable (dani laktacije; dnevni prinos mleka; starost pri prvom teljenju i sezona kontrole mlečnosti) uključene u statistički model statistički visoko značajan efekat ($p<0,001$) uticale su na analizirane osobine (dnevni sadržaj laktoze i log vrednost somatske ćelije broj, logSCC). Najveći dnevni sadržaj laktoze zabeležen je u prolećnoj sezoni (4,590%), a najmanji (4,513%) u jesenjim mesecima. Nasuprot tome, log vrednosti somatskih ćelija bile su najniže u proleće (16,017), a najviše u jesen (16,180). Rezultati istraživanja ukazuju na to da varijabilnost dnevnog sadržaja laktoze i broja somatskih ćelija, kao i rizik od prevalencije mastitisa kod prvotelki simentalske rase zavise od dnevne proizvodnje mleka, stadijuma laktacije, starosti pri prvom teljenju i sezone kontrole mlečnosti. Zbog toga bi te efekte trebalo uzeti u obzir u statističkom modelu za procenu rizika prevalencije mastitisa kod muznih krava na osnovu podataka o kontroli mlečnosti.

Ključne reči: mastitis, laktoza, snimanje mleka, godišnja doba, indirektna procena

References

ALHUSSIEN M. N., DANG A. K. (2018): Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview, Veterinary World, 11(5), 562–577.

BEWLEY J. (2010): Precision dairy farming: Advanced analysis solutions for future profitability, in The First North American Conference on Precision Dairy Management, 16.

BOTTON F. S. et al. (2019): Relationship of total bacterial and somatic cell counts with milk production and composition – Multivariate analysis, Acta Scientiarum - Animal Sciences, 41(1).

COSTA A. et al. (2019): Milk lactose—Current status and future challenges in dairy cattle, Journal of Dairy Science, 102(7), 5883–5898.

GANTNER V. et al. (2011): Temperature-humidity index values and their significance on the daily production of dairy cattle, Mljekarstvo, 61(1), pp. 56–63.

GANTNER V. et al. (2017): Differences in response to heat stress due to production level and breed of dairy cows, International Journal of Biometeorology, 61(9), 1675–1685.

GRÁFF M., MIKÓ J. E. (2015): Analysis of mastitis in holstein-fresian cows and economics effects of mastitis, Agricult Manage/Lucrari Stiintifice, 17(1), 294–297.
HASAN M. M. et al. (2018): Status of milk production and subclinical mastitis in dairy cows along with socioeconomic condition of the farmers, Bangladesh Journal of Veterinary Medicine, 16(1), 71–79.
HAYGERT-VELHO I. M. P. et al. (2018): Multivariate analysis relating milk production, milk composition, and seasons of the year, Anais da Academia Brasileira de Ciencias, 90(4), 3839–3852.
IBRAHIM N. (2017): Review on Mastitis and Its Economic Effect, Canadian Journal of Scientific Research, 6(1), 13–22.
ICAR (2017): Guidelines for Dairy Cattle Milk Recording, Guidelines.
NÓBREGA D. B., LANGONI H. (2011): Breed and season influence on milk quality parameters and in mastitis occurrence, Pesquisa Veterinaria Brasileira, 31(12), 1045–1052.
PETROVSKI K. R., TRAJCEV M., BUNESKI G. (2006): A review of the factors affecting the costs of bovine mastitis, Journal of the South African Veterinary Association, 52–60.
PYORALA S. (2003): Indicators of inflammation in the diagnosis of mastitis, Veterinary Research, 34(5), 565–578.
SAS Institute Inc. (2019): SAS User ' s Guide , Version 9.4. SAS Institute Inc. Cary, NC.
SAYEED M. A. et al. (2020): Prevalence of sub-clinical mastitis and associated risk factors at cow level in dairy farms in Southwestern part of Bangladesh, Advances in Animal and Veterinary Sciences, 8, 112–121.
SEEGERS H., FOURICHON C., BEAUDEAU F. (2003): Production effects related to mastitis and mastitis economics in dairy cattle herds, Veterinary Research, 475–491.
SHARIF A. et al. (2014): Lactose as an Indicator of Udder Health Status under Modern Dairy Production, International Journal of Agriculture Innovations and Research, 2(6), 971–975.
WEBER C. T. et al. (2020): Season effects on the composition of milk produced by a Holstein herd managed under semi-confinement followed by compost bedded dairy barn management, Semina:Ciencias Agrarias, 41(5), 1667–1678.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

INVESTIGATIONS ON HAEMATO-BIOCHEMICAL INDICATORS IN ROMANIAN BLACK AND SPOTTED DAIRY COWS WITH RETAINED PLACENTA – PRELIMINARY RESULTS

Marinela Enculescu

Research and Development Institute for Bovine, 21, Bucuresti-Ploiesti, 077015, Balotesti, Romania
Corresponding author: Marinela Enculescu, marinelaenculescu2006@yahoo.com

Abstract: This study was carried out to investigate the effects of retained placenta (RP) in dairy cows on haemato-biochemical indicators. Experiments were conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti using 40 multiparous Romanian Black and Spotted dairy cows, which were divided into two groups, cows with retained placenta (experimental, n=16) and the control group (n=24), between 2019 and 2020. Each group was fed with the same diet and had free access to water and mineral blocks. Results were expressed as mean±standard deviation (SD) and minimum-maximum range values. The independent means t-test was applied for comparisons between the control (C) group and the retained placenta (RP) group. Pearson's coefficients were utilized to test the correlations between the main blood indicators. Cows with retained placentas showed a significant decrease in RBC (p=0.0451), HGB (p=0.0000), MCH (p=0.0000), MCHC (p=0.0012) and WBC (p=0.0001), compared with the control group. Whereas the LY (p=0.0551), MO (p=0.0000) and NE (p=0.0273) concentrations of cows with retained placentas were significantly higher than those of healthy cows. The AST and ALT serum concentrations of cows with retained placenta were significantly higher than those of cows from the control group (p=0.0000). Regarding urine analysis, no significant changes were found between the two groups studied. In conclusion, the obtained preliminary results suggest that blood indicators could be useful for risk prediction of retained placenta in dairy cows, while further research is needed on correlations between blood constituents and retained placenta.

Key words: dairy cow, haematology, retained placenta, transaminase.

Introduction

Retained placenta (RP) is one of the main reproductive disorders affecting dairy cows. RP usually is defined as failure to expel the fetal membranes within 24 h after parturition (*Sheldon, 2009*). RP in cows causes considerable economic losses (*Haile, 2014*), especially when the incidence exceeds 5-10% in the herd (*Maldonado and Shreif, 2010*). There are important relationships between metabolic disorders, immune function and retained placenta, with further consequences on the overall reproductive efficiency (*Trevisi et al., 2008; Dubuc et al., 2010*), such as reduction in pregnancy rates, increased number of services per conception (*Sheldon et al., 2006; Wathers, 2010; LeBlanc, 2012*). The common risk factors that predispose for retention placenta, are the following: age, parity, cow's body weight, repeatability (past RTs), stillbirth, calves' birth weight, abortion, twin birth, dystocia, cesarean section, nutritional deficiencies, metabolic disorders, bovine viral diarrhea, brucellosis, leptospirosis, vibriosis, listeriosis, etc. (*Han and Kim, 2005; Le Blanc, 2010*). The main cause of retained placenta is due to a failure of the timely (more than 24 hours) breakdown of the caruncle-cotyledon attachments after calving, induced by infectious and/or noninfectious factors (*Martin, 2006; Le Blanc, 2008; Moizur et al., 2013*). Cows that had once retained placenta are at an increased risk of recurrence in future parturitions, with a significant negative effect on milk yield for several weeks after calving (*Rajala and Grohn, 1998*), and increased risk of developing fatty liver syndrome and ketosis (*Shiferaw et al., 2005*). Metabolic, immunologic and endocrine changes (correlated or independently), are potential predictors for retained placenta (*Beagley et al., 2010*). The aim of this study was to investigate the effects of retained placenta (RP) in dairy cows on haemato-biochemical indicators.

Materials and Methods

Forty multiparous Romanian Black and Spotted dairy cows were investigated for haemato-biochemical and urine parameters, during the first 2 weeks *post-partum* in relation to retained placentas. The cows were divided into two groups: retained placenta (RP: n=16 heads/group), when fetal membranes not being released within the first 24 h *post-partum*, and the control (C: n=24 heads/group), which consisted out of clinically healthy cows, without any *post-partum* uterine disorders. The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, Romania (44°36′46″N 26°4′43″E),

The age of studied cows ranged between 5 and 8 years. The cows were housed under tied stanchion barn conditions. All cows were fed twice daily, the diets being formulated to meet nutrient requirements at the stage of their lactation (the diet/head/day consisting of 7 kg alfalfa hay, 25-28 kg corn silage, 6 kg concentrates, 80-100 g *Saccharomyces cerevisiae,* mineral blocks and water *ad libitum).*

Blood samples were taken from the jugular vein of each cow, in the first 2 weeks *post partum* in the morning, being collected in vacutainer tubes with EDTA/K_3 for hemoleukogram (1-2 ml/tube, and chilled to +4 °C), and vacutainer tubes (6 ml/tube, centrifuged at 3000 rpm for 15 minutes and stored at –20 °C till further testing) for biochemical examination. Urine samples were collected in 50 ml sterilized vials, as free catch during micturition. Red blood cells count, hemoglobin concentration, hematocrit percentage, mean platelets volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, the total white blood cells count, lymphocytes percentage, monocytes percentage, neutrophil percentage were performed using an automated hematology analyzer (Abacus Junior Vet 5). Aspartate transaminase and alanine aminotransferase were determined using a semiautomated biochemical analyzer (StarDust MC 15), and DiaSys reagents in dedicated kits. Urine examination (bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrites, leukocytes, specific gravity) was determined with the DocUReader urine analyzer.

Statistical analyses were carried out using Microsoft Excel Statistical Software. The data were expressed as mean±standard deviation (SD), minimum (min.) and maximum (max.) range values, and the independent means t-test was applied for comparisons between the control group and the retained placenta group. Pearson's coefficients were utilized to reveal the correlations between neutrophiles and the transaminase indicators. Differences were considered statistically significant at $p<0.05$.

All experimental procedures were performed in accordance with the practices and standards approved by the *Romanian Law no. 43/2014* and the *Council Directive 2010/63/EU.*

Results and Discussion

The effects of retained placenta on blood parameters in dairy cows are being presented in Table 1. Statistical evaluations revealed significant differences in red blood cells-RBC (6.18±0.59 10^6/µl, ranges 5.32-6.94 10^6/µl in RP group vs. 6.65±0.58 ranges 5.86-8.27 in C group; $p<0.05$), hemoglobin-HGB (8.97±0.47 g/dl, ranges 8.8-10.3 in RP group vs. 10.11±0.78 g/dl, ranges 8.6-11.9 in the C group; $p<0.001$), mean corpuscular hemoglobin-MCH (13.83±0.59 pg, ranges 13-14.9 in RP group vs. 15.23±1.02 pg, ranges 13.7-17.4 in the C group; $p<0.001$), and mean corpuscular hemoglobin-MCHC (31.43±2.33 g/dl, ranges 30.3-37.4 in the RP group vs. 35.57±1.44 g/dl, ranges 32.6-40.4 in the C group; $p<0.01$). There were no significant differences ($p>0.05$) for hematocrit (HTC) and mean platelets volum (MPV) concentrations. For HTC, the obtained results were below the reference limits (by 30-32%) for both groups studied, a decrease of the HTC possible indicating anemia and impaired ability to carry oxygen from red blood cells. In the case of MCH and MCHC, significant differences ($p<0.001$) between the retained placenta group and the control group were observed, while for MCV no significant differences ($p>0.05$) were found.

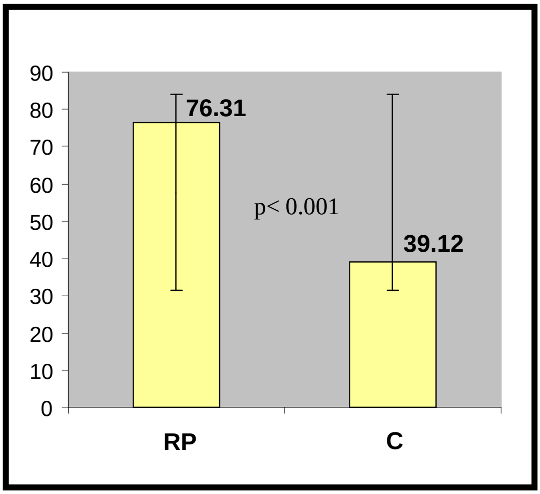
A lower level of total white blood cells (WBC) was found in the retained placenta group (7.68±1.07 10^3/µl, ranges 6.25-9.63) compared with the control group (9.51±1.71 10^3/µl, ranges 6.55-12.35). The leukogram showed leucocytosis accompanied by lymphopenia and monocytosis. The condition may be attributed to inflammation and increase of monocytes for scavenging of cell debris. For neutrophil (NE), dairy cows with retained placentas showed significant differences compared to the control group ($p<0.05$), however, with minimum and maximum ranges being in physiological reference limits.

Table 1. Haematological parameters in cows with retained placenta (RP), compared with the control group (C)

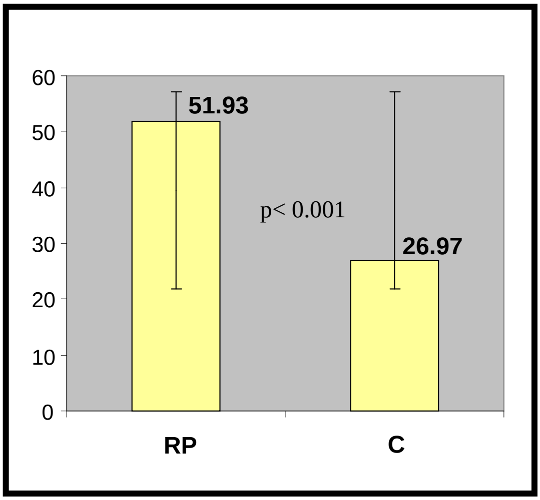
Parameters	RP			C			p-value
	X±SD	min	max	X±SD	min	max	
RBC, 10^6/µl	6.18±0.59	5.32	6.94	6.65±0.58	5.86	8.27	0.0451
HGB, g/dl	8.97±0.47	8.8	10.3	10.11±0.78	8.6	11.9	0.0000
HTC, %	28.89±1.08	27.44	30.85	28.47±2.52	23.71	34.66	0.2616
MCV, fl	43±2.06	40	46	42.94±3.50	34	53	0.4754
MCH, pg	13.83±0.59	13	14.9	15.23±1.02	13.7	17.4	0.0000
MCHC, g/dl	31.43±2.33	30.3	37.4	35.57±1.44	32.6	40.4	0.0012
MPV, fl	6.47±0.56	5.9	7.5	6.72±0.49	6.1	7.6	0.0634
WBC, 10^3/µl	7.68±1.07	6.25	9.63	9.51±1.71	6.55	12.35	0.0001
LY, %	68.40±11.13	43.2	89.6	63.45±7.68	51.2	76.5	0.0551
MO, %	7.80±2.33	1	10.8	1.31±0.35	0.8	1.9	0.0000
NE, %	29.81±7.82	16.6	39.6	24.91±8.37	16.1	46.8	0.0273

RBC=red blood cells count, HGB=hemoglobin concentration, HCT=hematocrit percentage, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration, MPV=mean platelets volum, WBC=total white blood cells count, LY=lymphocytes percentage, MO=monocytes percentage, NE=neutrophil percentage.

Changes in liver enzymes, such as asparagine aminotransferase (AST) and alanine aminotransferase (ALT) during *post-partum* periods in cows with retained placenta and the controls (C) are shown in Figure 1. The concentration of these enzymes was statistical significantly higher ($p<0.001$) in the retained placenta group, compared to the control group. In this study, the mean activity of AST was 76.31±9.27 U/L (interval values 58-91) in the retained placenta group vs 39.12±4.39 U/L (interval values 32-47) in the control group. For ALT, the obtained results were 51.93±12.70 U/L (interval values 77-35) in the retained placenta group vs 26.97±4.02 U/L (interval values 20-37) in the control group. The obtained values revealed higher enzyme concentration in the retained placenta group comparative with the control group. Increases in AST and ALT activities in dairy cows with retained placenta were found also by *Djokovic et al. (2019)* and *Sahinduran et al. (2010). Hassan et al. (2020)* obtained average values of 81.1 IU/L (RP) vs. 72.17 IU/L (C) for AST and values of 43.97 IU/L (RP) vs. 34.84 IU/L (C) for ALT. *Semcan and Sevinc (2005)* showed values of 120.6±19 (RP) and 78.5±9 0.045 (C) for AST.



a



b

Figure 1 a.b. Mean±SD of serum AST (a) and ALT (b) levels (U/I) in dairy cows with retained placenta (RP) compared to the control (C)

The ALT is the most sensitive targeted diagnosis for acute liver damage, whereas AST is more sensitive in reflecting the degree of damage (*Kew, 2000*). *Winnicka (2011)* showed that ranges between 68-100 mmol/l for serum AST may be helpful during potential biochemical blood metabolic monitoring in dairy cows, in terms of retained placenta. Retained placenta is mediated by impaired migration of neutrophils to the placental interface in the *pre-partum* period. The impaired neutrophil function extends into the *post-partum* period and probably mediates the recognized complications of risk retained placenta. *Cai (2002)* and *Moretti et al. (2015)* suggested that a relationship between neutrophil functions and *pre-partum* disorders in cows exists, while neutropenia and increased aspartate transaminase (AST) activity representing important co-factors connected with the pathogenesis of retained placenta in cows. In this study, there was a non-statistical positive correlation between the blood NE concentrations and AST concentrations ($r=0.0505$; $r^2=0.0026$; $p=0.8526$), with the same pattern for the blood concentrations of NE and ALT ($r=0.2046$; $r^2=0.0419$; $p=0.4471$).

However, except for WBC and NE, haematological parameters should be used in correlations with other biochemical indices such as the mineral, vitamins, and other biochemical indicators, as well as gamma-glutamyltransferase (GGT) serum enzyme.

Urine parameters in cows with retained placenta and control group are presented in Table 2. Urine examination showed no significant changes ($p>0.05$)

between the two groups studied. However, fluctuations for urine proteins (30-100 mg/dl) for the retained placenta group were found.

Table 2. Urine parameters in the retained placenta group (RP), compared with the control group (C)

Parameters	RP	C
Bilirubin, mg/dl	Negative	Negative
Urobilinogen, mg/dl	Normally	Normally
Ketones, mg/dl	Negative	Negative
Glucose, mg/dl	Negative	Negative
Protein, mg/dl	30-100	30
Blood, ery/µl	Negative	Negative
pH	6-8	5-6
Nitrite	Negative	Negative
Leukocytes, leu/µl	Negative	Negative
Specific gravity	1.010-1.035	1.015-1.025

Conclusions

The obtained values were significantly higher for lymphocytes (LY), monocytes (MO), neutrophil (NE), and significantly lower for red blood cells (RBC), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total white blood cells (WBC) concentrations in cows with retained placenta, compared with the control group. The asparagine aminotransferase (AST) and alanine aminotransferase (ALT) serum concentrations of cows with retained placenta were significantly higher than the control cows, which indicated that retained placenta might be associated with fatty liver disease.

This study shows that haematological indicators could be used as parameters for risk prediction of retained placenta.

Further research on a higher number of animals in order to establish connections between blood indicators and retained placenta is required. Early detection and diagnosis of cows at increased risk of developing retained placenta immediately after calving could improve treatments efficiency, milk yield and reproductive performances.

Istraživanja o hemato-biohemijskim pokazateljima kod rumunskih crnih i šarenih krava sa zadržanom placentom - preliminarni rezultati

Marinela Enculescu

Rezime

Ovo istraživanje je sprovedeno radi ispitivanja uticaja zadržane placente (RP) kod muznih krava na hemato-biohemijske pokazatelje. Eksperimenti su sprovedeni na Eksperimentalnoj farmi Instituta za istraživanje i razvoj u govedarstvu, Balotešti, koristeći 40 rumunskih crnih i šarenih muznih krava, koje su podeljene u dve grupe, krave sa zadržanom placentom (eksperimentalno, n = 16) i kontrolnu grupu (n = 24), između 2019. i 2020. godine. Svaka grupa je hranjena istim obrokom i imala je slobodan pristup vodi i mineralnim blokovima. Rezultati su izraženi kao srednja ± standardna devijacija (SD) i minimalno-maksimalne vrednosti opsega. Za poređenje između kontrolne (C) grupe i eksperimentalne (RP) primenjen je nezavisni t-test. Pearsonovi koeficijenti su korišćeni za testiranje korelacije između glavnih pokazatelja krvi. Krave sa zadržanom placentom pokazale su značajno smanjenje RBC (p=0,0451), HGB (p=0,0000), MCH (p=0,0000), MCHC (p=0,0012) i WBC (p=0,0001), u poređenju sa kontrolnom grupom. Dok su u grupi krava sa zadržanom posteljicom koncentracije LY (p=0.0551), MO (p=0.0000) i NE (p=0.0273) bile značajno veće od koncentracija zabeleženih u grupi zdravih krava. Koncentracije AST i ALT u serumu krava sa zadržanom placentom bile su značajno više od koncentracije krava iz kontrolne grupe (p=0,0000). Što se tiče analize urina, nisu pronađene značajne promene između dve ispitivane grupe. U zaključku, dobijeni preliminarni rezultati ukazuju na to da bi krvni pokazatelji mogli biti korisni za predviđanje rizika zadržavanja posteljice kod krava muzara, dok su potrebna dalja istraživanja o korelacijama između sastojaka krvi i zadržane posteljice.

Ključne reči: mlečne krave, hematologija, zadržana placenta, transaminaza.

Acknowledgements

This study was supported by Project No. 4469/2018 “Research on the development and implementation of a new dairy cows feeding technology using natural protein and mineral additives”.

References

BEAGLEY J.C., WHITMAN K.J., BAPTISTE K.E., SCHERZER J. (2010): Physiology and treatment of retained fetal membranes in cattle, Journal of Veterinary Internal Medicine, 24, 261–268.

CAI T.Q., WESTON P.G., LUND L.A., BRODIE B., MCKENNA D.J., WAGNER W.C.I. (2002): Association between neutrophil function and periparturient disorders in cows, American Journal of Veterinary Research, 55: 934-943.

DIRECTIVE 2010/63/EU regarding handling and protection of animals used for scientific purposes, OJEU L 276/33 IA.

DJOKOVIC R., CINCOVIC M., IIIC Z., KURCUBIC V., ANDJELIC B., PETROVIC M., LALIC N., JASOVIC B. (2019): Estimation of metabolic status in high yielding dairy cows during transition period and full lactation, Acta Scientific Veterinary, 47, 1667.

DUBUC J., DUFFIELD T.F., LESLIE K.E., WALTON J.S., LEBLANC S.J. (2010): Risk factors for postpartum uterine diseases in dairy cows, Jornal of Dairy Science, 93, 5764–5771.

HAILE A., TSEGAYE Y., TESFAYE N. (2014): Assessment of major reproductive disorders of dairy cattle in urban and per urban area of Hosanna, Southern Ethiopia, Animal and Veterinary Science, 2(5): 135-141.

HAN I. K., KIM I. H. (2005): Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows, Journal of Veterinary Science, 6(1):53-59.

HASSAN M.S., ABD-ALLAH E.A. (2020): Egypt Metabolites and hormones can predict postpartum uterine disorder during transition period of dairy cows, Journal Multidisciplinary Scientific, 2(1), 1-9.

KEW M.C. (2000): Serum aminotransferase concentration as evidence of hepatocellular damage, Lancet, 355:591-592.

LAW no. 43/2014 regarding handling and protection of animals used for scientific purposes, OM no.326/Part I.

LeBLANC S.J. (2012): Interactions of metabolism, inflammation and reproductive tract in postpartum period in dairy cattle, Reproduction Domestic Animals, 47 (5): 18-30

LeBLANC S. (2010): Monitoring metabolic health of dairy cattle in the transition period. J. Reproduction Development, 56, S29-S35.

LeBLANC S.J (2008): Postpartum uterine disease and dairy herd reproductive performance: A review, The Veterinary Journal, 176: 102-114.

MALDONADO J., SHREIF S. (2010). Livestock and Product Annual Global Agricultural International Network USDA Foreign Agricultural Service.

MARTIN G. J. (1996): Ethnobotany; People and Plant Conservation Manuals. Worldwide Fund for Nature International, Chapman and Hall, London.

MOIZUR R. M., ZAHAN M., KADER M.D., MOZAFFOR HOSSAIN K.M. (2013): Retained placenta of dairy cows associated with managemental factors in Rajshahi, Bangladesh, Veterinary World, 64:180-184, doi: 10.5455/vetworld.2013.180-184.

MORETTI P., PROBO M., MORANDI N., TREVISI E., FERRARI A., MINUTI A., VENTURINI M., PALTRINIERI S., GIORDANO A. (2015): Early post-partum hematological changes in Holstein dairy cows with retained placenta, Animal Reproduction Science, 152: 17-25

RAJALA P.J., GROHN Y.T. (1998): Effects of dystocia, retained placenta, and metritis on milk yield in dairy cows, Journal of Dairy Science, 81: 3172–3181.

SAHINDURAN S., SEZER K., BUYUKOGLU T., ALBAY M.K., KARAKURUM M.C. (2010): Evaluation of some haematological and biochemical parameters before and after treatment in cows with ketosis and comparison of different treatment methods, Journal of Animal and Veterinary Advances, 9, 266-271.

SHELDON I.M., CRONIN J., GOETZE L., DONOFRIO G., SCHUBERTH H.J. (2009): Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle, Biological Reproduction, 81(1), 1025-1032.

SHELDON I.M., LEWIS G.S., LeBLANC S., GILBERT R.O. (2006): Defining postpartum uterine disease in cattle, Theriogenology, 61: 1516-1530.

SEMACAN A., SEVINC M. (2005): Liver Function in Cows with Retained Placenta, Turk Journal of Veterinary Animal Science, 29: 775-778.

SHIFERAW Y., TENHAGEN B.A., BEKANA M., KASSA T. (2005): Reproductive disorders of crossbred dairy cows in the central highlands of Ethiopia and their effect on reproductive performance. Tropical Animal Health and Production, 37: 427- 41.

TREVISI E., FERRARI A. R., BERTONI G. (2008): Productive and metabolic consequences induced by the retained placenta in dairy cows, Veterinary Research Communication, 32 (1), 363–366.

WATHERS D.C. (2010): Interactions between energy balances, the immune system end the reproductive tract with influence on dairy cow fertility, Cattle Practice, 18: 19-26.

WINNICKA A. (2011): Reference values of basic laboratory tests in veterinary medicine, Edition V, amended SGGW, Warsaw, p 40-72.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

ASSESSMENT OF THE RISK STATUS OF FOUR LOCAL BULGARIAN BREEDS BASED ON THEIR GEOGRAPHIC DISTRIBUTION

Zhivko I. Duchev

Institute of Animal Science – Kostinbrod, 2232 Kostinbrod, Bulgaria
Corresponding author: Zhivko I. Duchev, zhivko.duchev@agriacad.bg

Abstract: The aim of the study was to assess the breeds risk status based on their geographic distribution. Four local Bulgarian breeds were included - Rhodope Shorthorn Cattle, Rhodope Tsigai sheep, Central Rhodope sheep, and Bulgarian screw-horned longhaired goat. For each breed, the identification of the farm, its location, and the number of animals were collected, and the geographic distribution was analyzed by GIS methods. The location of each farm was geocoded using two approaches - by the village coordinates, and by the municipality center coordinates. The percentage of the population within 25 and 50 km distance from the weighted mean center of the farms, and the radius of the circle enclosing 75% and 100% of the population were calculated. In all the breeds, except the goats, more than 75% of the population was concentrated in less than 50 km from the weighted mean center, thus their status is “vulnerable”, and they should be closely monitored. The Bulgarian screw-horned longhaired goat was distributed in three distant regions, there additional study has to be conducted on sub-population level. Farms located far away from the original area of the breed were observed in all breeds. The results when using the municipality coordinates were similar to those produced using village coordinates. For practical purposes, as a simplification, the total number of animals per municipality can be used.

Key words: livestock biodiversity, local breeds, GIS, risk status.

Introduction

The effective management and conservation of the livestock biodiversity is one of the key elements for ensuring food security. The local breeds, well adapted to the climate conditions in the country, are important part of this biodiversity. The importance of their biodiversity is recognized in the United Nations Sustainable Development Goals, more precisely in the Target 2.5 of Goal 2 – “By 2020, maintain the genetic diversity of seeds, cultivated plants and farmed and domesticated animals and their related wild species, including through soundly managed and diversified seed and plant banks at the national, regional and international levels, and promote access to and fair and equitable sharing of benefits arising from the utilization of genetic resources and associated traditional knowledge, as internationally agreed" *(United Nations General Assembly, 2015)*. Indicator 2.5.2 to this Target is "Proportion of local breeds classified as being at risk, not-at-risk or at unknown level of risk of extinction", which again stresses the importance of regular assessment of the risk status.

Many systems for assessment the risk status of a breed were developed at national and international level during the years, e.g. (*Loftus and Sherf, 1993; Simon and Buchenauer, 1993; Reist-Marti et al., 2003; Gandini et al.,2004; Duchev et al., 2006; Alderson, 2009; Verrier et at., 2015*), most of which were based mainly on population data. The geographical distribution of the breeds as a component of the breed risk status was included in some of these systems (*Reist-Marti et al., 2003; Alderson, 2009*) and included by the Food and Agriculture Organization of the United Nations in their "In vivo conservation of Animal Genetic Resources guidelines" *(FAO, 2013)*. The concentration of the population in a restricted area, presents a threat to significant reduction in the size of the breed, or even its extinction, by catastrophic events like disease outbreaks. Following the proposal of *Alderson (2009)* the breed is assigned to category "critical" if 75% of the population is concentrated within a circle with radius 12.5 km and to category "endangered" if 75% of the population is within a circle with 25 km radius. Alderson, proposes also "vulnerable" category if the 75% of the population is within a circle with 50 km radius.

In a study funded by the European Regional Focal Point for Animal Genetic Resources, *Sturaro et al. (2013)* analyzed, using GIS methods, the geographic distribution of 8 local breeds from UK, Greece, Slovenia and Italy, assessing the concentration of the 75% of the population. They noted that there are some non-trivial cases, like breeds with large herds, geographically wide distributed, but in a small number of farms, or breeds geographically concentrated in more than one region.

The aim of this study is to assess the risk status of four Bulgarian local breeds based on their geographic concentration.

Materials and Methods

Four local breeds were included in the study, one cattle - Rhodope Shorthorn Cattle (RKG) (the population kept by the Association for breeding of local cattle breeds), two sheep - Rhodope Tsigai (RTs), Central Rhodope sheep

(SR), and one goat - Bulgarian screw-horned longhaired goat (BVDK). For each breed the identification of the farm, its location (village, municipality and province), and the number of animals were collected in year 2020 from the respective breeding societies looking after these breeds.

The location of each farm was then geocoded by the village coordinates, using the Nominatim Geocoder service provided by the OpenStreetMap project. The data was loaded and analyzed in the free and open source software QGIS *(QGIS.org, 2021)*.

For each breed, the location (latitude and longitude) of its weighted mean center (WMC) was calculated, using the number of animals in each farm as weights. The radiuses of the circles with this center and enclosing 75% and 100% of the population were calculated. The percentage of the population within 25 and 50 km distance from the weighted mean center were also calculated.

To assess whether simplification can be used for the routine estimation of the risk status, all farms on the territory of the same municipality were merged in one ‘virtual’ farm located in the center of the municipality. The same calculations as in previous paragraph were performed and the results compared.

Results and Discussion

The four breeds (Table 1.) represent various cases found in Bulgaria. The Bulgarian screw-horned longhaired goat is the smallest in number of animals from the four, located in only 20 farms in 19 villages. The two sheep breeds are similar in number of farms and animals, each breed distributed in only 4 provinces. The Rhodope Shorthorn Cattle is a typical mountainous breed, with large number of small farms.

Table 1. Number of animals and farms by breed

Breed	Farms No.	Provinces No.	Municipalities No.	Villages No.	Animals No.
RKG	136	9	21	86	5243
RTs	45	4	13	42	5850
SR	44	4	12	35	8164
BVDK	20	6	11	19	3107

The WMC of RKG farms is located in the typical area for this breed – the East Rhodope Mountains (Figure 1.), where are also most of the farms. There are single herds outside this area, however, the largest farm rearing RKG is among them.

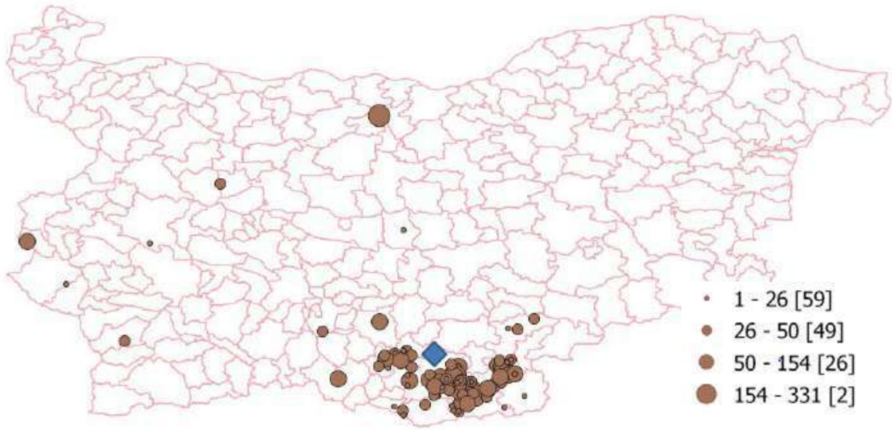


Figure 1. Location of RKG farms, members of the Association for breeding of local cattle breeds. The farms are depicted by circles proportional to the number of animals. The weighted mean center of the farms is depicted by a diamond. In the brackets are the number of farms within the respective size.

The RTs breed is also located mainly in the Rhodope Mountains, with single farms outside the region (Figure 2.). A cluster of farms was observed in the southeast part, where 3328 animals (57% of the population) are within a circle with 18 km radius. In this case additional study is needed, in order to estimate the potential genetic loss, if all the animals within the cluster get extinct due to an epidemiological outbreak.

Figure 2. Location of RTs farms. The farms are depicted by circles proportional to the number of animals. The weighted mean center of the farms is depicted by a diamond. In the brackets are the number of farms within the respective size.

The situation with SR breed (Figure 3.) is similar to RTs, and even the WMCs of the two breeds are less than 20 km apart. Only one SR farm is outside the Rhodope region.

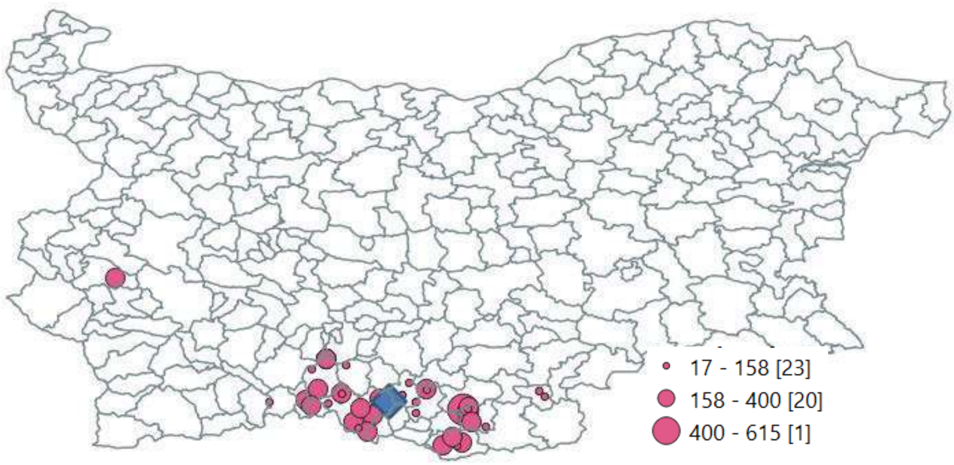


Figure 3. Location of SR farms. The farms are depicted by circles proportional to the number of animals. The weighted mean center of the farms is depicted by a diamond. In the brackets are the number of farms within the respective size.

The WMC of the BVDK farms (Figure 4.) is outside the typical area of the breed and visually, there are no farms in near distance to the center. This breed is distributed in three, distant from each other, regions of the country.

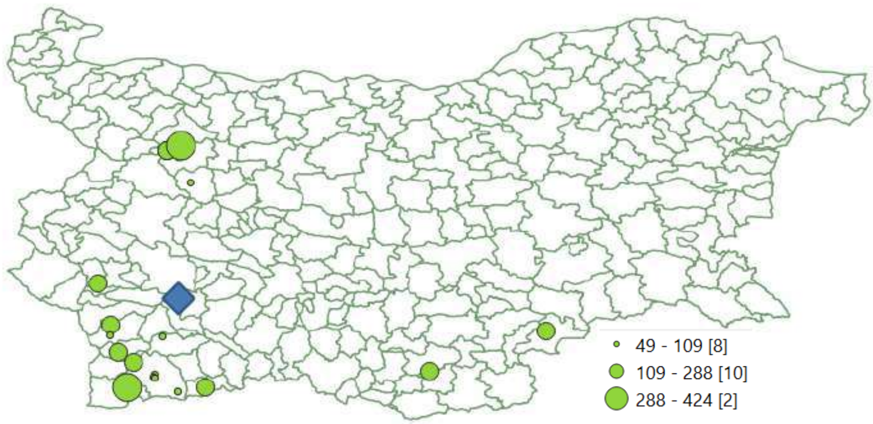


Figure 4. Location of BVDK farms. The farms are depicted by circles proportional to the number of animals. The weighted mean center of the farms is depicted by a diamond. In the brackets are the number of farms within the respective size.

The largest part of the population (62%) is located in the South-West part of the Bulgaria, another 30% are kept around the region of Vratsa, North from the Balkan, and the rest 8% of the population is kept in two farms in South, in the Rhodope and Sakar mountains.

As shown in Table 2., in all four breeds less than 31% of the population is concentrated within 25 km of the WMC of the farms, which is well under the 75% threshold. Thus, not any of these breeds should be considered endangered because of concentrated population. For RKG, RTs and SR breeds more than 75% of population is located in a circle with radius 50 km, which implies increased risk due to geographic concentration and a need for more detailed monitoring.

Table 2. Breed concentration (actual farms)

Breed	Percentage of the population within distance of		Radius of the circle enclosing 75% of the population (km)	Radius of the circle enclosing 100% of the population (km)
	25 km	50 km		
RKG	30.94	84.49	46	254
RTs	14.96	75.01	50	149
SR	29.45	92.71	47	176
BVDK	0.00	13.68	122	223

In all these breeds, the large distances to the furthest farm (the radius of the circle enclosing 100% of the population) are also indication that there are farms outside the typical area of the breed, something observed by the maps in Figures 1, 2, and 3.

As noted before, one extreme case is the Bulgarian screw-horned longhaired goat. There are no farms within 25 km from the MWC, and less than 14% of the population is within 50 km distance. The radius of the circle enclosing 75% of the population is 122 km, which implies that the breed is not at risk due to its geographic concentration. However, additional research is needed to clarify if the sub-populations in these regions possess unique genetic features. If this is the case, the concentration in one of the regions presents also a threat to the biodiversity of the whole breed.

Table 3. Breed concentration (virtual farms by municipalities)

Breed	Percentage of the population within distance of		Radius of the circle enclosing 75% of the population (km)	Radius of the circle enclosing 100% of the population (km)
	25 km	50 km		
RKG	38.91	83.62	41	250
RTs	8.70	84.09	50	166
SR	37.40	94.05	48	185
BVDK	0.00	22.95	130	224

When using the centers of the municipalities, instead of the villages, the results are similar (Table 3.). Some of the numbers had slightly increased, others decreased, depending on the position of the farms villages within the municipalities. Nevertheless, the proportion of the population, concentrated within 25 km of the WMC of the farms, is still well under the 75% in all breeds. For RKG, RTs and SR more than 75% of population is concentrated again within a circle with radius 50 km, corresponding to status “vulnerable”. This observation is in line with the conclusions of Sturaro et al., 2013 that the simplest option seems to be the use of total number of individuals per municipality.

Conclusion

RKG (the population kept by the Association for breeding of local cattle breeds), RTs and SR breeds are vulnerable to risk of extinction with 75% of the population within 50 km distance from the weighted mean center.

The BVDK breeds should be studied at subpopulation level in the different regions.

The results obtained when using geocoding by village and geocoding by municipality are very similar in relation to the concentration thresholds.

For practical purposes, total number of animals per municipality can be used, placing all the animals in a virtual farm in the municipality center.

Procena statusa rizika četiri lokalne Bugarske rase na osnovu njihove geografske rasprostranjenosti

Zhivko I. Duchev

Rezime

Cilj studije je bio da se proceni status rizika rasa na osnovu njihove geografske rasprostranjenosti. Uključene su četiri lokalne bugarske rase - rodopska kratkoroga goveda, ovce rase rodopska cigaja, centralno rodopske ovce i bugarska dugodlaka koza. Za svaku rasu prikupljena je identifikacija farme, njena lokacija i broj životinja, a geografska rasprostranjenost je analizirana GIS metodama. Lokacija svake farme geokodirana je pomoću dva pristupa - prema koordinatama sela i koordinatama centra opštine. Izračunat je procenat stanovništva na 25 i 50 km udaljenosti od ponderisanog srednjeg središta farmi i radijus kruga koji obuhvata 75% i 100% populacije. U svim rasama, osim kod koza, više od 75% populacije bilo je koncentrisano na manje od 50 km od ponderisanog srednjeg centra, pa je njihov status „ranjiv“, pa ih treba pažljivo pratiti. Bugarska dugodlaka koza bila je rasprostranjena u tri udaljena regiona, gde se moraju sprovesti dodatna istraživanja na nivou subpopulacije. Farme koje se nalaze daleko od izvornog područja rase primećene su kod svih rasa. Rezultati pri korišćenju opštinskih koordinata bili su slični onima dobijenim korišćenjem koordinata sela. U praktične svrhe, kao pojednostavljenje, može se koristiti ukupan broj životinja po opštini.

Ključne reči: biodiverzitet domaćih farmskih životinja, lokalne rase, GIS, status rizika

Acknowledgment

Research was financed by the Agricultural Academy of Bulgaria, project Zh156

References

ALDERSON L. (2009): Breeds at risk: Definition and measurement of the factors which determine endangerment. Livestock Science,123, 23–27.

DUCHEV Z., DISTL O., GROENEVELD E. (2006): Early warning system for loss of diversity in European livestock breeds, Archiv fur Tierzucht, 49, 6, 521-531

FAO (2013): In vivo conservation of animal genetic resources, FAO Animal Production and Health Guidelines No. 14, Rome

GANDINI G.C., OLLIVIER L., DANELL B., DISTL O., GEORGOUDIS A., GROENEVELD E., MARTYNIUK E., VAN ARENDONK J.A.M., WOOLLIAMS J.A. (2004): Criteria to assess the degree of endangerment of livestock breeds in Europe. Livestock Production Science, 91,1-2,173–182.

LOFTUS R., SCHERF B. (eds.) (1993): World watch list for domestic animal diversity. 1st edition. Rome, FAO.

QGIS.ORG (2021): QGIS Geographic Information System. QGIS Association. http://www.qgis.org

REIST-MARTI S.B., SIMIANER H., GIBSON J., HANOTTE O., REGE J.E.O. (2003): Weitzman's Approach and Conservation of Breed Diversity: an Application to African Cattle Breeds, Conservation Biology, 17, 1299–1311.

SIMON D.L., BUCHENAUER D. (eds.) (1993) Genetic diversity of European livestock breeds. Results of Monitoring by the EAAP Working Group on Animal Genetic Resources; EAAP Animal Genetic Data Bank, Institute of Animal Breeding and Genetics, School of Veterinary Medicine, Hannover, EAAP Publication, No. 66, Wageningen Pers, Wageningen, 591 p.

STURARO E., KOMPAN D., ALDERSON L., LIGDA CH. (2013): Assessment of breeds risk status by investigating their geographic distribution, Agricultural Sciences, 13, 147-150

UNITED NATIONS NATIONAL ASSEMBLY, (2015): Resolution adopted by the General Assembly on 25 September 2015: 70/1 - Transforming our world: the 2030 Agenda for Sustainable Development.

VERRIER E, AUDIOT A, BERTRAND C, CHAPIUS H, CHARVOLIN E, DANCHIN-BURGE C, DANVY S., GOURINE J.L, GAULTIER P., GUEMENE D., LALOE D., LENOIR H., LEROY G., NAVES M., PATIN S., SABBAGH M. (2015): Assessing the risk status of livestock breeds: a multi-indicator method applied to 178 French local breeds belonging to ten species. Animal Genetic Resources, 57, 105–118.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

PREDICTION OF AMMONIUM EMISSION FROM DAIRY CATTLE BASED ON MILK UREA NITROGEN USING THE PRECISION FARMING METHODOLOGY

Tamara Papović, Denis Kučević, Miroslav Plavšić, Snežana Trivunović, Ksenija Čobanović

University of Novi Sad, Faculty of Agriculture, Trg D. Obradovića 8, 21000 Novi Sad, Serbia
Corresponding author: Tamara Papović, tamara.papovic@stocarstvo.edu.rs

Abstract: Currently the livestock production sector causes the production of huge amounts of manure, which is a source of ammonia. Ammonia is dangerous to human, animal and environmental health. According to some studies, livestock is responsible for 76% of global nitrogen emissions, with cattle production accounting for 36% of ammonia emissions in Europe. An important challenge during the increase and strengthening of the livestock production sector is the impact of livestock on the environment - especially on the climate and ecosystems. Reducing greenhouse gas emissions and ammonia emissions from dairy farms is one of the main goals in achieving environmentally sustainable milk production. A prerequisite for the reduction is a quick, simple and accurate assessment based on already available data, which in the case of dairy farms means the use of milk control data (control day data). By feeding lactating cows balanced meals in terms of protein and energy content, the urea content in milk (MUN) ranges from 15 to 30 mg/dl, which is a general recommendation. When the urea content in milk increases by 20 and 30 mg/dl, the ammonia emission increases by 2.5%, ie. 3.5%. The urea content in milk is highly correlated with daily milk yield, the increase in production also increases the urea content in milk. The low content of urea in milk indicates a lack of ammonia in the rumen, ie. to the insufficient amount of proteins degradable and unfavorable conditions for the growth of microorganisms in the rumen. High values of MUN content are an indicator of excessive formation of ammonia in rumen, as a consequence of high content of proteins degradable in rumen and small amounts of carbohydrates. The high concentration of ammonia in the rumen burdens the liver, which synthesizes urea. The amount of ammonia in the barn increases, which negatively affects the welfare of animals and humans. Many researchers have proven that the properties of milk content can be used not only to assess animal productivity, but also to characterize metabolic processes in the animal body, and thus to predict possible diseases in time (ketosis, acidosis) and control production efficiency. Determination of urea content in milk using IR spectrophotometry in milk analysis during regular monthly control of milk yield of cows provided new opportunities in monitoring milk quality, through which it is possible to assess the quality of nutrition on dairy farms, especially in terms of feed balance in terms of crude protein content and energy in the meal. This indicates the possibility of applying the urea content in milk in practice, in order to determine the efficiency of protein utilization from meals. A precise system of protein nutrition in lactating cows must provide a meal in accordance with the level of milk production, but also avoid consuming too much protein, which results in their inefficient use, adverse effects on energy balance of animals, and increased nitrogen excretion into the environment, but also unnecessary increases meal price.

Key words: milk urea nitrogen (MUN), ammonia emission, dairy farming

Introduction

FAO indicate that the world's population will increase from the current 7.2 billion to 9.6 billion by 2050. The combination of population growth, income growth and urbanization poses challenges to agricultural production and the food industry. The question arises as to how to ensure a healthy diet globally, with protein foods playing a key role. By 2050, the demand for milk and meat is projected to increase by about 58%. At the same time, consumers prefer that they be produced in the most sustainable way, with growing demand. Even with calls for more balanced consumption of animal proteins and replacement with plant alternatives, the demand for animal proteins will continue to grow. A significant problem at the time of intensification and consolidation of livestock production is its impact on the environment, especially on the climate and ecosystems. Livestock production will continue to be the most dynamic agricultural subsector as global milk production is projected to increase from 580 to 1,043 million tonnes in 2050. Such a significant increase in production must be accompanied by appropriate environmental protection measures. Many studies emphasize well-balanced agricultural production, looking for a way to achieve optimal animal productivity with minimal environmental pollution. At the same time, livestock, and especially cattle production, are mostly responsible for the emission of ammonia into the atmosphere. In European countries, 15 to 75% of the total ammonia released into the atmosphere goes to cattle production *(Rösemann et al., 2019)*. Ammonia poses a possible danger from the aspect of human, animal and environmental health. Reducing greenhouse gas emissions, especially ammonia emissions from dairy farms, is one of the main goals in achieving environmentally sustainable milk production. For the dairy cows, the source of ammonia is urea from urine, which is hydrolyzed to ammonia and CO_2, by the activity of microbiological urease present in the feces *(Jonker et al., 2002)*. This leads to the need to reduce the negative impact on the environment, which can be achieved through adequate nutrition and improving the efficiency of food consumption. Urea, formed in the liver by detoxification of ammonia, is a molecule of small molecular weight soluble in water and diffuses through the bloodstream through cell membranes and enters body fluids, including milk. There is a high positive correlation between the content of urea in the blood, urine and milk of cows. The content of urea in blood and milk is considered to be a useful indicator of protein metabolism and nutritional status of lactating cows. Milk sampling is a far more affordable method compared to the complex and invasive method of blood sampling. A number of researchers *(Duinkerken et al. 2011; Spek et al. 2013)* suggest determining the urea content in milk samples, as a routine method for monitoring the efficiency of protein feeding in cows because they believe that it is possible to predict urea excretion into the atmosphere based on MUN content, because it is a useful indicator of NH_3 emissions from the barn. The Netherlands is already conducting long-term monitoring and assessment of environmental threats *(Bijgaart, 2003)* based on the parameters of the MUN content. The obtained results by *(Ruska et al., 2017)* show how easily the urea content in milk enables the assessment of the efficiency of the use of protein in each feed dose and the identification of potential threats to environmental pollution. After evaluating the results of the research and their connections, the use of MUN content parameters together with traditional parameters such as fat and crude protein content is proposed for successful herd management. Monitoring change is necessary to help establish a design for practical action to achieve specific goals in an economically and environmentally sustainable way. At the same time, precision agriculture can increase safety and reduce the impact of agriculture and agricultural practices on the environment, thus contributing to the sustainability of agricultural production *(European Commission, 2020)*. The application of precise technologies in dairy cattle breeding enables the collection of a large amount of individual information about each animal, supplementing the daily observation of the herd. By properly processing this data in combination with decision support systems, the application of these technologies improves animal surveillance and decision making.

Significant correlation between blood urea nitrogen (BUN), MUN and urinary N

Urea in milk is a very reliable indicator of urea concentration in blood and urine *(Kohn et al., 2004)*. Since there is a significant correlation between urea content in milk and nitrogen content in urine and manure of animals *(Burgos et al., 2010; Eckersall and Bell, 2010; Spek et al., 2013)* in optimizing dairy farm management, urea in milk should be used as a good indicator of nutrition efficiency, also for assessing the impact of the farm on the environment *(Godden et al., 2001; Haig et al., 2002)*. Urea is a naturally occurring ingredient in milk as part of non-protein nitrogen. During the decomposition of crude proteins from food in the digestive system, ammonia is released, which the microorganisms of the rumen use for the synthesis of their proteins. Excess ammonia is translated in the liver into urea, which is released into body fluids (blood, milk, urine). The concentration of ammonia in the rumen and the pH of the rumen are the two main factors that affect the level of ammonia. Excess amino acids and peptides are released in the liver and nitrogen is converted to urea. Ammonia is toxic to animals and is rapidly converted to urea in the liver *(Swenson and Reece, 1993)*. The optimal urea content in milk is 10-30 mg/ml (2.5 - 5.0 mmol/l) of milk. The urea content in milk mostly depends on the ammonia content in the rumen and as such is a good indicator of meal quality *(Eicher, 2004)*. MUN concentration increases by 0.06 or 0.03 mg / 100 ml, for every 1 kg increase in daily milk yield of cows of 5-29 kg and 30-59 kg, respectively *(Wattiaux et al., 2005)*. Low urea content in milk causes low ammonia content in rumen juice. Too low ammonia level is the cause of inadequate conditions for the growth of rumen microorganisms, prolonged degradation of nutrients in the rumen and reduced synthesis of microbial proteins in the rumen. The lower supply of animals with energy and microbial proteins is a consequence of the above, and for cows with a particularly low urea content, this results in a reduction of milk yield and the content of milk fat, protein and lactose in milk. The high content of urea in milk is an indicator of the high content of ammonia in the rumen. Cows with too high level of urea in milk must use a large amount of energy to excrete nitrogen from the body, or to synthesize urea from ammonia. Also, the conversion of ammonia into urea puts a lot of strain on the liver. Elevated levels of nitrogen in the urine cause elevated levels of ammonia in the air and thus the microclimate of the facility becomes inadequate for human and animal health. Urea consists of small neutral molecules, which are transferred to body fluids by diffusion and across the cell membrane. Because a very large amount of blood passes through the mammary gland, urea diffuses into. In this way, a balance is established with the concentration of urea in the blood. This is precisely the reason why the amount of nitrogen in milk is proportional to the blood urea concentration *(Roseler et al., 1993)* and can be used as an economic and non-invasive process for monitoring protein in the herd *(Ropstad and Refsdal, 1987)*. Significant correlation between blood urea nitrogen (BUN), MUN and urinary N is evident, and the possibility for its application, not only as performance indicator, but also in the possible reduction environment N emissions in dairy farms.

MUN as a useful indicator for prediction of ammonium emission from dairy cattle

Issue on food supply becomes increasingly more topical as earth population number is growing. Many researches emphasizes well-balanced agricultural production, seeking for a way to achieve optimum animal productivity with minimum environmental pollution. Interest towards environmental pollution has been growing. Accordingly, in Europe several regulatory enactments are controlling possible environmental pollution that may arise when performing agricultural activities. In some countries, farms are controlled on the basis of urea content in milk. This makes it possible to define potential sources of pollution and inform farms about precautionary measures *(Bijgaart, 2003)*. *Hristov et al. (2011)* state that an important part of the nitrogen of cattle manure, especially from urinary urea, is converted into ammonium and finally lost in the atmosphere as ammonia. The different nature of the factors that control ammonia evaporation, such as manure management, ambient temperature, wind speed, and manure composition and pH, complicate the definition of ammonia emissions from cattle *(Ross et al., 2002)*. Data from the National Research Council show that the amount of nitrogen in the diet of dairy cows is exceeded by close to 6.6%, consequently, the nitrogen content in urine increases by 16%, and in manure by 2.7%. The urea content in milk is used to calculate the amount of nitrogen used, because it is easy to determine, and the collection and testing of special samples (urine or feces) is not necessary *(Broderick and Huhtanen 2013; Jonker et al., 2002)*. Studies show that urea in milk identifies the content of urea in the blood and urine. Furthermore, the urea content in milk can be used to assess environmental contamination and digestive efficiency because it shows a lack of crude protein for dairy cows, especially an excess in the digestive tract *(Broderick and Clayton 1997; Hof et al. 1997; Burgos et al., 2010)*.

Netherlands already monitoring and evaluating environmental threats basing on urea content parameter acquired from cow milk monitoring data. Measures taken since 1998, covering monitoring of legislation and farmer control over and correction of fodder protein and energy amount, have produced good result. Many researchers emphasize that use of urea content is not unambiguous, and it may not be used separately without considering factors influencing changes thereof – not only physiological, but also time, when milk samples were taken, and testing method, as well as laboratory in which testing was performed. Therefore scientists and feeding specialists suggest basing regular herd control on average results calculated that were obtained from individual animals instead of urea parameter found for total milk produced *(Bijgaart, 2003; Ingvarsten, 2006)*. Each farmer has to evaluate advantages and disadvantages, andmby using all available milk productivity and quality traits, has to make a decision on the most efficient and environmentally friendly farming method.

Possible ammonium pollution could be evaluated with the help of calculations based on model developed in University of California. With data which determine the variability of daily milk production, daily urea and urea nitrogen content in milk, it is possible to estimate the daily ammonia emission of dairy cows through dairy control data. The ammonia emission (AM-EMISS) can be calculated using milk urea nitrogen (MUN) accordingly to the following equation: AM-EMISS (g/cow daily) = 25.0 + 5.03 * MUN *(Burgos et al., 2010)*.

All agricultural holdings of milk producing should control urea content in milk yielded from each individual cow on regular basis, while farms engaged in milk monitoring should find out milk urea content for all cows within monthly control. MUN allows farmers to make sure that fodder that fodder doses are efficient and ensures that possible problems are discovered and solved in time.

Conclusion

Precise system of protein nutrition of lactating cows must provide a meal in accordance with the specific level of milk production, but also avoiding the consumption of excess protein, which affects their inefficient use, adversely affects the energy balance of animal production, leads to increased nitrogen excretion and unnecessarily increases meal. There is a pronounced need to monitor the adequacy of the protein diet of lactating cows. By determining the nitrogen content of urea in milk, it is possible to adjust the meal in terms of protein content, protein-energy ratio, efficient use of consumed protein and energy, meal economy, optimal reproductive performance of dairy cows, more profitable overall production, and what is especially significant is the reduction of nitrogen emissions into the environment. Based on the assumed, a model of optimization of feed composition for dairy cows can be developed, which can influence the reduction of ammonia emissions from dairy farms, the application of which can be realized ecologically and economically more sustainable management of dairy farms.

Predviđanje emisije amonijaka od mlečnih goveda na bazi uree u mleku korišćenjem metoda preciznog stočarstva

Tamara Papović, Denis Kučević, Miroslav Plavšić, Snežana Trivunović, Ksenija Čobanović

Rezime

Kao prateći proizvod stočarske proizvodnje, pojavljuju se velike količine stajskog đubriva koje su izvor amonijaka. Amonijak je opasan kako za zdravlje ljudi i životinja tako i za kontaminaciju životne sredine. U skladu sa pojedinim istraživanjima, stočarstvo je odgovorno za 76% globalnih emisija azota, pri čemu govedarska proizvodnja čini 36% emisija amonijaka u Evropi. Važan izazov tokom povećanja i jačanja sektora stočarske proizvodnje je uticaj stočarstva na životnu sredinu - posebno na klimu i ekosisteme. Smanjenje emisije gasova staklene bašte, te emisije amonijaka s farmi mlečnih krava predstavlja jedan od glavnih ciljeva u postizanju ekološki održive proizvodnje mleka. Preduslov za smanjenje je brza, jednostavna i tačna procena na osnovu već dostupnih podataka, što u slučaju farmi mlečnih krava znači upotrebu podataka o kontroli mlečnosti (podaci o kontrolnim danima). Ishranom krava u laktaciji izbalansiranim obrocima u pogledu sadržaja proteina i energije, sadržaj uree u mleku se kreće od 15 do 30 mg/dl, što je i opšta preporuka. Pri povećanju sadržaja uree u mleku od 20 i 30 mg/dl, emisija amonijaka se povećava za 2,5% tj. 3,5%. Sadržaj uree u mleku visoko je koreliran s dnevnom mlečnošću, odnosno povećanjem proizvodnje povećava se i sadržaj uree u mleku. Nizak sadržaj uree u mleku ukazuje na nedostatak amonijaka u rumenu, tj. na nedovoljnu količinu proteina razgradivih u rumenu i nepovoljne uslove za rast mikroorganizama u rumenu. Visoke vrednosti sadržaja MUN pokazatelj su prekomernog formiranja amonijaka u rumenu, kao posledica visokog sadržaja proteina razgradivih u rumenu i male količine ugljenih hidrata. Visoka koncentracija amonijaka u rumenu opterećuje jetru, koja sintetiše ureu. U štali se povećava količina amonijaka što negativno utiče na dobrobit životinja i ljudi. Mnogi istraživači su dokazali da se osobine sadržaja mleka mogu koristiti ne samo za procenu produktivnosti životinja, već i za karakterizaciju metaboličkih procesa u životinjskom telu, a time i za predviđanje mogućih bolesti na vreme (ketoza, acidoza) i kontrolu efikasnosti proizvodnje. Određivanje sadržaja uree u mleku primenom IR spektrofotometrije u analizi mleka prilikom redovne mesečne kontrole mlečnosti krava pružilo je nove mogućnosti u praćenju kvaliteta mleka, preko kojeg je moguće sagledavanje kvaliteta ishrane na farmama mlečnih krava, a pre svega po pitanju izbalansiranosti obroka u pogledu sadržaja sirovih proteina i energije u obroku. Navedeno ukazuje na mogućnost primene sadržaja uree u mleku u praksi, sa ciljem utvrđivanja efikasnosti iskorišćavanja proteina iz obroka. Precizan sistem proteinske ishrane krava u laktaciji mora obezbediti obrok u skladu sa nivoom proizvodnje mleka, ali i izbegavanje konzumiranja prevelike količine proteina, što za posledicu ima njihovo neefikasno iskorišćavanje, nepovoljno dejstvo na energetski bilans životinja, i povećano izlučivanje azota u okolinu, ali i nepotrebno poskupljenje obroka.

Ključne reči: urea u mleku, emisija amonijaka, mlečno govedarstvo

Acknowledgment

Research will be financed by short-term project of special interest for sustainable development in the Autonomous Province of Vojvodina in 2021 (No: 142-451-2310/2021-01/01).

References

BIJGAART D. (2003): Urea: New applications of mid-infra-red spectrometry for the analysis of milk and milk products. Bulletin - International Dairy Federation, 383, 5-15.

BRODERICK G.A., CLAYTON M.K. (1997): A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. Journal of Dairy Science, 80, 2964–2971.

BRODERICK G., HUHTANEN P. (2013): Application of milk urea nitrogen values. US Dairy Forage Research Center Agricultural Research Service, USDA Madison, Wisconsin and Department of Animal Science Cornell University and MTT Agrifood Research Finland.

BURGOS S.A., EMBERTSON N.M., ZHAO Y., MITLOEHNER F.M., DEPETERS E.J., FADEL J.G. (2010): Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to ammonia emissions. Journal of Dairy Science, 93(6), 2377–2386.

CASTILLO A.R., KEBREAB E., BEEVER D.E., FRANCE J. (2000): A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. Journal of Animal and Feed Sciences 9, 1–32.

DUINKERKEN VAN G., SMITS M. C. J., ANDRÉ G., ŠEBEK L. B. J., DIJKSTRA J. (2011): Milk urea concentration as an indicator of ammonia emission from dairy cow barn under restricted grazing. Journal of Dairy Science (94) 321–335.

ECKERSALL P.D., BELL R. (2010): Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Vet. J. 185, 23–27.

EICHER R., BOUCHARD E., BIGRAS-POULIN M. (1999): Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. Prev.Vet.Med. 39: 53-63.

EICHER R. (2004): Evaluation of the metabolic and nutritional situation in dairy herds: Diagnostic use of milk components. 23rd World Buiatrics Congress, Quebec City, Canada.
GODDEN S.M., LISSEMORE K.D., KELTON D.F., LESLIE K.E., WALTON J.S., LUMSDEN J.H. (2001): Factors Associated with Milk Urea Concentrations in Ontario Dairy Cows. Journal of Dairy Science Volume 84, Issue 1, 107-114.
GRESSLEY T.F., ARMENTANO L.E. (2007): Effects of Low Rumen–Degradable Protein or abomasal Fructan Infusion on Diet Digestibility and urinary Nitrogen Excretion in Lactating Dairy Cows. Journal of Dairy Science 90(3), 1340–1353.
HAIG P.A., MUTSVANGWA T., SPRATT R., MCBRIDE B.W. (2002): Effects of Dietary Protein Solubility on Nitrogen Losses from Lactating Dairy Cows and Comparison with Predictions from the Cornell Net Carbohydrate and Protein System. Journal of Dairy Science, 85, 5, 1208-1217.
HOF G., VERVOORN M.D., LENAERS P.J., TAMMINGA S. (1997): Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. Journal of Dairy Science (80) 3333 - 3340.
HRISTOV A. N., HANIGAN M., COLE A., TODD R., MCALLISTER T. A., NDEGWA P., ROTZ A. (2011): Review: Ammonia emissions from dairy farms and beef feedlots. Can. J. Anim. Sci. 91: 1-35.
INGVARSTEN K.L. (2006): Feeding and management related diseases in the transition cow physiological adaptations around calving and strategies to reduce feeding–related diseases. Animal Feed Science and Technology 126, 175–213.
JONKER J.S., KOHN R.A., HIGH J. (2002): Use of Milk Urea Nitrogen to Improve Dairy Cow Diets. Journal of Dairy Science, Volume 85, Issue 4, 939-946.
KIRCHGESSNER M., KREUZER M., ROTH-MAIER D. (1986): Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. Archiv Tiernährung, 36: 192-198.
KOHN R. A., FRENCH K. R., RUSSEK-COHEN E. (2004): A Comparison of Instruments and Laboratories Used to Measure Milk Urea Nitrogen in Bulk-Tank Milk Samples. Journal of Dairy Science, 87, 6, 1848-1853.
LEIP A., BILLEN G., GARNIER J., GRIZZETTI B., LASSALETTA L., REIS S., SIMPSON D., SUTTON M.A., DE VRIES W., WEISS F., WESTHOEK H. (2015): Impacts of European livestock production: nitrogen, sulphur, phosphorus and greenhouse gas emissions, land-use, water eutrophication and biodiversity. Environ. Res. Lett., 10, Article 115004.
ROSELER D.K., FERGUSON J.D., SNIFFEN C.J., HERREMA J. (1993): Dietary protein degradibility effects on plasma and milk urea nitrogen and milk non-protein nitrogen in Holstein cows. Journal of Dairy Science 76: 525.

ROSS C.A., SCHOLEFIELD D., JARVIS S.C. (2002): A model of ammonia volatilisation from a dairy farm: an examination of abatement strategies. Nutrient Cycling in Agroecosystems 64, 273– 281.

ROPSTAD E., REFSDAL A.O. (1987): Herd reproductive performance related to urea concentration in bulkmilk. Acta Veterinarian Scandinavia 28, 55.

RÖSEMANN C., HAENEL H.D., DÄMMGEN U., DÖRING U., WULF S., EURICH-MENDEN B., FREIBAUER A., DÖHLER H., SCHREINER C., OSTERBURG B., FUß R. (2019): Calculations of gaseous and particulate emissions from German agriculture 1990 – 2017: Input data and emission results. Open Agrar Repository, Göttingen, Germany.

RUSKA D., JONKUS D., CIELAVA L. (2017): Monitoring of ammonium pollution from dairy cows farm according of urea content in milk. Agronomy Research, Vol.15, No.2, 553-564.

SHINGFIELD K.J., JAAKKOLA S., HUHTANEN P. (2001): Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage–based diets. Animal Science: an International, Journal of Fundamental and Applied Research 73(3), 541–554.

SPEK J.W., DIJKSTRA J., VAN DUINKERKEN G., HENDRIKS W.H., BANNINK A. (2013): Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis. Journal of Dairy Science (96) 4310–4322.

SWENSON M.J., REECE W.O. (1993): Water balance and excretion. In: Duke's Physiology of Domestic Animals, 11th ed. Cornell University Press, Ithaca, NY, USA, 573–604.

WATTIAUX M.A., KARG K.L. (2004): Protein level for alfalfa and corn silage-ba-sed diets: I. Lactational response and milk urea nitrogen. J. Dairy Sci., 87, 3480-3491.

HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS OF PIROT PRAMENKA - ENDANGERED SHEEP POPULATION

Dragana Ružić-Muslić[1], Bogdan Cekić[1], Ivan Ćosić[1], Ivan Pavlović[2], Nevena Maksimović[1], Violeta Caro Petrović[1], Zorica Bijelić[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Republic of Serbia
[2]Scientific Veterinary Institute of Serbia, Belgrade, Serbia
Corresponding author: Dragana Ružić-Muslić, muslic.ruzic@gmail.com

Abstract: Pirot breed is a highly endangered autochthonous population of sheep in Serbia, from which several important national brands originate: Pirot lamb, Pirot carpet and Pirot hard cheese. In the available literature, there are no data on hematological and biochemical parameters of the blood of this population.
The aim of this paper, which represents the beginning of the research, is to determine the above indicators in the blood of Pirot Pramenka, which will contribute to the preservation of this population. Since the breed affects the value of blood parameters, the obtained results will be used to define reference intervals for this population and compare with others. The study included 30 clinically healthy sheep, 2-3 years old, from the area of Stara Planina. Blood samples were taken by puncture to c. jugularis from which, after coagulation and centrifugation, blood serum was extracted. Haematological analyzes were performed in the next 24 hours on a Siemens Advia 120 analyzer. The following parameters were determined: total number of leukocytes, erythrocytes, platelets, hemoglobin content, hematocrit value, MCV, MCH, PLT and MPV. Biochemical blood tests were performed using Olympus AU 400 analyzers.The following parameters were determined: total protein, albumin, globulin, urea, creatinine, glucose, total bilirubin, cholesterol. Also, the following minerals were determined - calcium, phosphate and magnesium, and following enzymes: AST, GGT and CK. Descriptive statistical analysis was performed using the statistical package STATISTICA (Version 8). Hematological indicators of Pirot pramenka are in reference intervals for sheep, which indicates good animal health status. Biochemical parameters of Pirot pramenka blood were within defined physiological limits, except for creatinine and phosphorus levels, which were below the lower limit, which is a consequence of dehydration and reduced alimentary intake. In accordance with the obtained results, it is necessary to correct the diet.

Key words: Pirot pramenka, hematological parameters, biochemical indicators

Introduction

Pramenka (Zapfel) belongs to the autochthonous (locally adapted) breeds of sheep that represent a unique genetic heritage created thousands of years ago. They are an important element of the regional agro-biodiversity, tradition and cultural heritage of Serbia. Different climatic and nutritional conditions, together with animal migrations, have influenced the formation of different strains of Pramenka, which differ in morphological and production characteristics: Sjenica, Svrljig, Liplje, Krivovir, Karakačan, Pirot, Vlach Vitoroga and Bardoka. They possess sets of genes responsible for good adaptability, disease resistance, longevity, and pronounced maternal instinct. However, the more productive, more intense breeds whose primary goal is profit, win. Therefore, the biological survival of autochthonous populations is endangered, which caused some strains of pramenka to acquire the status of "critical" and "endangered" population. (*Ružić-Muslić et al.,2015*).

Pirotska pramenka is the most endangered population of sheep in Serbia. According to the **Domestic Animal Diversity Information System (DAD-IS),** for 2021, 207 females and 25 males are bred in Serbia. Most of this population is grown in the area of the Stara Planina National Park and southeastern Serbia. The effective population size is 89, which is highly endangered and at risk of extinction. On the other hand, with good management, locally adapted genotypes could serve as a promoter of sustainable development, to reduce the pressure on regional agro-biodiversity and costs of production since these are genotypes of combined production traits, and represent a potential source of meat, wool, milk, manure and hides (*Gorkhali et al., 2015; Cekić et al., 2018*). Pirot Pramenka has spawned several important national brands: Pirot lamb, Pirot carpet and Pirot hard cheese, which indicates that its preservation is imperative. Its survival should be sought in increasing the economic attractiveness of breeding, which will encourage the affirmation of this sheep. Therefore, it is necessary to explore the possibility of maximum production, while monitoring the metabolism of nutrients in the body and maintaining health. The key indicators of nutritional status and animal health are the metabolic profile and hematological picture of sheep (*Klinkon and Zadnik, 1997; Herdt et al., 2000; Van Saun, 2000; Antunović et al., 2002; Antunović et al. 2007; Antunović et al., 2014)*. Determining and monitoring the values of metabolic profile parameters show whether homeostatic mechanisms maintain blood composition within physiological limits, depending on different rearing conditions (*Prodanović et al., 2012*).

In the available literature, there are no data on hematological and biochemical parameters of Pirot Pramenka. Given that breed has an impact on the value of blood parameters (*Dias et al., 2010; Addass, 2011; Vojta et al., 2011*), defining the reference range of these parameters for Pirot Pramenka will have significant implications for determining similarities and differences between different breeds, strains as well as healthy and diseased individuals.

The aim of this work, initial research, is to determine the hematological and metabolic profile in the blood of Pirot pramenka, which will contribute to the preservation of this highly endangered sheep.

Material and Method

The research was conducted on 30 sheep of Pirot Pramenka, reared in the area of Stara Planina, with an average age of 2-3 years, during the winter season. The sheep were healthy, in the last third of pregnancy. The animal diet per individual animal consisted of meadow hay (ad libitum) and 0.3 kg of maize, per head/day. Water and mineral blocks were constantly available. Sheep blood sampling was performed in the morning. Blood was taken by puncture to c. jugularis in 5 ml glass tubes, with a gray colour stopper (with anticoagulant EDTA, for glucose), yellow colour (for biochemical analysis) and purple colour, for complete blood picture. After labeling, the samples were stored at 4°C in the refrigerator, and subsequently transferred to the laboratory. Haematological analyses were performed in the next 24 hours on a Siemens Advia 120 analyzer, which is equipped with software. The following parameters were determined: total number of leukocytes (WBC), erythrocytes (RBC), platelets (PLT), hemoglobin content, hematocrit value, MCV average volume erythrocytes in a liter of blood, MCH-average amount of hemoglobin in erythrocyte, MCHC-average concentration of hemoglobin in a liter of blood, PLT-number of platelets, MPV-average volume of platelets. Since the total number of leukocytes represents the sum of neutrophilic, eosinophilic and basophilic ganulocytes, lymphocytes and monocytes, differentiation of white blood cells was performed and shown as a percentage. Biochemical analyzes of the blood were performed using an Olympus AU 400 analyzer. The blood was centrifuged for 7 minutes at 3600 rpm, during which the serum was separated, used in the analyses. The following parameters were determined: total protein, albumin, globulin, urea, creatinine, glucose, total bilirubin, cholesterol. Also, following mineral substances were determined: calcium, phosphate, magnesium, as well as following enzymes: AST (aspartate aminotransferase), GGT (glutamyl transferase) and CK (creatine kinase). Reference values of the examined biochemical parameters were taken from *Baumgartner and Wittek (2017).* Descriptive statistical analysis was performed using the statistical package STATISTICA (Version 8).

Results and Discussion

Hemogram (total number of leukocytes, erythrocytes and thrombocytes/platels) in the blood of Pirot Pramenka is presented in Table 1. In addition to the above parameters, the relative share of individual fractions of leukocytes (neutrophils, lymphocytes, monocytes, eosinophils, basophils), hemoglobin content, hematocrit value and erythrocyte constants: MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin - average amount of hemoglobin in a single erythrocyte) and MCHC (Mean Corpuscular Hemoglobin Concentration: concentration of hemoglobin in one liter of blood). Sheep erythrocytes are among the smallest of all mammals (*Kramer, 2000*). Thrombocytes or platelets are discoidal cells that form in the bone marrow of mammals from the cytoplasm of megakaryocytes, do not contain a nucleus and cannot multiply (*Guyton and Hall, 2006*).

Table 1. Hematological parameters of blood in Pirot Pramenka sheep population

Hematological parameters	Units	Mean value ± SD	Minimum	Maximum	Reference value*
WBC leukocytes	10^9/L	6.93±1.30	4.24	8.45	4.0-12.0
NE-neutrophils	10^9/L	2.13±0.99	0.76	4.27	2.0-4.0
LY-lymphocytes	10^9/L	4.05±0.94	2.56	5.46	3.5-6.9
MO-monocytes	10^9/L	0.16±0.07	0.07	0.27	0.0-0.4
EO- eosinophils	10^9/L	0.40±0.18	0.18	0.74	0.0-0.6
BA-basophils	10^9/L	0.09±0.02	0.07	0.15	0.0-0.2
NE- neutrophils	%	30.0±10.77	18.00	54.10	10.0-50.0
LY- lymphocytes	%	59.25±11.38	32.40	69.80	40.0-75.0
MO- monocytes	%	2.45±1.16	1.10	3.80	0.0-6.0
EO- eosinophils	%	5.74±1.96	2.80	9.40	0.0-10.0
BA-basophils	%	1.42±0.21	1.10	1.70	0.0-3.0
Erytrocytes-RBC	10^{12}/L	9.84±0.88	7.41	10.14	9.0-15.0
Hemoglobin	g/L	103.11±10.99	86.00	125.00	90.0-150.0
Hematocrit	%	30.37±3.20	26.00	35.20	27.0-45.0
MCV	fL	34.4±2.35	29.60	37.20	28.0-40.0
MCH	pg	11.68±0.81	10.30	13.20	8.0-12.0
MCHC	g/L	339.0±17.58	302.00	361.00	310.0-340.0
RDW	%	15.96±0.61	15.10	16.70	12.0-27.0
Platelet count-PLT	10^9/L	382.72±18.94	340.50	390.54	250.0-750.0

WBC - leukocytes, MCV - average volume of erythrocytes in a liter of blood, MCH - average amount of hemoglobin in a erythrocyte, MCHC - average concentration of hemoglobin in one liter of blood, RDW - width of red cell distribution, PLT - platelet count * W.*Baumgatner and Wittek., (2017)* A complete blood count or hemogram is a basic laboratory blood test and an important diagnostic "tool" used to monitor general animal health, nutritional status, hematopoietic disorders, viral and bacterial diseases and to define a reference interval for hematological parameters in different sheep breeds, in different rearing conditions, which is especially important for the preservation of indigenous breeds. Analyzing the obtained hematological indicators of Pirot Pramenka, it is noticeable that there were no major deviations from the established reference values for sheep. Our results indicate good health status, in agreement with the results of authors who have studied other strains of Pramenka (*Šimpraga et al., 2013; Antunović et al, 2011; Shek Vugrovečki et al., 2017*).

Table 2. Mean value and intervals of variation of hematological parameters of different strains of Pramenka from the relevant literature

Hematological parameters	Units	Dubrovnik Pramenka[1] (mean)	Zeta Žuja[2] (mean)	Lika Pramenka[3]	Dalmatian Pramenka[4]
Leukocytes	10^9/L	8.11	8.05	5.6-17.0	3.02-15.64
Eritrocytes	10^{12}/L	9.53	8.97	6.6-9.9	7.81-12.77
Platelet	10^9/L	195.70	404.10	75-807	0-731
Hemoglobin	g/L	107.40	100.30	74.55-104.7	78.1-134.7
Hematocrit	%	0.43	0.38	0.22-0.31	0.24-0.41
MCV	fL	45.72	42.15	28.80-35.80	28.60-34.80
MCH	Pg/L	11.32	11.19	9.9-11-9	9.54-11.00
MCHC	g/L	248.00	266.30	320-353	284-362

[1,2] *Antunović et al. (2014)*; [3] *Shek Vugrovečki et al. (2017);* [4] *Šimpraga et al. (2013)*

Variations in the number of leukocytes and platelets, between individual strains of Pramenka, can be associated with pregnancy. It is known that the number of leukocytes decreases in pregnancy, which is related to the involution of the uterus (*Antunović et al., 2011*). Also, the same group of authors report a lower platelet count in highly pregnant sheep compared to non-pregnant and lactating sheep. The analysis of the differential blood picture, in terms of the relative share of lymphocytes (59.25%), shows similarity with the results of *Antunović et al. (2011)* in Dubrovnik ruda sheep (57.40%), which is an indicator of the immunomodulatory response of the organism, initiated by pregnancy, considering that Pirot pramenka and Dubrovnik sheep were in the second half of pregnancy. Eosinophils are an important indicator of parasite infestation, which correlates their high value with increased resistance to endoparasites (*Pfeffer et al., 1998; Balic et al., 2000).* Erythrocytes or red blood cells (RBCs) are responsible for oxygen transport, carbon dioxide and hydrogen ion buffering. They have a diameter of 3.2-5μm and a lifespan of 70-150 days (*Bórnez et al., 2009; Šimpraga et al., 2013*), do not aggregate and deform as easily as erythrocytes of others species. Erythrocyte indices (MCV, MCH, MCHC) provide information on average cell size, hemoglobin content and concentration (*Polizopoulou, 2010*). The values for MCH and MCHC in our studies are relatively high. Higher values for MCH may indicate the presence of reticulocytes or hemolysis. MCHC is considered to be the most accurate erythrocyte constant and can be increased in the case of hemolysis (*Polizopoulou, 2010*). Table 3 shows the biochemical parameters of Pirot Pramenka blood. In general, biochemical analyzes are an important part of laboratory diagnostics that assess the work of internal organs, measure electrolyte levels and identify enzyme concentrations (*Čepelak and Strauss, 2009*), and above all reflect the nutritional status of the individual animal. In order to monitor the health and nutritional status of sheep, it is necessary to establish reference physiological values, which is of particular importance in the case of endangered breeds. Knowing the quality of some biochemical parameters could improve our knowledge about the specifics of sheep metabolism, but at the same time the production abilities of this endangered population. Blood biochemical parameters are the most important indicators used to determine the energy, protein, enzyme, hormone and mineral profile, as well as to assess nutritional status, milk production and animal health (*Antunović et al., 2011; Milošević-Stanković, 2020*).

Table 3. Biochemical parameters of blood in the population of Pirot pramenka

Biochemical parameters	Unit	Mean ± SD	Minimum	Maximum	Reference value
Total protein	g/L	72.09±2.86	68.40	77.40	59.0-78.0
Albumin	g/L	31.64±1.60	29.10	34.50	27.0-37.0
Globulin	g/L	40.45±2.22	37.40	43.00	32.0-50.0
Urea	mmol/L	4.57±0.70	3.40	5.70	3.70-9.30
Creatinine	µmol/L	59.18±8.59	47.50	73.90	75.8-174.3
Glucose	mmol/L	3.05±0.59	2.40	4.00	2.40-4.50
Total bilirubin	µmol/L	2.46±1.50	1.30	6.20	0.70-8.60
AST	IU/L	117.67±9.76	101.10	130.50	49.0-123.3
GGT	IU/L	41.19±10.01	20.50	53.90	19.60-44.10
CK	IU/L	46.16±7.03	16.3	67.50	7.70-101.0
Cholesterol	mmol/L	1.99±0.36	1.50	2.60	1.10-2.30
Calcium	mmol/L	2.49±0.11	2.40	2.70	2.30-2.90
Phosphate	mmol/L	1.28±0.22	1.00	1.60	1.30-2.40
Magnesium	mmol/L	0.86±0.06	0.80	1.00	0.8-1.10

For the analysis of sheep's protein supply, a combination of different indicators in the blood is used, which includes determining the concentration of total proteins, albumin, urea, creatinine and the activity of creatine kinase enzymes. All of these parameters, except creatinine, ranged in the physiological range. Creatinine is an indicator of kidney function. The literature states that creatinine excretion in sheep averages about 10.7 mg, with variations of 5.0-13.6 mg creatinine/kg body weight/day (*Liu and McMeniman, 2006*), depending on the type of diet and energy : protein ratio. At the optimal E : P ratio, lower creatinine values are most often the result of dehydration of the animal, due to reduced water intake. The established content of total proteins of 72.08 g/L is within the reference values. In the sheep's body, proteins are constantly synthesized and broken down. In healthy animals, a balance is established between the intake and synthesis of amino acids, on the one hand, and the breakdown and excretion of excess nitrogenous substances, in the form of urea, on the other hand. The obtained average urea concentrations of 4.57 mmol/L do not deviate from the given physiological values, but are lower compared to the values stated by *Shek Vugrovečki et al., (2017)* in the research on Lika Pramenka (6.7-10.9 mmol), which are conducted in the summer period. Of the total plasma proteins, 52-62% are albumins and represent a reserve of proteins in the body. Since albumins have the ability to reversibly bind many organic compounds, they represent an important transport system in blood plasma (*Jovanović, 1983; Milošević-Stanković, 2020*). Total albuminemia ranged from 29.10-34.50 g/L, which is in the reference range, but is slightly higher than the results of *Kramer (2000)* and *Kaneko et al. (2008),* who report values of 24-30g/L. The decrease in albumin concentration in the metabolic profile should always be observed together with the urea concentration. If the urea concentration is normal or elevated in hypoalbuminemia, then it is probably liver disease, while if hypoalbuminemia is associated with low blood urea concentration, then it is a protein deficiency in the diet (*Kaneko et al., 2008*). The concentration of glucose in the serum of Pirot Pramenka ranged from 2.4-4.0 mmol/L, which does not deviate from the given reference values, but is somewhat higher in relation to the results of research by *Antunović et al. (2009)* and *Shek Vugrovečki et al. (2017).* The "mirror" of liver function are the enzymes: AST (aspartate amino transferase), GGT (gamma glutathione transferase). CK (creatine kinase) also plays an important role. Analysis of enzymes determined in the serum of the examined sheep shows that their values range within physiological values: 117.67, 41.19, 46.16 IU/L, respectively. Normal values for these enzymes, in the relevant literature, are higher compared to our results, which may be due to the chemical composition and nutritional value of nutrients from different areas. The variability of nutrient composition is related to climate, nutrient type, soil type and climatic conditions defined by different altitudes (*Hrković et al., 2009*). The established cholesterol content of 1.99 mmol/l is in accordance with our and reference values stated by other authors, in other strains of pramenka (Dubrovnik, Lika). Cholesterol concentration is a direct reflection of the physiological status of animals, so in the late phase of pregnancy, its content may be higher compared to the lactation stage *(Antunović et al., 2002*). Cholesterol is a precursor of vitamin D, steroid hormones and bile acids, and is an integral part of cell membranes. It enters the body through food or is synthesized in the body itself, mainly in the liver. Cholesterol metabolism is physiologically in a state of equilibrium. Depending on whether the body receives a higher or lower amount of cholesterol (exogenous) through food, more or less endogenous cholesterol will be synthesized in the liver. Synthesis in the liver is stimulated by its reduced level in the blood and insulin, and is inhibited by elevated plasma cholesterol, glucagon and glucocorticoids (*Bruss, 1997)*. The concentration of calcium in sheep serum averaged 2.49 mmol/L and was within the reference values, while phosphorus levels (1.28 mmol/L) were below the physiological range. Hypophosphatemia in the serum of Pirot Pramenka is most likely the result of reduced alimentary intake, which is in accordance with the results of *Stefanović et al. (2015)* who conducted research on Karakačan Pramenka. Similar results are stated by *Antunović et al. (2009)* who in research on Dubrovnik pramenka, record the concentration of inorganic phosphorus in the blood from 1.05-1.75 mmol/L, which can be linked to phosphorus deficiency of pastures. Also, the reduced level of mineral elements in the blood may be a consequence of the inability of animals to fully utilize the mineral substances from the diet, which results in a milder form of deficiency, which is not manifested as a major metabolic disorder (*Hrković et al., 2009*). The concentration of magnesium in the blood plasma ranged from 0.80 to 1.10 mmol/l, and the obtained values in the serum of the examined sheep were from 0.80-1.0 mmol/l.

Table 4. Intervals of variations of biochemical parameters of different strains of Pramenka as reported in the relevant literature

Hematological parameters	Units	Dubrovnik pramenka[1]	Karakačan[2]	Lika pramenka[3]	Dalmatian pramenka[4]	Textbooks[5]
Total protein	g/L	72.70-86.70	49.00-76.00	66.7-91.0	66.8-87.4	60-79
Albumin	g/L	31.70-32.80	26.50-47.30	35.4-47.5	28.5-44.7	24-30
Urea	mmol/L	4.30-5.60	-	6.70-10.9	3.50-7.80	2.86-7.14
Creatinine	µmol/L	86.0-97.0	-	74.5-103.2	98-144	106-168
Glucosa	mmol/L	2.60-3.30	-	1.40-3.70	2.90-4.30	2.78-4.44
Total bilirubin	µmol/L	0.01-4.00	-	5.00-11.00	-	1.71-8.55
AST	IU/L	-	65.00-172.00	110.7-241	66.2-129.3	60-280
GGT	IU/L	9.00-69.00	25.60-86.90	14.3-80.0	31.7-71.7	20-52
Cholesterol	mmol/L	1.90-2.70	-	0.74-2.47	-	1.35-1.97
Calcium	mmol/L	2.92-3.14	2.15-3.22	2.15-2.76	-	-
Phosphate	mmol/L	1.05-1.75	0.89-2.18	1.31-2.39	-	-

[1]Antunović et al. (2009); [2]Stevanović et al. (2015); [3]Shek Vugrovečki et al. (2017); [4]Šimpraga et al. (2013); [5]Kramer (2000) and Kaneko et al. (2008)

Deviations of some parameters in relation to the reference values, from the relevant literature, are most likely a consequence of the feeding conditions of the animals. Therefore, we believe that in order to better understand the characteristics of the metabolic profile of Pramenka, it is necessary to expand the research to the examination of the floristic composition of the soil at the places of cultivation of individual strains.

Conclusion

Pramenka (Zapfel) belongs to the autochthonous (locally adapted) breeds of sheep that represent a unique genetic heritage created thousands of years ago and an important element of the regional agro-biodiversity, tradition and cultural heritage of Serbia. Pirot Pramenka is the most endangered population of sheep in Serbia. It has produced several important national brands: Pirot lamb, Pirot carpet and Pirot hard cheese, which indicates that its preservation is imperative. Therefore, it is necessary to explore the possibility of maximum production, while monitoring the metabolism of nutrients in the body and maintaining health. The key indicators of the nutritional status as well as the health condition of the individual animal are the metabolic profile and the hematological picture of the sheep. In the available literature, there are no data on hematological and biochemical parameters of Pirot pramenka. Hematological indicators of Pirot Pramenka are in reference intervals for sheep, which indicates good animal health status. Biochemical parameters of Pirot pramenka blood were within defined physiological limits, except for creatinine and phosphorus levels, which were below the lower limit, which is a consequence of dehydration and reduced alimentary intake.

Hematološki i biohemijski parametri krvi pirotske pramenke - ugrožene populacije ovaca

Dragana Ružić-Muslić, Bogdan Cekić, Ivan Ćosić, Ivan Pavlović, Nevena Maksimović, Violeta Caro Petrović, Zorica Bijelić

Rezime

Pirotska ovca je visoko ugrožena autohtona populacija ovaca u Srbiji, od koje potiče nekoliko važnih nacionalnih brendova: pirotsko jagnje, pirotski ćilim i pirotski kačkavalj.U dostupnoj literaturi, nema podataka o hematološkim i biohemijskim parametrima krvi ove populacije.

Cilj ovog rada, koji predstavlja početak istraživanja, je utvrđivanje navedenih pokazatelja u krvi pirotske pramenke, što će doprineti očuvanju ove populacije. Obzirom da rasa utiče na vrednost parametara krvi, dobijeni rezultati će poslužiti za definisanje referentnih intervala za ovu populaciju i upoređivanje sa drugim.Ispitivanjem je obuhvaćeno 30 klinički zdravih ovaca,starosti 2-3 godine, sa područja Stare planine. Uzorci krvi uzimani su punkcijom v. jugularis iz kojih je, nakon koagulacije i centrifugovanja, izdvojen krvni serum. Hematološke analize su izvršene u naredna 24 sata na analizatoru Siemens Advia 120.Određeni su sledeći parametric:ukupan broj leukocita, eritrocita, trombocita, sadržaj hemoglobin,vrednost hematokrita, MCV,MCH,PLT i MPV.Biohemijske analize krvi su izvršene korišćenjem Olympus AU 400 analizatora.Utvrđeni su sledeći parametri:ukupan protein,albumin, globulin, urea, kreatinin, glukoza, ukupan bilirubin, holesterol. Od mineralnih materija su određeni: kalcijum,fosfat i magnezijum, a od enzima:AST,GGT i CK.Deskriptivna statistička analiza je urađena korišćenjem statističkog paketa STATISTICA(Version 8). Hematološki pokazatelji pirotske pramenke su u referentnim intervalima za ovce što ukazuje na dobar zdravstveni status grla.Biohemijski parametri krvi pirotske pramenke su se kretali u definisanim fiziološkim granicama, osim nivoa kreatinina i fosfora, koji su bili ispod donje granice, što je posledica dehidratacije i smanjenog alimentarnog unosa. U skladu sa dobijenim rezultatima, neophodno je izvršiti korekciju obroka.

Ključne reči: pirotska pramenka, hematološki parametri, biohemijski pokazatelji

Acknowledgement

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022

References

ANTUNOVIĆ Z., NOVOSELEC J., SAUERWEIN H., ŠPERANDA M., VEGARA M. (2011): Blood metabolic profile and some of hormones concentration in ewes during different physiological status. Bulgarian Journal of Agricultural Science, 17(5): 687-695.

ANTUNOVIĆ Z., MARIĆ I.,STEINER Z., VEGARA M., NOVOSELEC J. (2011): Blood Metabolic Profile of the Dubrovnik Sheep - Croatian Endangered Breed, Maced. J. Anim. Sci. 1, 35-38.

ANTUNOVIĆ Z., NOVOSELEC J., ŠPERANDA M.,VEGARA M., PAVIĆ V., MIOČ B., DJIDARA M. (2011):Changes in biochemical and hematological parameters and metabolic hormones in Tsigai ewes blood in the first third of lactation. Archives Animal Breeding, 54(5): 535-545.

ANTUNOVIĆ Z., SENIĆ Đ., ŠPERANDA M., LIKER B. (2002): Influence of the season and the reproductive status of ewes on blood parameters. Small Rum. Res., 45, 39-44.

BALIC A., BOWLES V.M., MEEUSEN E.N.T. (2000): The immunology of gastro-intestinal nematodes in ruminants. Adv Parasitol. 45: 181-241.

BAUMGARTNER W., WITTEK T. (2017): Klinische Propädeutik der Haus- und Heimtiere, p 424, ISBN: 9783132402744.

BÓRNEZ R., LINARES M. B., VERGARA H. (2009): Haematological, hormonal and biochemical blood parameters in lamb: effect of age and blood sampling time. Livest. Sci., 121: 200-206.

BRUSS M.L. (1997): Lipids and Ketones.U:Clinical biochemistry of domestic animals, 5th ed. (J. J. KANEKO, J. W. HARVEY, M. L.BRUSS, ur.) Academic Press. San Diego, 83 – 115.

CEKIĆ B., PETROVIĆ P.M., RUŽIĆ MUSLIĆ D., MAKSIMOVIĆ N., CARO PETROVIĆV., ŽIVKOVIĆ V., MARINKOVIĆ M. (2018): Genetički resursi u ovčarstvu i kozarstvu centralne Srbije. Selekcija i semenarstvo, XXIV, 1. doi: 10.5937/SelSem1801047C

ČEPELAK I., ŠTRAUS B. (2009): Uvodni dio u Štrausova medicinska bikemija. Medicinska naklada – Zagreb, 6.

DIAS I.R, VIEGASC A., SILVA A.M., PEREIRA H.F., SOUSA C.P., CARVALHO P.P., CABRITAP.J., FONTESS.R., SILVAJ M., AZEVEDOT (2010): Haematological and biochemical parameters in Churra-da-TerraQuente ewes from the northeast of Portugal. Arq. Bras. Med. Vet. Zootec. 62, 265-272.

DOMESTIC ANIMAL DIVERSITY INFORMATON SYSTEM (2012): http://dad.fao.org/

GORKHALI N.A., HAN J.L., MA Y.H. (2015): Mitochondrial DNA variation in indigenous sheep (Ovis aries) breeds of Nepal. Tropical Agricultural Research, 26(4): 632.

GUYTON A. C., HALL J. E. (2006): Blood cells, immunity and blood clotting in: Guyton and Hall Textbook of Medical Physiology (11th ed.). Philadelphia: Elsevier Saunders., 429 – 457.

HERDT T.H., RUMBEIHA W., BRASELTON W.E. (2000): The use of blood analyses to evaluate mineral status in livestock. Vet. Clin. North Am. Food Anim. Pract., 16, 423–444.

HRKOVIĆ A., HODŽIĆ A., HAMAMDŽIĆ M., VEGARA M., SARIĆ ZAHIROVIĆ A., JUHA S., PAŠIĆ E, KRNIĆ J. (2009): Karakteristike biokemijskog statusa bosansko-hercegovačke pramenke. Krmiva, 51, 2, 117-128.
JOVANOVIĆ J.M., STAMATOVIĆ S., ŠAMANC H., IVANOV I., RADOJIČIĆ B., ARSIĆ B., JONIĆ B., GLIGORIJEVIĆ M. (1983): Izučavanje značajnih parametara za dobijanje metaboličkog profila u ovaca. Veterinarski glasnik, 37 (8), 575-586.
KANEKO J., HARVEY J.W., BRUS M.L. (1997): Clinical Biochemistry of Domestic Animals. Academic Press, 932.
KANEKO J., HARVEY J.W., BRUSS M.L. (2008): Clinical Biochemistry of Domestic Animals. 6th ed., Academic Press, Inc., San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, 882 – 888.
KLINKON M., ZADNIK T. (1997): An outline of the metabolic profile test (MPT) in small ruminants. Stočarstvo, 51 : 449–454.
KRAMER J.W. (2000): Normal hematology oc cattle, Sheep, and Goats In: Schalm's Veterinary Hematology (B. F. KRAMER, J. G. ZINKL, N. C. JAIN, ur.), 5. iz., Baltimore, Lippincot Williams& Wilkins, 1057-1084.
KRAMER J. W. (2010): Normal hematology of sheep and goats. In: Weiss, D. J. & Wordrop, K.J.,Schalm's Veterinary Hematology. 6th Ed., WileyBlackwell Publishing Ltd, Ames. U.S.A, Iowa. PP. 836-842.
LIU Z.J., McMeniman N.P. (2006): Effect of nutrition level and diets on creatinine excretion by sheep. Small Ruminant Research 63, 265–273.
MILOŠEVIĆ-STANKOVIĆ I., HRISTOV S., MAKSIMOVIĆ S., POPOVIĆ N., DAVIDOVIĆ V., MEKIĆ C., DIMITRIJEVIĆ B., CINCOVIĆ M., STANKOVIĆ B. (2020): Energy metabolism indicators and body condition in peripartal period of Alpine goats. Large Animal Review 26: 13-18.
PFEFFER P.G., DOUH R.J., SHAWT P.K., GATEHOUSE B., RABEL R.S., GREEN C.L., SHIRER W.E., JONAS BISSTE S. (1998): Sequential cellular and Humoral responses in the abomasal mucosa and blood of Romany sheep dosed with Trichostrongylus axei. Internatinal Journal Parasitology, 26: 765-773.
POLIZOPOULOU Z.S. (2010): Haematological test in sheep health management. Small Ruminant Research, 92: 88-91.
PRODANOVIĆ R., KIROVSKI D., ŠAMANC H., VUJANAC I., IVETIĆ V., SAVIĆ B., KURELJUŠIĆ B. (2012): Estimation of herd-basis energy status in clinically healthy Holstein cows: practical implications of body condition scoring and shortened metabolic profiles. African Journal of Agricultural Research, 7, 3, 418-425
RUŽIĆ MUSLIĆ D., BIJELIĆ Z., PETROVIĆ C.V., ŠKRBIĆ Z., CIVIDINI A., BOJKOVSKI D., SIMČIČ M., KOMPAN D. (2015): Conservation of autochthonous sheep breeds in Serbia and Slovenia. Proceedings of the 4th

International Congress New Perspectives and Challenges of Sustainable Livestock Production, Belgrade, 7-9. October 2015, 83-103.

SHEK VUGROVEČKI A., VOJTA A., ŠIMPRAGA M. (2017): Establishing reference intervals for haematological and biochemical blood variables in Lika pramenka sheep. Veterinarski arhiv, 87, 487-499.

ŠIMPRAGA M., ŠMUC T., MATANOVIĆ K., RADIN L., SHEK-VUGROVEČKI A., LJUBIČIĆ A.,VOJTA A. (2013): Reference intervals for organically raised sheep: Effects of breed, location and season on hematological and biochemical parameters. Small Ruminant Res. 112, 1-6.

STATISTICA-StatSoft,Inc. version 8,0. 2008, www.statsoft.com

STEVANOVIĆ M., STOJILJKOVIĆ D., NEDIĆ D., RADOJA V., NIKOLIĆ R., PRODANOVIĆ S., IVANOV S., VUJANAC I. (2015): Variability of blood serum biochemical parameters in karakachan sheep. Biotechnology in Animal Husbandry 31 (1), 55-62.

VAN SAUN R. (2000): Blood profiles as indicators of nutritional status. Proceedings of 18th Annual Western Canadian Dairy Seminar, Red Deer, Alberta, Canada, 1–6 .

VOJTA A., SHEK-VUGROVEČKI A., RADIN L., EFENDIĆ M., PEJAKOVIĆ J., ŠIMPRAGA M. (2011): Hematological and biochemical reference intervals in Dalmatian pramenka sheep estimated from reduced sample size by bootstrap resampling. Vet. arhiv 81, 25-33.

IMPORTANCE, PRODUCTIVITY AND POTENTIALS OF LOCAL SERBIAN SHEEP BREEDS

Bogdan Cekić[1], Dragana Ružić Muslić[1], Nevena Maksimović[1], Violeta Caro Petrović[1], Ivan Ćosić[1], Tamara Stamenić[1], Madlena Andreeva[2]

[1]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[2]Institute of Biology and Immunology of reproduction "Acad. K. Bratanov", BAS, Sofia, Bulgaria
Corresponding author: Bogdan Cekić, bcekic@istocar.bg.ac.rs

Abstract: Due to the political, demographic and economic situation, agriculture in Serbia changed, including also the structure in sheep breeds. Traditional sheep breeding centers with large pasture areas and large number of local breeds and strains are deserted, and, consequently, sheep production focused on meat production, in intensive systems, but in expensive and inadequate ways. This led to decreasing number in local breeds (pramenka) with its strains. In this research, two most numerous pramenka strains were observed, Sjenica and Svrljig strains, concerning their number, productive and reproductive parameters. Population of registered and active heads in 2020. was 139,319 for Sjenica and 19585 for Svrljig strain. Weight on birth for Sjenica and Svrljig lambs were 3.20 and 3.15, while weight after 30 days and on weaning was 11.06, 11.42 and 27.02 and 24.97, respectively. Lambing index was 1.27 for Sjenica and 1.29 for Svrljig strain. Svrljig strain had longer lactation (104 vs. 88 days), with higher milk production (64.05 kg vs. 55.01 kg), but protein content was higher in Sjenica sheep's milk (5.08% vs. 4.19%). Besides their importance on agro-biodiversity and genetic pool, these strains are best adapted for breeding conditions that are in hilly and mountainous parts of Serbia. As triple production strains, their milk, meat and wool could be used in processing to traditional, high-quality products with additional value, so can provide extra income for the farmers which leads to survival and sustainable development of rural areas.

Key words: Indigenous breeds, genetic resources, sustainable development, milk, wool

Introduction

The changes in the political and economic situation that have affected Serbia in the last few decades have led to a series of adjustments in the agricultural sector, including the breeding of small ruminants. Due to the pronounced trend of deagrarization and demographic depopulation of the villages, there was a decrease in the number of sheep *(Petrović et al., 2011; Petrovic et al., 2013)*. Traditional sheep breeding centers in the hilly mountainous area have been deserted, and small ruminants have moved to lower areas. Under the influence of western European trends, sheep are raised in certain lowland areas, primarily for meat production, but in an expensive and inadequate way. Large financial resources are given for the import of different breeds of sheep. This neglects the fact that this production would be more profitable, and the products are of incomparably better quality in ecologically preserved areas of hills and mountains by growing indigenous populations *(Caro Petrovic, 2014)*. Indigenous breeds are less demanding, sturdier than imported genotypes, and with lower production inputs *(Cekic et al., 2018)*. Also, local sheep breeds are very important as genetic resources. Variability of the breed guarantees the sustainability of production in times of possible climate change, the emergence of new diseases and other reasons why commercial the race will not be able to achieve the expected production *(Stojanović, 2019)*.

The objective of this study was to determine current state, number and level of productivity of two very important local populations from the Pramenka breed group. These are the Sjenica and Svrljig strains, which are also the most numerous local sheep in Serbia. Sjenica strain is named after town of Sjenica, in western part of Serbia, which is characterized with plateau at more then 1000 m above sea level. Population of this strain is widespread mainly on western and south-western part of Serbia. The color of wool is white, but there are specific black patches of hair around eyes, mouth, and half of the ears. Ewes are polled, rams have large and strong horns. The tail is long, often reaching the ground.

Svrljig strain is named after village in south-eastern part of Serbia, characterized with hilly-mountainous landscape. Majority of population is spread in eastern part of Serbia. The color of wool is white, but the hair color can be white with dark and black dots, with black mouth. Most recognizable characteristic for this strain is white tuft of wool above forehead. Ewes are polled, rams can be with or without horns. Tail is also long, reaching the ground.

Both strains are entering reproduction cycle quite late, with more than 12-18 months of age, depending on the breeding conditions. They are finishing growth after 3 years, and they are triple production strains, used for milk, wool and meat.

Material and Methods

Research included active ewes and rams, both Sjenica and Svrljig strains. Number of heads represents the number of animals registered in the main book (Herdbook), which are active and productive in the last five years Number of animals is taken from the Annual Report of the Institute for Animal Husbandry

"Activities report and results of the control of realization of breeding programs in 2020" for sheep in Central Serbia.

All measurements and record keeping were performed in period from 01.10.2018. to 30.09.2019. The live weight of mature animals was measured at the beginning of the mating season, while the live weight of lambs were measured at birth, after 30 days of life and on weaning, which was around 90 days in the case of this genotypes.

The index of lambing is calculated as the index of the total number of descendants based on the total number of animals that gave birth. In addition to these parameters, yield of raw wool were measured, which was done right after the shearing.

Examined milk parameters were: lactation duration, milk yield for lactation after the weaning of lambs, average daily milk production, and average protein content and average milk fat content. Milk control was carried out by a modified absolute (AT) method, carried out at intervals of 28-34 days, alternating in the morning and evening *(ICAR, 2018)*. The first measurement was done after the weaning of lambs, and the milk components (proteins and milk fat) were determined by Ekomilk and Milkoscan apparatus.

The collected data were processed using the statistical package Statistica for Windows *(Stat. Soft Inc. 7)*, and the basic parameters of descriptive statistics (arithmetic mean, minimum and maximum values, standard error of the mean and variation coefficient) were calculated.

Results and Discussion

In table 1 number of active heads of both strains in last five years is shown. Sjenica population is expanding more quickly than Svrljig population. One of the major reason for population growth are stimulative measures of the government in terms of subsidies. Although number of animals under control of productive parameters is increasing, it is still insufficient for stable and constant production. These strains are reared in extensive and semi-extensive systems, where large part of nutrition system in spring-autumn represents grazing on non-cultivated pastures and hay-based nutrition in winter months. Also, the size of flock is still in intervals 10-50 heads per household or farms, which is not enough for demands of the market. The structure of the farms and the current number of heads do not allow a stable distribution of products on the market.

Table 1. Number of active and controlled heads of Sjenica and Svrljig sheep in last five years

Genotype	Year				
	2016	2017	2018	2019	2020
Sjenica strain	32,751	38,313	74,587	105,573	139,319
Svrljig strain	9,314	12,981	14,781	18,166	19,585

Productive parameters of two strains are showed in table 2. Live weight of Sjenica lambs on birth, after 30 days and on weaning were 3.20, 11.06 and 27.02, respectively. *Petrović et al. (2011)* reported similar weights at birth and after 30 days (3.09 and 11.82 kg, respectively), while weaning weight was lower (21.16 kg).

Live weight of Svrljig lambs were 3.15, 11.42 and 24.97, respectively. Although birth weight and weight after one month of age are similar to Sjenica strain, Svrljig lambs were lighter on weaning than Sjenica lambs. Lambing weight was slightly lower than in 3.38 and 3.27 reported in previous researches *(Petrović et al. 2011; Cekic et al., 2018*, respectively), while weight after first month of life was slightly higher than previously reported (10.99 and 10.56, respectively). In research reported by *Caro Petrović et al. (2012)*, slightly heavier lambs on birth was reported with 3.89, but live weight after month was 10.29. Weight on weaning was accordant to 24.65 reported in *Cekic et al. (2018)* but lower than 26.66, reported in *Petrović et al. (2011).* As milk in the first month is the major feed in lambs nutrition, their weight is highly correlated with milk production of their mothers. Both Sjenica and Svrljig lambs are weaned at the roughly age of three months.

Weight of the adult animals is highly correlated with quality of feed and current condition of the animals at the moment of measurement, so this parameter varies the most *(Cekic et al., 2019)*. Weight of adult Sjenica ewes was 58.23 kg, which was higher than 52 kg reported in *Mekić et al. (2007)*, and similar to 57.98, reported in *Petrović et al. (2011)*. Weight of adult Svrljig ewes was 56.31 kg, slightly higher than 54.53 reported in *Petrović et al. (2011)*, and 55.84, reported in *Cekic et al. (2019).*

Yield of raw wool for Sjenica and Svrljig strain was 2.29 and 2.84, respectively. Those results are similar to the previous research, which reported 2.32 and 2.79 *(Cekic et al. 2018; Petrović et al., 2011).*

Table 2. Productive parameters of Sjenica and Svrljig sheep

Genotype	No. of controlled heads	Statistical parameter	Lambing index	Weight on birth (kg)	Weight on 30 days (kg)	Weight at weaning (kg)	Weight of adults (kg)	Wool yield (kg)
Sjenica strain	55,794	Mean	1.27	3.20	11.06	27.02	58.23	2.29
		Min	1.00	2.38	7.23	19.95	42.53	1.41
		Max	1.78	4.04	15.43	32.13	69.00	3.29
		Se	0.02	0.05	0.22	0.35	1.18	0.07
		CV (%)	11.77	11.38	14.80	9.71	14.99	22.84
Svrljig strain	14,157	Mean	1.29	3.15	11.42	24.97	56.31	2.84
		Min	1.09	2.47	8.10	47.64	35.44	1.21
		Max	1.64	3.91	15.42	29.65	66.79	4.45
		Se	0.03	0.07	0.31	0.58	1.10	0.11
		CV (%)	11.62	11.59	14.43	12.45	10.34	21.18

*Mean – arithmetic mean; Min – smallest value; Max – largest value; Se – standard error of the mean; CV – variation coefficient

Lambing index of autochthonous genotypes is on average 1 - 1.3 (Cekic et al., 2018), and values for Sjenica and Svrljig strains were 1.27 and 1.29, respectively, and in line with this values.

Milk production control was performed on a total of 3,825 Sjenica and 3,810 Svrljig ewes, and results are shown in table 3. Lactation lasted on average 88 and 104 days, respectively, and ewes gave an average of 55.01 and 64.05 kg of milk without the amount lambs consumed, and daily production was 0.63 kg and 0.62 kg. Protein and fat content was 5.08% and 6.75% for Sjenica sheep, and 4.20% and 6.71% for Svrljig sheep, respectively.

Table 3. Control of milk production of Sjenica and Svrljig sheep

Genotype	Number of heads	Statistical parameter	Lactation period (days)	Total milk yield (kg)	Daily milk production (kg)	Average milk fat content (%)	Average protein content (%)
Sjenica strain	3,825	Mean	88	55.01	0.63	6.75	5.08
		Min	65	38.32	0.44	5.71	3.08
		Max	136	86.50	0.85	8.20	5.85
		Se	4.34	2.88	0.03	0.21	0.15
		CV (%)	18.77	19.74	17.28	12.47	11.88
Svrljig strain	3,810	Mean	104	64.05	0.62	6.71	4.19
		Min	64	34.64	0.48	3.09	2.44
		Max	144	115.27	1.19	8.39	5.64
		Se	3.33	3.53	0.03	0.23	0.15
		CV (%)	16.61	24.47	22.53	16.67	17.76

*Mean – arithmetic mean; Min – smallest value; Max – largest value; Se – standard error of the mean; CV – variation coefficient

Results for Svrljig strain differ from previous research, where higher parameters were reported *(Mekić et al., 2005)*. Lactation lasted for 165 days, with a yield of 128.2 kg milk consisted from 6.54% milk fat content. Amount of milk production is acceptable, but not sufficient for autochthonous breeds. From the results, it is evident that Svrljig strain had longer lactation than Sjenica strain, but daily production was similar. Sjenica strain showed higher protein content than Svrljig, but fat content was similar. Among the various components of the milk, proteins and fat are of fundamental importance, due to their contribution to the yield, flavour and sensory features of dairy products *(Scintu et al., 2007)*. Variability in milk traits can be exploited in positive direction throughout planed selection and better rearing *(Maksimović et al., 2019)*, and further work is needed to improve those traits.

By processing milk, highly valuable, famous products such as white Sjenica cheese, or Svrljig belmuz could be obtained. On the other hand, Sjenica and Svrljig lamb are famous delicacies. Currently, the wool market is not satisfactory, and the price of wool is very low, it often cannot even cover the cost of shearing. However, wool of local breeds is suitable for the production of traditional products (socks, vests) or souvenirs, which can contribute to added value. All of these traditional, high quality products, provide additional value and extra profit for breeders and contribute to the sustainability of sheep production.

These breeds have triple production (meat, milk and wool).

Conclusion

Population of Sjenica sheep is larger then Svrljig, but their productive parameters are similar, except for the weight of lambs after weaning, which was higher for Sjenica strain. Although there is increasing trend in number of Sjenica and Svrljig sheep, number is still insufficient. Concerning milk production, Svrljig strain was more productive, but average protein content was lower then in Sjenica strain. In comparison to imported, more productive and specialized genotypes, production of these strains is lower, but, given the fact that they are productive in extensive systems, with harsh environmental factors, their production is satisfactory. These strains are best adapted to the conditions in which they are reared, and with very modest inputs, they give high-value products. By processing them, the high-quality, traditional products with additional value are obtained. These strains, as two largest representatives of localy-adapted, autochtonous strains, have a huge importance on the agro-diversity of the Republic of Serbia, but also for the whole Balkan peninsula, and even Europe.

With modest production and insufficient number of animals, productive and processing potential is still low. Work on the popularization of these genotypes and their traditional products is imperative, because they are very important for the survival and sustainable development of rural areas.

Značaj, produktivnost i potencijali autohtonih rasa ovaca u Srbiji

Bogdan Cekić, Dragana Ružić Muslić, Nevena Maksimović, Violeta Caro Petrović, Ivan Ćosić, Tamara Stamenić, Madlena Andreeva

Rezime

Usled političke, demografske i ekonomske situacije, poljoprivreda u Srbiji se promenila, uključujući i rasni sastav ovaca. Tradicionalni ovčarski centri sa velikim pašnim prostranstvima i velikim brojem autohtonih rasa i sojeva su napušteni, i posledično je fokus ovčarske proizvodnje prebačen na intenzivne sisteme proizvodnje mesa, koji su skupi i neodgovarajući. Ovo je dovelo do smanjenja broja pramenki sa svojim sojevima. U ovom istraživanju, praćena su dva soja pramenke: sjenički i svrljiški, uključujući njihov broj, produktivne i reproduktivne parametre. Kontrolisana populacija ovih životinja u 2020. godini je bila 139319 sjeničke i 19585 svrljiške pramenke. Prosečna telesna masa na rođenju jagnjadi sjeničke pramenke je iznosila 3,20, a svrljiške 3,15, dok je telesna masa nakon trideset dana i na odlučenju iznosila 11,06 i 11,42, odnosno 27,02 i 24,97, redom. Indeks jagnjenja je bio 1,27 za sjeničku pramenku i 1,29 za svrljišku pramenku. Svrljiška pramenka je imala dužu laktaciju od sjeničke (104 i 88 dana), veću proizvodnju mleka (64,05 kg i 55,01 kg), ali je sadržaj proteina bio veći kod sjeničke pramenke (5,08% i 4,19%). Pored značaja koji imaju na agro-biodiverzitet i genetički pul, autohtone rase i sojevi su adaptirani na uslove gajenja koji preovladavaju u brdsko-planinskim delovima Srbije. Kao predstavnici trojnog smera proizvodnje, njihovo mleko, meso i vuna mogu da se koriste za dobijanje tradicionalnih, kvalitetnih proizvoda dodate vrednosti, te mogu da obezbede dodatni prihod odgajivačima, što doprinosi opstanku i održivom razvoju ruralnih područja.

Ključne reči: Autohtone rase, genetički resursi, održivi razvoj, mleko, vuna

Acknowledgement

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200022.

References

CARO PETROVIĆ V. (2014): Influence of sheep crossing system on neonatal development yield and quality of lamb meat. Doctoral dissertation. University of Kosovska Mitrovica, Faculty of Agriculture, Lesak.

CARO PETROVIĆ V., PETROVIĆ M.P., ILIĆ Z., MAKSIMOVIĆ N., RUŽIĆ MUSLIĆ D., STOLIĆ N. (2012): Estimation of phenotypic and genetic trends of the growth traits in lipska and svrljig sheep. Biotechnology in Animal Husbandry 28(4):743-749.

CEKIC B., RUZIC-MUSLIC D., MAKSIMOVIC N., CARO PETROVIC V., COSIC I., BIJELIC Z., STANOJKOVIC A. (2019): Productivity of Krivovir strain and its importance on sheep farming in Republic of Serbia. Acta Universitatis Agriculturae et Silviculturae mendelianae brunensis, 67:1427-1431.

CEKIC B., RUZIC-MUSLIC D., MAKSIMOVIC N., CARO PETROVIC V., ZIVKOVIC V., MARINKOVIC M., COSIC I. (2018): Productivity of local sheep and goat breeds in small farm households and their role in sustainability in Central Serbia. In: Volconovici, V. (Ed.), Proceedings of the International Scientific

Symposium Modern agriculture – Achievements and Prospects. Chisinau, Moldova, pp. 10-15.

INSTITUTE FOR ANIMAL HUSBANDRY. 2021. Annual report of activities and results of the control of realization of breeding programs in 2020. Insitute for Animal Husbandry, Serbia.

INTERNATIONAL COMMITTEE FOR ANIMAL RECORDING (ICAR) (2018): Available at: https://www.icar.org/Guidelines/16-Dairy-Sheep-and-Goats.pdf, accessed 25.02.2021.

MAKSIMOVIĆ N., RUŽIĆ-MUSLIĆ D., CARO PETROVIĆ V., PANTELIĆ V., CEKIĆ B., ĆOSIĆ I., LAZAREVIĆ M. (2019): Performance records of autochthonous goats in central Serbia. Biotechnology in Animal Husbandry 35(4):357-365.

MEKIĆ C., LATINOVIĆ D., GRUBIĆ G. (2007): Breeding, reproduction, selection and nutririon of sheep. Monography. Faculty of Agriculture, University of Belgrade, Serbia.

MEKIĆ C., PETROVIĆ M.P., TRIFUNOVIĆ G., ŽUJOVIĆ M., PERIŠIČ P. (2005): Milk production of svrljiška stock of domestic tuft sheep, Biotechnology in Animal Husbandry 21(5-6):85-88.

PETROVIC M., ILIC Z., CARO PETROVIC V. (2013): Sheep and Goat breeding. Institute for Animal Husbandry, Belgrade., 500pp.

PETROVIĆ, M., ILIC, Z., CARO PETROVIC, V., PAVLOVIC, I. (2021): Successful and profitable sheep breeding. Russian Academy of Natural Sciences. Balkan Science Center, Belgrade., 350 pp.

PETROVIĆ M.P., PETROVIĆ M.M., RUŽIĆ-MUSLIĆ D., CARO-PETROVIĆ V., MAKSIMOVIĆ N., ILIĆ Z., VUČKOVIĆ S. (2011): Opportunities and challenges for sustainable sheep production in Serbia. Biotechnology in Animal Husbandry 27(3):463-472.

SCINTU M.F., PIREDDA G. (2007): Typicity and biodiversity of goat and sheep milk products. Small Ruminant Research 68:221-231.

STOJANOVIĆ S. (2019): The state of animal genetic resources in republic of Serbia. Proceedings of the 2nd Symposium “Agrobiodiversity protection and preservation indigenous breeds of domestic animals”, June 28-30, Dimitrovgrad, 2-9.

STUDY OF THE SPERM PARAMETERS OF RAMS' EJACULATES OBTAINED IN THE BREEDING AND NON- BREEDING PERIOD

Rossen Stefanov, Madlena Andreeva

Institute of Biology and Immunology of Reproduction, BAS,1113 Sofia, Bulgaria
Corresponding author: Rossen Stefanov, stefanovrossen@gmail.com

Abstract: The aim of the study is to show some of the main sperm parameters in ejaculates obtained in and out of the reproductive period change. The experiment was conducted with 11 clinically healthy rams of Northeast Bulgarian fine-fleece breed. The collection of ejaculates took place during the natural breeding season (June-July) and out of the natural breeding season (October-November). A total of 22 ejaculates were examined for the experiment. Each ejaculate was tested for indicators - motility, concentration and velocity. A higher percentage of total motility and progressively motile sperm is observed in ejaculates obtained during the breeding season. In ejaculates obtained during the breeding season, the percentage of rapid-moving sperm was higher, while in those from the non-breeding period, a high percentage of slow-moving and medium-moving sperm was observed.

Keywords: ram, spermatozoa, motility, velocity parameters

Introduction

The sheep are seasonal breeders, the beginning of sheep reproduction is regulated by reducing the photoperiod, transformed to a physiological signal by pineal neurological hormone (*Malpaux et al., 1996; Heiliwell and Williams, 1992*). In rams, fluctuations in reproductive capacity are assessed during the different seasons of the year (*Boland et al., 1985)*. Sexual activity in rams changes with seasonality, influenced by changes in light periods, age, and social hierarchy (Mickelsen et al., 1982). It has been proven in them that sexual behavior and sperm quality vary with age and breed (*Aisen, 2004; Pelayo, 2019*). The libido of the ram may be affected more by season than by age (*Aisen and Venturino, 2004*), given the reduced sperm production in weeks and even months outside the reproductive season. Although the ram retains some fertility in the non-breeding season, this period is characterized by a decrease in libido, testicular size, quality and quantity of sperm, which subsequently leads to a period of reduced productivity (*Langford et al., 1987; Lincoln et al., 1990*). On the other hand, sperm quality is also affected by sexual activity or the frequency of the semen collection. The seasonality of reproduction in the ram remains an obstacle in sheep farming systems, as it limits the reproductive potential and thereby the production.

The aim of the study is to show some of the main sperm parameters in ejaculates obtained in and out of the reproductive period change.

Materials and Methods

Animals

The experiment was conducted with 11 clinically healthy rams of Northeast Bulgarian fine-fleece breed. The rams were bred in the Scientific center for agriculture (SCA) -Targovishte. The animals were aged 2-5 years, placed under the same conditions of rearing, feeding and breeding, in accordance with the regulatory requirements. The collection of ejaculates took place during the natural breeding season- of the breed (June-July) and out of the breeding season (October-November). From each ram by the artificial vagina method were obtained two consecutive ejaculates. A total of 44 ejaculates were examined for the experiment. Each ejaculate was tested for indicators - motility, concentration and velocity. All ejaculates were diluted with extender 6A which consist sodium citrate, sucrose and lactose, prepared at the Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov" - BAS.

Sperm Analysis

The analysis of sperm was performed in a specialized laboratory of the Institute of Biology and Immunology of Reproduction, Sofia, Bulgaria. The evaluation of sperm quality parameters - concentration, motility and various kinematic parameters of sperm was performed with the CASA system (Sperm Class Analyzer [SCA] 5.0. Microptic, Barcelona, Spain). Sperm concentration was adjusted to SCA assays by dilution, loaded into a Leja 20 chamber (Leja Products B.V., Nieuw-Vennep, The Netherlands) and examined using a warm-stage microscope 37°C (Nikon, Tokyo, Japan). Velocity parameters that the SCA software measured are:

- Rapid, % - Rapid motility spermatozoa;
- Medium, % - Medium motility spermatozoa;
- Slow, % - Slow motility spermatozoa;

- VCL, μm/s - The curvilinear velocity (The average path velocity of the sperm head along its actual trajectory);

- VSL, μm/s - The straight-line velocity (the average path velocity of the sperm head along a straight line from its first to its last position);

- VAP, μm/s - The average path velocity (the average velocity of the sperm head along its average trajectory);
- LIN,% - The percentage of linearity (the ratio between VSL and VCL);

- STR,% - The percentage of straightness (the ratio between VSL and VAP).

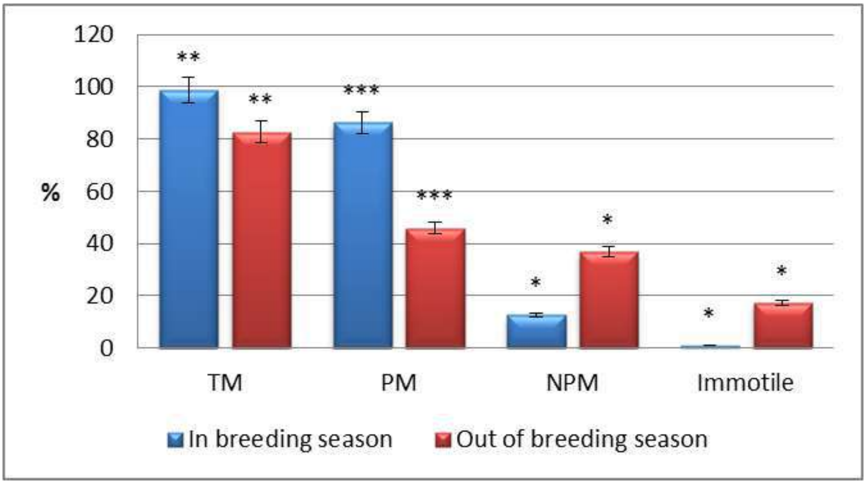
- WOB,% - The percentage of the wobble (which reflects the measure of oscillation of the actual path about the average path);

Statistical analysis

The analysis of all results was performed with a specialized statistical analysis package IBM SPSS Statistics, 23 (SPSSInc., Chicago, USA). The comparison of sperm characteristics was performed by statistical tests of ANOVA. Significance of group differences was assessed with One Sample T-Test. Results were presented as average value ± standard error (Mean ±SE).

Results and Discussion

Figure 1 shows the data on sperm motility in ejaculates obtained in both the reproductive and non-reproductive periods. As with total motility, a high percentage of motile sperm is observed in ejaculates obtained during the breeding season. As with total motility, a high percentage of motile sperm was observed in ejaculates obtained during the reproductive period. As well as in terms of the indicator - progressively motile sperm, and here we have a high degree of reliability ($p \leq 0.001$). Whereas in non-progressively motile and static sperm the percentage is higher in ejaculates obtained in the non-breeding season.



***p ≤0.001 ** p≤0.01 *p≤0.05

Figure 1. Sperm motility in ejaculates obtained during the reproductive and non-reproductive period

Table 1 presents the values for sperm velocity parameters in ejaculates obtained in and out of the reproductive period. Here, highly reliable differences (p ≤0.001) were observed between the percentage of rapidly moving sperm, and this percentage is significantly higher in ejaculates obtained in the breeding season. In terms of the percentage of medium and slow-moving sperm, higher values observed in ejaculates obtained out of the reproductive period. In all other sperm parameters, no significant differences were observed between the breeding and non-breeding periods.

Table 1. Velocity parameters of sperm in ejaculates obtained during the reproductive and non-reproductive period (Mean±SE)

Parameters	In breeding season	Out of breeding season
Rapid, %	78.96±5.89***	18.90±3.18***
Medium, %	16.15±3.62**	49.56±2.97**
Slow, %	4.79±2.56*	25.71±4.78*
VSL, µm/s	27.82±1.10***	31.26±1.19***
VCL, µm/s	102.44±5.15***	115.45±6.72***
VAP, µm/s	59.20±2.20**	60.39±2.79**
STR, %	51.68±0.43***	52.21±0.95***
LIN, %	27.47±0.61*	27.63±1.05*
WOB, %	53.07±0.79*	52.75±0.96*

***p ≤0.001 ** p≤0.01 *p≤0.05

The obtained results confirm the connection established by us in previous studies between sperm motility and the breeding season, namely that the age of rams affects the motility of sperm in the ejaculate only in the non reproductive period (*Andreeva and Stefanov, 2020*). Similar seasonal variations in sperm motility have been found by other authors (*Obrest et al., 2011*) in ejaculates of Lacaune breed.

Regarding the influence of the breeding season on the velocity parameters, our conclusions from previous studies were also confirmed (*Abadjieva et al., 2014*), namely, small differences were observed between the ejaculates obtained in the reproductive and non-reproductive periods.

Conclusion

In ejaculates obtained in and out of the reproductive period, the following differences were observed in some basic sperm parameters.

1. A higher percentage of total motility and progressively motile sperm were observed in ejaculates obtained during the reproductive period.
2. In ejaculates obtained during the breeding season, the percentage of rapid-moving sperm is higher, while in those from the non-breeding period, a high percentage of slow-moving and medium-moving sperm is observed.

Ispitivanje parametara sperme ejakulata ovnova dobijenih u i izvan sezone parenja

Rossen Stefanov, Madlena Andreeva

Rezime

Cilj ispitivanja je bio da pokaže kako se menjaju neki od glavnih parametara spermatozoida u ejakulatu dobijenih u reproduktivnom periodu i van njega. Eksperiment je izveden sa 11 klinički zdravih mužjaka rase severoistočna bugarska ovca. Sakupljanje ejakulata odvijalo se tokom kampanje osemenjavanja (jun-jul) i van kampanje osemenjavanja (oktobar-novembar). U okviru studije su ispitana ukupno 22 ejakulata. Svaki ejakulat je testiran na sledeće indikatore - pokretljivost, koncentraciju i brzinu. Veći procenat ukupne pokretljivosti i progresivno pokretljive sperme primećen je u ejakulatima dobijenim tokom kampanje osemenjavanja. Kod ejakulata dobijenih tokom sezone parenja, procenat brzo pokretnih spermatozoida je veći, dok se kod onih van sezone parenja primećuje visok procenat sporo pokretnih i srednje pokretnih spermatozoida.

Ključne reči: ovan, spermatozoidi, pokretljivost, parametri brzine

References

ABADJEVA D., CHERVEMKOV M., STEFANOV R., METODIEV M., KISTANOVA E., KACHEVA D., RAYCHEVA E. (2014): Effect of breeding season on the kinematic parameters and morphology of ram's sperm from Synthetic population Bulgarian milk sheep breed. BJAS, 20 (4) 967-972.

AISEN E.G. (2004): Reproducción ovina y caprina. 1 ed. Buenos Aires: Inter-medica, pp. 87-98.

AISEN E.G., VENTURINO A. (2004): Recolección y evaluación de semen. En Reproducción ovina y caprina. Ed. Aisen, E. Inter-Medica. Buenos Aires, Argentina. pp. 55 – 69.

ANDREEVA M., STEFANOV R. (2020): Study of relationship between the ace of the rams and the quality of their ejaculates obtained outside the breeding season. Biotechnology of Animal Husbandry, 36 (4) 437-435.

BOLAND M., AL-KAMALIA A., CROSBYA T., HAYNESB B., HOWLESB C., KELLEHERA D., GORDONA I. (1985): The influence of breed, season and photoperiod on semen characteristics, testicular size, libido and plasma hormone concentrations in rams Anim. Reprod. Sci., 9, 241 - 252.

HEILIWELL R. J. A., WILLIAMS L. M. (1992): Melatonin Binding Sites in the Ovine Brain and Pituitary: Characterization During the Oestrous Cycle. Journal of Neuroendocrinology 4, 287–294.

LANGFORD G. A., AINSWORTH L., MARCUS G. J., SHRESTHA J. N. B. (1987): Photoperiod Entrainment of Testosterone, Luteinizing Hormone, Follicle-Stimulating Hormone, and Prolactin Cycles in Rams in Relation to Testis Size and Semen Quality. Biology of Reproduction, 37 (2) 489-499 https://doi.org/10.1095/biolreprod37.2.489 .

LINCOLN G. A., LINCOLN C. E., MCNEILLY A. S. (1990): Seasonal cycles in the blood plasma concentration of FSH, inhibin and testosterone, and testicular size in rams of wild, feral and domesticated breeds of sheep. Journal of the Society for Reproduction and Fertility 22, 623–633.

MALPAUX B., VIGUIÉ C., SKINNER D.C., THIÉRY J.C., PELLETIER J., CHEMINEAU P. (1996): Seasonal breeding in sheep: Mechanism of action of melatonin. Animal Reproduction Science, 42, 109–117 .

MICKELSEN W.D., PAISLY L. G., DAHMEN J. J. (1982): Seasonal variation in scrotal circumference, sperm quality, and sexual ability in rams. J. Am. Vet. Med. Assoc., 181: 376 – 380.

OBERST E. R., SMIRDELE A., BRITO M., MARSCHNER T.R., RIBEIRO L. A., MATTOS R. C. (2011): Variação estacional na qualidade do sêmen de carneiros Lacaune no Brasil. Braz. J. Vet. Res. Anim. Sci., Sao Paulo, 48, 4, 319-324,

PELAYO R., RAMÓN M., GRANADO-TAJADA I., UGARTE E., SERRANO M., GUTIÉRREZ-GIL B., ARRANZ J.J. (2019): Estimation of the Genetic Parameters for Semen Traits in Spanish Dairy Sheep. Animals (Basel), 9(12): 1147.

EFFECTS OF GENOTYPE AND PROTEASE ENZYME SUPPLEMENTATION ON THE PRIMAL CARCASS CUTS OF CHICKENS

Vladimir Dosković[1], Snežana Bogosavljević-Bošković[1], Božidar Milošević[2], Zdenka Škrbić[3], Miloš Lukić[3], Simeon Rakonjac[1], Veselin Petričević[3]

[1] Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia
[2] Faculty of Agriculture, University of Pristina, Kosovska Mitrovica-Zubin potok-Lešak, 38219 Lešak
[3] Institute for Animal Husbandry, Autoput 16, P. Fah 23, 11080, Belgrade–Zemun, Serbia
Corresponding author: Vladimir Dosković, vladosko@kg.ac.rs

Abstract: This study compared weight and percent yields of primal carcass cuts of commercial fast-growing broiler - Cobb 500 and medium-growing broiler genotype - Master Gris. Three hundred Cobb 500 chickens and three hundred Master Gris chickens were used for the experiment. Feed and water were provided *ad-libitum.* Treatments were: T1 - corn-soybean meal based control diet; T2: T1 − 4.0% crude protein than in the control diet + 0.2% protease and T3: T1 − 6.0% crude protein than in the control diet + 0.3% protease. Chickens were slaughtered at 49 days of age. Medium-growing hybrid Master Gris had a lower weight of all primal carcass cuts and breast yield (as a percentage of ready-to-grill carcass weight), as well as higher thighs, drumsticks, wings and pelvis yields in comparison with the Cobb 500 broilers (P<0.05), except back yield (P>0.05). Also, the addition of protease to broiler feed had no significant effect on the weight and percent yields of primal carcass cuts (P>0.05).

Keywords: hybrids, broilers, protease enzyme, primal carcass cuts

Introduction

In the production of chicken meat in recent decades, increasing attention is paid to minimizing the cost of broiler fattening and reducing the environmental pollution. Several factors such as genotype, diet composition, digestible nutrient content, energy to protein ratio, feed form, feed processing, environment, and disease could affect the cost of production and poultry product quality through influencing feed intake, body weight gain and feed conversion ratio (*Ahiwe et al., 2018*).

Today, technologies supplemented by exogenous enzymes (xilanase, phytase, glucanase, protease, amylase,…) in the corn-soybean based diet are most commonly used to achieve these goals. Aiming to increase the efficiency of rations, the usage of exogenous enzymes in the feeding of broilers is gaining more space and has become a great alternative, since it enhances food digestibility, minimizing the anti-nutritional effects and promoting the productivity indexes **(***Hooge et al., 2010*). These enzymes can be included in the diet of livestock to improve digestibility, reduce nitrogen and phosphorus pollution, reduce diet cost, and improve the uniformity of animal growth **(***Cowieson et al., 2019*).

Proteases may have an 'extra-proteinaceous' advantage for broilers reared under high-temperature conditions by improving gut health (*Cowieson et al., 2016*). Single exogenous protease, when added to the diet, effectively increased the apparent amino acid and protein digestibility in the broiler chicken's diet, even in a low protein diet (*Romero et al., 2014; Law et al., 2015; Bertechini et al., 2020; Da Silva et al., 2021,…*). Some researchers (*Sabino et al., 2004; Kamran et al., 2008;…*) have examined the effects of reducing the levels of crude protein in the diet with exogenous enzyme supplementation on carcass yields and cuts of chickens.

The objective of this study was to compare the carcass characteristics and meat quality of a medium-growing broiler - Master Gris with a fast-growing commercial Cobb 500 broiler, both grown in identical housing conditions and fed the same diet (a diet based on maize and soybean meal, with or without protease enzymes).

Materials and Methods

A total of 300 Cobb 500 broiler chickens (1 day old, 44.73 g weight) and 300 Master Gris chickens (1 day old, 43.03 g weight) were used in the experiment. Diets were provided in phases: starter (day 1-21), grower (day 22-35) and finisher (day 35-49). Respective diets (mash feed) and water were provided *ad libitum* from day 1 to 49.

Diet treatments were T1: control diet (based on maize and soybean meal, starter phase 22% crude protein, grower phase 19% crude protein, finisher phase 17% crude protein), T2: T1 − 4.0% crude protein than in the control diet + 0.2% protease and T3: T1 − 6.0% crude protein than in the control diet + 0.3% protease. The enzyme preparation used in this study was a commercial product (Ronozyme ProAct®, produced by DSM, The Netherlands) produced through submerged fermentation of Bacillus licheniformis.

At the end of the experimental period (day 49), a total of 20 birds (10 male and 10 female) were randomly selected from each treatment. Chickens were weighed and slaughtered and then the ready-to-grill carcass weight. After that, the carcasses are cut into primal cuts - breast, drumsticks, thighs, wings, back, pelvis and abdominal fat. Primal cuts is the measured weights and the proportions of primal cuts in relation to the ready-to-grill carcass weight were calculated.

Statistical analysis data were subjected to two-way factorial ANOVA (hybrids, diet treatments). The significance of differences between means was determined by the LSD test and differences were considered significant at P<0.05. Statistical analyses were performed using the Stat Soft Inc Statistica For Windows, Version 7.0., 2006.

Results and Discussion

Table 1 presents weight of primal carcass cuts - breast, drumsticks, thighs, wings, back and pelvis in broilers two hybrids.

Table 1. Effect of hybrids and diet on weights of primal carcass cuts across treatments, g

Hybrid	Diet		Breast	Drumsticks	Thighs	Wings	Back	Pelvis
Cobb 500	T1	$\bar{x}$	798.68^{a}	326.45^{a}	376.97^{a}	261.88^{a}	283.53^{a}	246.21^{a}
		Sd	80.28	43.93	43.89	27.22	33.25	31.64
	T2	$\bar{x}$	791.36^{a}	312.57^{a}	372.29^{a}	252.15^{a}	274.78^{a}	244.96^{a}
		Sd	91.38	34.71	37.13	27.11	51.96	30.86
	T3	$\bar{x}$	764.78^{a}	307.96^{a}	361.19^{a}	253.83^{a}	279.95^{a}	252.22^{a}
		Sd	88.92	40.65	39.29	30.17	34.80	41.06
Master Gris	T1	$\bar{x}$	524.51^{b}	277.48^{b}	303.78^{b}	221.74^{b}	222.64^{b}	211.47^{b}
		Sd	38.66	28.44	31.98	19.49	27.09	16.44
	T2	$\bar{x}$	509.90^{b}	264.66^{b}	293.25^{b}	219.16^{b}	209.10^{b}	200.89^{b}
		Sd	48.88	32.09	26.78	20.41	19.00	18.15
	T3	$\bar{x}$	515.70^{b}	265.04^{b}	297.08^{b}	219.18^{b}	216.55^{b}	205.55^{b}
		Sd	50.58	31.26	28.49	23.41	24.80	22.06
p-value								
Source of variation								
Hybrid			0.000	0.000	0.000	0.000	0.000	0.000
Diet			0.394	0.113	0.347	0.491	0.332	0.552
Hybrid x Diet			0.554	0.921	0.633	0.799	0.950	0.608

$\bar{X}$ -Average, Sd - Standard deviation
a-b Means followed by different superscript letters within columns differ significantly (P<0.05)

The results presented in Table 1 showed a significant effect of genotype, ie hybrid on the weight of primal carcass cuts in chickens (P<0.05). Average weight of primary cuts were higher in broilers Cobb 500, which was expected, according to a higher body mass chickens of hybrids at the end of the experiment. Also, the results of the experiment show that lowering crude proteins levels, with the addition of protease enzymes, did not have a negative effect on examined carcass characteristics (P>0.05). The breast weight of fast-growing broilers (784.94 g) was 1.52 time higher compared to the breast weight (516.70 g) of medium-growth hybrids. *Aksoy et al. (2010)* also reported fast-growing genotypes showed higher breast, legs, wings, back and neck weight than medium growing birds (P<0.001). Commercial fast-growing chickens had higher live weight, carcass, breast and thigh-drumstick weights (P<0.05) compared to medium and slow-growing chickens (*Cömert et al., 2016*).

Data on percent yields of primal carcass cuts (breast, drumsticks, thighs, wings, back and pelvis) the factors analyzed (hybrids, dietary treatments) are given in Table 2.

Table 2. Effect of hybrids and diet on percentage yield of primal carcass cuts of broilers, %

Hybrid	Diet		Breast	Drumsticks	Thighs	Wings	Back	Pelvis
Cobb 500	T1	$\bar{x}$	33.60^{a}	13.69^{b}	15.83^{b}	11.02^{b}	11.92	10.34^{c}
		Sd	1.59	0.87	0.68	0.53	0.80	0.70
	T2	$\bar{x}$	33.93^{a}	13.38^{b}	15.96^{b}	10.79^{b}	11.68	10.47^{bc}
		Sd	3.59	0.90	1.17	0.65	1.46	0.76
	T3	$\bar{x}$	33.24^{a}	13.34^{b}	15.47^{b}	11.02^{b}	12.15	10.91^{b}
		Sd	2.29	0.67	1.19	0.62	0.66	0.96
Master Gris	T1	$\bar{x}$	28.62^{b}	15.10^{a}	16.53^{a}	12.08^{a}	12.11	11.54^{a}
		Sd	1.33	0.56	0.75	0.44	0.88	0.56
	T2	$\bar{x}$	28.90^{b}	14.97^{a}	16.62^{a}	12.42^{a}	11.86	11.40^{a}
		Sd	1.20	0.79	0.45	0.41	0.60	0.68
	T3	$\bar{x}$	28.78^{b}	14.77^{a}	16.58^{a}	12.22^{a}	12.08	11.47^{a}
		Sd	1.25	0.848	0.58	0.49	0.81	0.58
p-value								
Source of variation								
Hybrid			0.000	0.000	0.000	0.000	0.549	0.000
Diet			0.659	0.148	0.388	0.819	0.228	0.195
Hybrid x Diet			0.785	0.845	0.431	0.099	0.782	0.134

$\bar{X}$ -Average, Sd - Standard deviation
a-b Means followed by different superscript letters within columns differ significantly (P<0.05)

The results presented in Table 1 showed that chickens of the fast-growing hybrid had a higher percent yields of breasts, and a lower percent yields of drumsticks, thighs, wings and pelvis compared to the medium-growing genotype (P<0.05), with a similar percent yields of backs (P>0.05). Nutritive applied treatments did not affect the percentage yield of the primary carcass cuts, in addition to the percent yield of the pelvis in the genotype Cobb 500 (significant differences were observed between the groups T1 and T3, P<0.05).

Relative weights of parts to cold carcass weight fast-growing genotypes showed a higher average for breast only whereas medium-growing genotypes had higher values for other parts (*Aksoy et al., 2010*). A similar difference in carcass traits between fast- and medium-growing genotypes was also found *Santos et al. (2004), Fanatico et al. (2008)* and *Dal Bosco et al. (2014).* Higher relative breast weight in fast-growing broiler in comparison with slow-growing broiler were also cited by *Nielsen et al. (2003), Fanatico et al. (2005)* and *Jaspal et al. (2020). Cömert et al. (2016)* published that the effect of the genotype was important for the breast and thigh-drumstick yields (P<0.05), fast-growing hybrids had higher breast yield, whereas medium slow-growing chickens had higher thigh-drumstick yield (P<0.05).

Conclusion

Based on the findings of the present study, it may be concluded that the differences in weight and percent yields of primal carcass cuts between the two hybrids of different intensity of growth were significant, except for the share of the back. Namely, the weights of all primal carcass cuts and breast yield were greater in fast-growth broilers, while the percent yield of drumsticks, thighs, wings and pelvis were greater in medium growth broilers. Also, present results showed that it was possible to reduce crude protein level without any negative effect on the weight and percent yields of primal carcass cuts of commercial fast-growing broiler (Cobb 500) and medium-growing broiler genotype (Master Gris) by adding protease to the diet of broiler chickens.

Uticaj genotipa i dodatka enzima proteaze na karakteristike trupa pilića

Vladimir Dosković, Snežana Bogosavljević-Bošković, Božidar Milošević, Zdenka Škrbić, Miloš Lukić, Simeon Rakonjac, Veselin Petričević

Rezime

U radu je upoređena masa i udeo osnovnih delova trupa komercijalnog brzorastućeg hibrida pilića - Cobb 500 i srednje rastućeg linijskog hibrida - Master Gris. U ogledu je bilo tri stotine pilića Cobb 500 i tri stotine pilića Master Gris. Ishrana i napajanje su bili po volji. Tretmani ishrane su bili: T1 - kontrolni obrok na bazi kukuruza i sojine sačme; T2: T1 − 4,0% sirovih proteina u odnosu na kontrolni obrok + 0,2% proteaze i T3: T1 − 6,0% sirovih proteina u odnosu na kontrolni obrok + 0,3% proteaze. Klanje pilića obavljeno je u uzrastu od 49 dana. Medium-growing hibrid Master Gris je imao manju masu svih osnovnih delova trupa i udeo grudi (u odnosu na masu trupa "spremno za roštilj"), kao i veći udeo bataka, karabataka, krila i karlice u poređenju sa pilićima Cobb 500 ($P<0.05$), izuzev udela leđa ($P>0.05$). Takođe, dodatak proteaze u hranu za piliće nije imao značajan uticaj na masu i udeo osnovnih delova trupa ($P>0.05$).

Ključne reči: hibridi, brojleri, enzim proteaza, osnovni delovi trupa.

Acknowledgment

The research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project ref. number 451-03-9/2021-14 and 451-03-9/2021-14/200022.

References

AHIWE E.U., OMEDE A.A., ABDALLH M.B., IJI P.A. (2018): Managing dietary energy intake by broiler chickens to reduce production costs and improve product quality. Animal Husbandry and Nutrition, Edited by Banu Yücel and Turgay Taşkin, IntechOpen Limited, London, United Kingdom, doi: 10.5772/intechopen.76972, 115-145.

AKSOY T., NARINE D., CUREK D.I., ONENC A., YAPICI N. (2010): Comparison of fast and medium- growing broiler genotypes raised in-doors: growth performance, slaughter results and carcass parts. Journal of Animal and Veterinary Advances, 9, 1485-1490.

BERTECHINI G.A., DE CARVALHO C.C.J., CARVALHO C.A., DALOLIO S.F., SORBARA O.B.J. (2020): Amino acid digestibility coefficient values of animal protein meals with dietary protease for broiler chickens. Transl. Anim. Sci., 4:1-11.

COWIESON A., ZAEFARIAN F., KNAP I., RAVINDRAN V.R. (2016): Interactive effects of dietary protein concentration, a mono-component exogenous protease and ascorbic acid on broiler performance, nutritional status and gut health. Anim. Produ. Sci., 57, 1058-68.

COWIESON A.J., SMITH A., SORBARA J.O.B., PAPPENBERGER G., OLUKOSI O.A. (2019): Efficacy of a mono-component exogenous protease in the presence of a high concentration of exogenous phytase on growth performance of broiler chickens. J. Appl. Poult. Res., 28, 638-646.

CÖMERT M., ŞAYAN Y., KIRKPINAR F., BAYRAKTAR Ö.H., MERT S. (2016): Comparison of carcass characteristics, meat quality, and blood parameters of slow and fast grown female broiler chickens raised in organic or conventional production system. Asian-Australas J. Anim. Sci., 29, 7, 987-997.

DA SILVA J.M.S., DE OLIVEIRA N.R., GOUVEIA A.B.V.S., VIEIRA R.A., DOS SANTOS R.O.F., MINAFRA C.S., DOS SANTOS F.R. (2021): Effect of protease supplementation on the digestibility of amino acids in animal-origin meals for broiler diets. Czech J. Anim. Sci., 66, 29-37.

DAL BOSCO A., MUGNAI C., GUARINO AMATO M., PIOTTOLI L., CARTONI A., CASTELLINI C. (2014): Effect of slaughtering age in different commercial chicken genotypes reared according to the organic system: 1. welfare, carcass and meat traits. Italian Journal of Animal Science, 13, 2, 467-472.

FANATICO A.C., CAVITT L.C., PILLAI P.B., EMMERT J.L., OWENS C.M. (2005): Evaluation of slower-growing broiler genotypes grown with and without outdoor access: Meat quality. Poult. Sci., 84, 1785-1790.

FANATICO A.C., PILLAI P.B., HESTER P.Y., FALCONE C., MENCH J.., OWENS C.M., EMMERT J.L. (2008): Performance, livability, and carcass yield of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci., 87, 1012-1021.

HOOGE D.M., PIERCE J.L., McBRIDE K.W., RIGOLIN P.J. (2010): Meta-analysis of broiler chicken trials using diets with or without allzyme SSF enzyme complex. International Journal of Poultry Science, 9, 9, 819-823.

JASPAL M.H., ALI S., RAJPUT N., NAEEM M., TALPUR F.N., REHMAN I. (2020): Fatty acid profiling and comparative evaluation of carcass cut up yield, meat quality traits of Cobb Sasso, commercial broiler and native aseel chicken. Pure Appl. Biol. (Pab) 9, 56-65.

KAMRAN Z., SARWAR M., NISA M., NADEEM M.A., MAHMOOD M.E. (2008): Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poult. Sci. 87, 468-474.

LAW F.L., ZULKIFLI I., SOLEIMANI A.F., HOSSAIN M., LIANG J.B. (2015): Nutrient digestibility of broiler chickens fed on a low-protein diet supplemented with mono-component proteases. Eur. Poult. Sci., 79, 107.

NIELSEN B.L., THOMSEN M.G., SØRENSEN J.P., YOUNG J.F. (2003): Feed and strain effects on the use of outdoor areas by broilers. Brit. Poul. Sci., 44, 2, 161-169.

ROMERO L.F., SANDS J.S., INDRAKUMAR S.E., PLUMSTEAD P.W., DALSGAARD S., RAVINDRAN V. (2014): Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn- and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. Poult. Sci. 93, 1-13.

SABINO H.F.N., SAKOMURA N.K., NEME R., FREITAS E.R. (2004): Protein levels in broiler feed during the growth phase. Brazilian Agricultural Research 39(5), 407-412.

SANTOS A.L., SAKOMURA N.K., FREITAS E.R., BARBOSA N.A.A., MENDONÇA M.O., CARRILHO E.N.V.M. (2004): Carcass yield and meat quality of three strains of broiler chicken. Proceedings of 22nd World's Poultry Congress, June 8-13, Istanbul, Turkey, pp.338.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

IMPACT OF INORGANIC PHOSPHORUS IN BROILERS DIET ON BONE MINERALIZATION

Marija Pavlović[1], Ksenija Nešić[1], Aleksandra Tasić[1], Nikola Čobanović[2], Mihajlo Vićentijević[1], Ivan Pavlović[1]

[1]Scientific Veterinary Institute Serbia, Janisa Janulisa 14, 11000 Belgrade, Serbia
[2]University of Belgrade, Faculty of Veterinary medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia
Corresponding author: Marija Pavlović, majaspavlovic@gmail.com

Abstract: The trial aimed to evaluate different inorganic phosphorus sources (P) in broilers feed, and its impact on growth, production performances and bone mineralization. 300 one-day-old broilers were randomly divided into the three groups, by one hundred birds in each. During the 42 experimental days birds were fed complete feed mixtures, formulated to meet or exceed the requirements of broilers, for all nutrients except for phosphorus. One group of broilers was fed a diet without the addition of inorganic P (basal diet), and the other two had a two different monocalcium phosphates as a source of inorganic P (MCP 1, MCP 2). Production results were monitored during the trial. At the end of the experiment by 12 birds from each group were slaughtered, tibia and tibiotarsus were collected, and linear bone parameters and bone density (radiodensity) were measured. Both experimental groups that was fed with the addition of inorganic source of P achieved significantly higher production results compared to the group of broilers fed with basal diet (P<0.001). Furthermore, production results, as well as bone tissue density differed significantly between MCP 1 and MCP 2 group (P<0.05). In the same group of broilers (MCP 2) bone length, cross sectional area and cortex thickness were higher, but without statistical significance (P>0.05). As opposed to, bone mass was higher in MCP 1 (P>0.05). The results of the experiment point out the significance of kind of MCP as inorganic P source in feed, and its impact on the ossification of bone tissue.

Key words: broilers, monocalcium phosphate, phosphorus, bone mineralization

Introduction

Phosphorus (P) is mineral involved in many metabolic functions and deposited in bone tissue. About 80% of total P in organism is present in bones, and along with calcium (Ca) has a significant role in bone mineralization (*Hemme et* *al., 2005; Scholey et al., 2018*). High growth rate and body weight gain achieved in short fattening time in poultry emphasize the impact of P from diet on proper body development (*Williams et al., 2000*). Phosphorus in diet is present as phytin P from plants, which is poorly digestible (50-60%) and inorganic P i.e. phosphate P from mineral sources (*Hyun An et al., 2020*). Most phosphate is derived from rock, which is a non-renewable resource, and current global reserves may be depleted in 50-100 years (*Cordell et al., 2009*). It is considered as the third most expensive nutrient after energy and protein (*Shastak and Rodehutscord, 2013*). Inorganic P sources contribute to adequate P supply, because they are highly digestible.

The aim of this study is to evaluate the impact of monocalcium phosphate (MCP), as a source of inorganic phosphorus in broilers diet, on production performances and bone mineralization.

Material and Methods

All the experimental procedures and animal handlings used in this study were in accordance with guidelines of the European Community (Directive86/609/EEC) and guidelines of the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade, Serbia.

The trial included 300 one-day-old broilers (Cobb 500), both sexes that were randomly divided into the three groups, by one hundred birds in each. During the 42 experimental days birds were fed complete feed mixtures that were formulated to meet or exceed the requirements of broilers set by NRC (1994), for all nutrients except for phosphorus (Table 1). One group of broilers was fed a diet without the addition of inorganic P i.e. basal diet (BD), and the other two had two different, commercially available monocalcium phosphates as a source of inorganic P. The MCP was added to diet by replacing the part of corn, at the rate of 14.3 g/kg of feed in starter, and 13.0 g/kg of feed for grower and finisher feed mixtures.

Table 1. Composition of experimental diets

Ingredient [g/kg]	Starter	Grower	Finisher
Corn	541.20	604.5	625.5
Soybean meal	230.0	160.00	100.0
Soy grits	180.0	190.00	230.00
MCP	14.30	13.00	13.00
Salt	3.50	3.50	3.50
Limestone	16.00	14.00	13.00
Mineral-vitamin premix*	15.00	15.00	15.00

* Mineral-vitamin premix does not contain phytase

Production results - average body weight, average body weight gain, feed intake and feed to gain ratio, were monitored during the trial. At the end of the experiment by 12 birds from each group male and female equally, were slaughtered, in order to collect bone samples. On the tibia and tibiotarsus were measured: bone mass, bone length, inner and outer radii of the cross-section at half of the diaphysis length. From obtained values for inner and outer radii, cross sectional area of medullar cavity and cortex thickness were calculated. Bone density was measured by computerized tomography (CT), on the mono-laser scanner for computerized tomography Somatom AR Star (used protocol: 110kV, 105 mA, slice thickness 5 mm) and the tissue density (radiodensity) was expressed in Hounsfield units [HU].

Data were analyzed by using Graph Pad Prism 6.0. Software (Graph Pad Software Inc., San Diego, CA, USA). Individual bird served as experimental unit. All values are expressed as means and standard deviation of means. One-way ANOVA with Tukey’s post test were performed to assess the significance of differences among experimental groups. Levels of $P < 0.05$ were considered as significant.

Results and Discussion

Production results are presented in Table 2. Initial body weight did not differ among groups. At the end of the trial, both experimental groups that received MCP as inorganic P source achieved higher body weight and body weight gain compared to the group fed with basal diet. Furthermore, in the group of broiler that was fed a diet with addition of MCP 2, was recorded higher body weight and body weight gain compared to the group fed with MCP 1 ($P < 0.05$). Feed consumption was the highest in the group MCP 1, and feed conversion was the lowest in the same group of broilers.

Table 2. Production results of broilers (n=100)

	Experimental period	BD	MCP 1	MCP 2	P value (ANOVA)
BW [g]	1. day	47±2.6	48±3.0	48±3.6	0.682
	21. day	505±131.9	854±96.8[a]	824±77.2[b]	< 0.0001
	42. day	1741±454.5	2443±273.5[a]	2519±249.1[b]	< 0.0001
BWG [g]	1-21. day	458±131.1	806±96.8	777±77.3	< 0.0001
	21-42. day	1236±452.8	1585±302.3	1695±260	< 0.0001
	1-42. day	1694±454.4	2396±273.2[a]	2471±249.1[b]	< 0.0001
DFC [g]	1-21. day	57±2.8	74±3.4	76±3.7	< 0.0001
	21-42. day	110.19±3.5[a]	149.6±6.2[b]	153±6.5[b]	< 0.05
	1-42. day	79±3.5	104±4.5	105±4.7	<0.0001
FCR	1-21. day	2.32±0.71	1.60±0.19	1.54±0.23	<0.0001
	21-42. day	1.87±0.61	1.98±0.24	1.89±0.23	<0.0001
	1-42. day	1.97±0.46[a]	1.82±0.2[b]	1.78±0.18	<0.0001

*Values are expressed as means ± SD
Means with different superscript within the row differ significantly a,b...P<0.05
BW-Body weight, BWG-Body weight gain, DFC-Daily feed consumption per bird, FCR-Feed conversion

Body weight, along with feed conversion has been used as a criterion to assess the relative bioavailability of phosphate sources. These parameters represent the indicators of growth rate, which is affected by adequate mineral supply (*Shastak and Rodehutscord, 2013*). Regarding that bioassays based on growth rate can provide the significant information about level of available P, thus can be used as a criterion for evaluation of P sources. Furthermore, broilers are often more sensitive in their performance to differences in mineral supply than other animals because they have low body reserves of minerals and relatively high growth rates (*Jongbloed and Kemme, 2002*). Obtained results point that P sources, as well as its production process and technology can influence the availability of P, which is in line with findings of some other authors (*De Bruyne and Von Felde, 2000; Pavlović et al., 2018*). However, the rate of growth is affected by several different factors, and controlled by several biological or biochemical events, thus growth data only cannot provide adequate information (*Adeola and Cowieson, 2011*). Bone criteria is considered to be the suitable test in evaluation of P availability, because about 80% of total P retention is in the skeleton in growing birds (*Scholey et al., 2018*). The level of biologically available minerals, especially Ca and P in diet, affects the ossification of bone tissue.

Bone length is presented in Table 3. There was significant differences between control group of broilers compared to other two experimental groups for tibia. In tibiotarsus length measured values was significantly higher for MCP 2 experimental group compared to BD group.

Table 3. Bone length and mass of bones (n=12)

	Tibia lenght [cm]	Tibiotarsus leght [cm]	Tibia mass [g]	Tibiotarsus mass [g]
BD	9.253 ± 0.850	6.067 ± 0.360	6.85± 1.19[a]	3.48±1.30[a]
MCP 1	10.650 ± 0.457	6.738 ± 0.558	9.32±1.76[b]	4.51±1.08
MCP 2	11.190 ± 0.661	6.990 ± 0.427	9.00 ± 1.66	5.09±0.75[b]

*Values are expressed as means ± SD
Means with different superscript within the column differ significantly a,b...P<0.05

Tibia mass was higher in MCP 1 group of broiler, and statistically higher compared to BD (P<0.05). As opposed to, tibiotarsus mass was higher, in the MCP 2 group of broiler (P<0.05) (Table 3). Mass of bones depends of individually factors such as growth rate and body weight. Furthermore, mass of bones can be expressed as mass of raw bones or mass of dried and defatted bone. The percent of fats in bone tissue can affect the accuracy of results (*Shim et al., 2012*). From measured linear bone parameters, cross-sectional areas were calculated (Table 4). Obtained values for cortex thickness show the similar trends as production results. The highest cortex thickness was in MCP 2, compared to other two groups, but without statistical significance.

Table 4. Cross sectional areas and bone density at half of diaphysis length (n=12)

Group	Total cross sectional area of tibia[mm^2]	Cross sectional area of medullar cavity of tibia[mm^2]	Cortex thickness [mm^2]	Tibia density [HU]	Tibiotarsus density [HU]
BD	48.76 ± 14.88	16.42 ± 6.35	32.35± 15.72	833.4 ± 166[a]	474±81.01[a]
MCP 1	52.55 ± 10.30	19.59 ± 4.75	32.96 ± 7.34	864.4±81.31[b]	533±52.2
MCP 2	54.58 ± 10.14	19.02 ± 4.97	35.56±10.13	1019±180.9[c]	662.5±149.5[b]

*Values are expressed as means ± SD
Means with same superscript within the column differ significantly a,b,c...P<0.05

Mineral density (i.e. radiodensity) of bone tissue was measured at the half of diaphysis length. Measured values showed similar trends compared with production results and linear bone parameters. Radiodensity of tibia was the highest for group of broilers fed with the addition of MCP 2 and significantly higher compared with the other two groups (P<0.05). Observed differences for tarsus were statistically significant between MCP 2 and BD group (P<0.05) (Table

4). CT allows three-dimensional visualization of a bone structure, consequently allows the volumetric bone density measurement (*Boivin and Meunier, 2002; Korver et al., 2004*). Thus, based on the CT expressed density, the impact of inorganic source on bone mineralization can be estimated. Significant differences of bone tissue radiodensity between BD and MCP groups of broilers, confirm that statement. Furthermore, from here presented results can be concluded that inorganic source of phosphorus has a crucial impact on level of digestibility of P, thus on ossification of bone tissue. MCP used in MCP 1 group of broilers shows the higher rate of phosphorus bioavailability that is expressed through production results and level of bone mineralization.

Conclusion

Type of phosphorus in feed, not only its chemical form, but also kind of inorganic source of phosphorus, has a significant impact on the level of its bioavailability to organism. Availability of phosphorus can affect growth rate, thus production results are considered one of the indicators of phosphorus digestibility. Being deposit in bone tissue phosphorus has a crucial role on bone mineralization, and bone parameters can be used in evaluation of mineral phosphorus sources. These results have applicability to farmers and producers, and can be used for formulating diets.

Uticaj neorganskog izvora fosfora u ishrani brojlera na mineralizaciju koštanog tkiva

Marija Pavlović, Ksenija Nešić, Aleksandra Tasić, Nikola Čobanović, Mihajlo Vićentijević, Ivan Pavlović

Rezime

U ogledu su ispitani različiti neorganski izvori fosfora (P) u hrani za brojlere i njihov uticaj na rast, proizvodne performanse brojlera i stepen mineralizacije koštanog tkiva. U ogled je uključeno 300 jednodnevne brojlerske piladi, nasumično podeljene u tri grupe, od po sto jedinki. Tokom 42 dana ogleda pilad je hranjena kompletnim smešama formulisanim prema potrebama brojlera, koje postavlja NRC (1994) za sve nutrijente, izuzev za fosfor. Jedna grupa brojlera nije dobila neorganski izvor P (kontrolna grupa), a druge dve su dobijale dva različita monokalcijum fosfata kao izvor neorganskog P (MCP 1, MCP 2). Tokom 42 dana trajnja ogleda praćeni su proizvodni rezultati, a 42-og oglednog dana žrtvovano je po 12 jedinki iz svake grupe. Uzorci tibije i tibiotarsus su uzeti, a u cilju merenja linearnih parametara kosti, kao i gustine koštanog tkiva (radio-gustina). Obe

ogledne grupe koje su dobijale neorganski izvor P kroz obrok postigle su značajno veće proizvodne rezultate u poređenju sa kontrolnom grupom brojlera (P <0,001). Dodatno, proizvodni rezultati, kao i gustina koštanog tkiva, značajno su se razlikovali između grupa MCP 1 i MCP 2 (P <0,05). U istoj grupi brojlera (MCP 2) dužina kostiju, površina poprečnog preseka i debljina korteksa bile su veće, ali bez statističke značajnosti (P> 0,05). Nasuprot tome, masa kostiju je bila veća kod MCP 1 (P> 0,05). Rezultati ogleda ukazuju na značaj vrste MCP kao neorganskog izvora P u hrani, i njen uticaj na stepen mineralizacije koštanog tkiva.

References

ADEOLA O., COWIESON A.J. (2011): Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. Journal of Animal Science 89, 3189-3218.

BOIVIN G., MEUNIER P.J. (2002): The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. Calcified Tissue International 70, 503-511.

CORDELL D., DRANGERT J.O., WHITE S. (2009): The story of phosphorus: Global food security and food for thought. Global Environmental Change 19, 292-305.

DE BRUYNE K., VON FELDE A. (2000): Quality feed phosphates – strategies for implementing digestible phosphorus. Kraftfutter/Feed Magazine 5, 210-215.

HYUN AN S., SUNG Y. J., KONG C. (2020): Ileal Digestibility and Total Tract Retention of Phosphorus in Inorganic Phosphates Fed to Broiler Chickens Using the Direct Method. Animals 10, 2167, 1-9.

HEMME A., SPARK M., WOLF P., PASCHERTZ H., KAMPHUES J. (2005): Effects of different phosphorus sources in the diet on bone composition and stability (breaking strength) in broilers. Journal of Animal Physiology and Animal Nutrition 89, 129-133.

JONGBLOED A.W., KEMME P.A. (2002): Terms and Methods to assess and evaluate the bioavailability of minerals for livestock: a general introduction. In: Bioavailability of major and trace elements Eds Jongobloed A.W., Kemme P.A., De Groote G., Lippens M. and Meschy F. EMFEMA, Brussels, Belgium., 5-16.

KORVER D.R., SAUNDERS-BLADES J.L., NADEAU K.L. (2004): Assessing bone mineral density in vivo: Quantitative computed tomography. Poultry Science 83, 222-229.

NRC (1994): Nutrient Requirement for Poultry, (9th ed.). Washington, DC: National Academy Press.

PAVLOVIĆ M., MARKOVIĆ R., RADULOVIĆ S., PETRUJKIĆ B., JOVANOVIĆ D., BALTIĆ M., ŠEFER D. (2018) Estimation of apparent and true total tract digestibility of phosphorus from monocalcium phosphate in broiler diets. European Poultry Science Journal 82, 1-13.

SCHOLEY D.V., MORGAN N.K., RIEMENSPERGER A., HARDY R., BURTON E.J. (2018): Effect of supplementation of phytase to diets low in inorganic phosphorus on growth performance and mineralization of broilers. Poultry Science 7, 7, 2435-2440.

SHASTAK Y., RODEHUTSCORD M. (2013): Determination and estimation of phosphorus availability in growing poultry and their historical development. World's Poultry Science Journal 69, 569-586.

SHIM M.Y., KARNUAH A.B., MITCHELL A. D., ANTHONY N.B., PESTI G.M., AGGREY S.E. (2012): The effects of growth rate on leg morphology and tibia breaking strength, mineral density, mineral content, and bone ash in broilers. Poultry Science 91, 1790-1795.

WILLIAMS B., SOLOMON S., WADDINGTON D., THORP B., FARQUHARSON C. (2000): Skeletal development in the meat-type chicken. British Poultry Science 41, 141-149.

SUGGESTED APPROACH TO RED POULTRY MITE CONTROL IN EXTENSIVE POULTRY PRODUCTION

Aleksandar Pavlićević[1], Ivan Pavlović[2], Nemanja Zdravković[2], Luis Francisco Angeli Alves[3]

[1] Aves Mit Llc, Bajmok, Serbia
[2] Scientific Veterinary Institute of Serbia, Belgrade, Serbia
[3] Western Parana State University, Cascavel Campus, Paraná, Brazil
Corresponding author: Ivan Pavlović, dripavlovic58@gmail.com

Abstract: The suggested control program approach in extensive poultry production is based on establishing technological order and introducing simple mechanical control of *D. gallinae*. Relevant part of the environment is separated from irrelevant and problematic parts by using physical positioning and barriers. Inert oil with long and efficient physical action is used for the barriers. Perches and nests are designed as movable objects, and they exclude overlapping spots and crevices. Separated from the environment, perches and nests provide protection from *D. gallinae* during egg-laying and sleeping. When the poultry does contract *D. gallinae*, the infestation is easily eliminated by mechanical means from the perches and nests, and then the barriers are refreshed in order to stop reinfestation. If necessary, the procedure is repeated. The suggested approach is an opportunity to control *D. gallinae* in extensive poultry production and provides the well-being of poultry, farmers and egg consumers in a simple, long-term and efficient way.

Key words: free range poultry breeding, nest, *Dermanyssus gallinae,* stres

Introduction

The external parasite red poultry mite, *Dermanyssus gallinae* (De Geer, 1778), poses significant health and economic impact on the poultry industry (*Flochlay et al., 2017; Van Emous, 2017*). Although the *D. gallinae* has infested most of farms now days (*Sparagano et al., 2014*), it has had been known in extensive poultry production for ages. About a century old papers about red poultry mite offered better understanding of biology (*Wood, 1917*) advised actions against (*Davidson, 1924)* and considered zoonotic transmission (*Wagner, 1873*). On average, it parasitizes on the host every 3-4 days, in order to feed on its blood. The rest of the time it stays in the environment where it reproduces. Frequent egg-laying and short reproductive cycle give it significant reproductive power. The infestation grows fast so there can be dozens or hundreds of mites per single hen

(*Van Emous, 2005)*. The mite upsets the poultry, causes stress, hinders the development of immunity, aggravates the existing health problems, transmits infectious diseases, and decreases the production results. *D. gallinae* can starve for longer than a year (*Pavlićević et al., 2007a*), but it can also find alternative sources of food. So far, it has been confirmed to parasitize on 30 species of birds and 20 mammals (*Nordenfors, 2000)*. In people, in addition to unpleasant sensation, it also causes localized changes on the skin where it stings, in the form of pruritus, erythematous papulae (*Pavlovic, 2004; Cafiero et al., 2017*). *D. gallinae* develops resistance to acaricides and insecticides (*Abbas et al., 2014*), and it poses a great challenge for all control methods used so far (figure 1).

Figure 1. ***Dermanyssus gallinae (Source: Pavlićević Aleksandar)***

In extensive poultry production, an especially problematic part of the control is the size (per hen) and complexity of the environment, as well as its hygienic circumstances. These circumstances increase the difficulty of control, they demand great effort, and result in low efficacy of control. In such situations, where veterinary medicine does not offer an adequate solution, in addition to regular health damage and economic loss, alternative control measures are emerging from research devoted to D. gallinae and its management (*Sparagano et al., 2014*).

Aim

To suggest a possibility of rational control program of the red poultry mite *D. gallinae* in extensive poultry production.

Situation analysis

Extensive, free range and organic poultry production is significantly different from the intensive one. Key differences are the number of housed poultry (flock size) and the method of housing. Directive 1999/74/EC prohibited use of traditional cages on egg production for poultry birds since 2012 (*Pavličević et al., 2019*). In extensive poultry production, a small number of poultry is allowed to move freely. Poultry sleeps and lays eggs in the poultry house or improvised places. The poultry house is a specially designed or improvised facility where each hen has a large part of the environment, which is complex and hygienically favourable for *D. gallinae* (*Sparagano et al.,* 2009). Red poultry mite control in this environment is difficult, since there is lack of sufficient contact of *D. gallinae* with opposing products and methods. Furthermore, there is plausibility of contact with wild birds, domestic poultry and other animals, which significantly increases the likeliness of reinfestation compared to intensive poultry farming (figure 2).

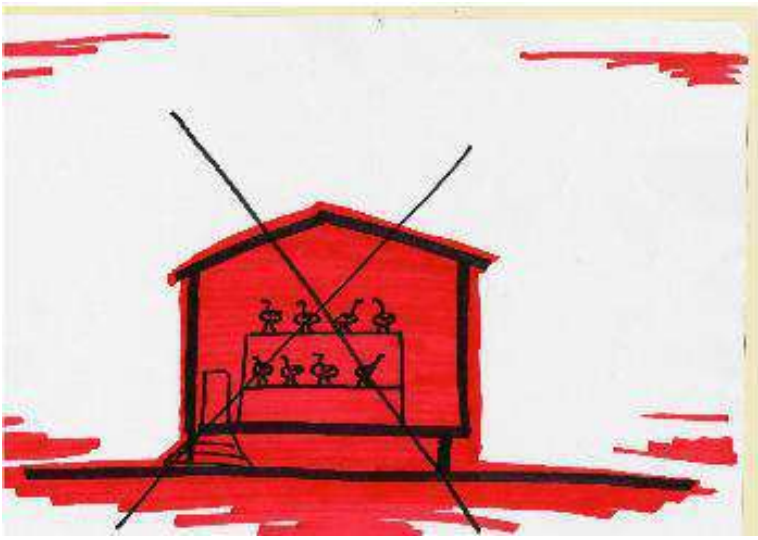


Figure 2. Complexity and hygienic conditions in the environment of extensive production make this problem troublesome, perhaps even impossible to solve by *D. gallinae* control. Therefore, in the control program we start with establishing technological order in the environment *(Source: Pavlićević Aleksandar)*

Suggested solution

In order to solve this seemingly insolvable situation, we will divide the environment into the relevant part which we can control and the other, which is irrelevant and cannot be controlled. Really relevant part of the environment in *D. gallinae* parasitism on layers are the perches where hens can sleep, and nests where they can lay eggs without disruption (figure 3).

Figure 3. Separating perches and nests from the rest of the environment prevents the mites as much as possible from feeding, reproducing growing, and continuing the infestation and adverse effect on the environment. This enables layers to sleep and lay eggs without disruption *(Source: Pavlićević Aleksandar)*

Perches and nests can be separated from the rest of the environment by efficient barriers and by physical separation. We suggest two types of barriers, which can be easily made, even improvised. Support for bearing constructions for perches and bearing constructions for nests are put in deeper dishes, the inner walls and bottoms of which are covered with an inert oil based preparations (suggested product Pulcap® Pulsil, Serbia) or a similar substance with long-term and efficient physical effect. Another, somewhat less practical, possibility is for a bell-shaped barrier to be built into the legs of the bearing construction for perches and nests. Its inner side is also covered with an inert oil or a similar substance with long-term and efficient physical effect (figure 4).

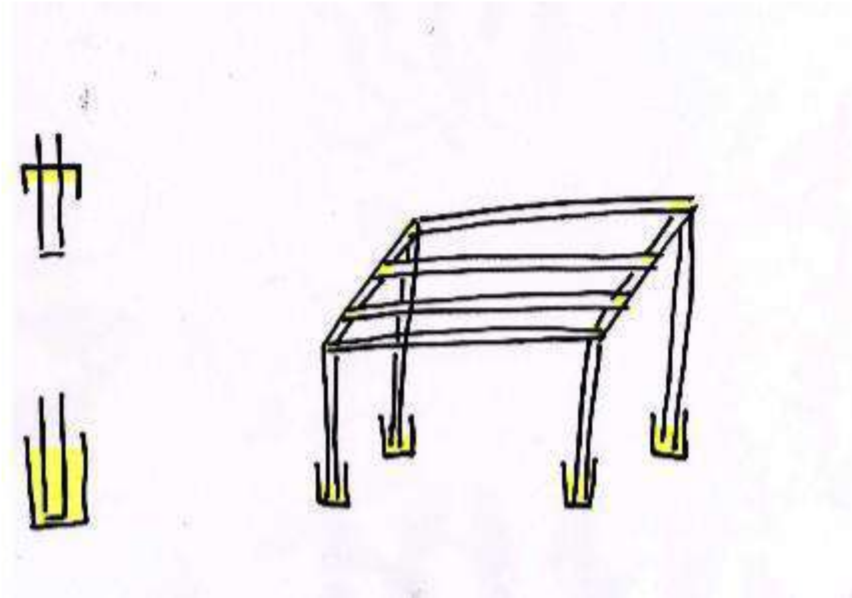


Figure 4. Barriers in the form of dishes or constructions in the shape of bells, with inert oil. In addition to this, spots and crevices need to be closed or avoided during construction ***(Source: Pavlićević Aleksandar)***

Heretofore there have been thought of consonantly barriers before (*Stang and Wirth, 1928*; *Thorsten 2011; Pritchard et al., 2016)*. However, it was important to offer a practical, feasible, and functional solution.

We believe that installing and maintaining bell-shaped barriers would be useful in intensive poultry production as well. For this purpose, already existing washers on bearing construction would have to be modified.

In addition to the barriers, their physical separation is also important. It means perches and nests not leaning against the wall or other objects, as well as the poultry on the perches and nests not touching the walls or other objects. If this condition was not fulfilled, the red poultry mite could go around the barriers and the separation of perches and nests from the rest of the environment would not be successful.

The third requirement is the elimination of overlapping spots and crevices on perches and nests. This can be done during the assembly or subsequently by closing.

The size of perches and nests should enable their occasional removing from poultry houses, in order for them to be washed, cleaned and for the straw in the nests to be changed.

Maintenance

Further procedure of maintenance, or red poultry mite control, in a technologically ordered environment of extensive poultry production is simple. It comes down to occasional mechanical cleaning and washing of perches and nests. In time, the flock will transmit the mites on the perches and nests. When farmers notice smaller clots, or that the mites are disrupting them or the poultry, the perches are washed by boiling water and barriers are refilled with said oil based preparations. The procedure is the same with nests. The straw is removed, they are thoroughly washed, dried, new straw is put, barriers are refreshed and everything is put back in place. In time, the existing red mite infestation will subside in a wider environment and the time span between two control procedures will get longer. If the suggested measures are carefully implemented, we expect the procedure of mechanical *D. gallinae* control to come down to 1-2 times a year (*Pavličević et al., 2007b*).

In case of great intensity and extensity of infestation in the poultry house (+++, ++++), we recommend (just for the beginning) a detailed hygienic preparation of the entire inner environment of the poultry house and systematic application of the chosen inert substances. In this way further pressure on the flock will be decreased, and mechanical control can be continued without disruption.

The very procedure of this approach to red poultry mite control is compatible with other products and methods of *D. gallinae* control, and therefore, if necessary, they can be combined.

Conclusion

In the above described way, by the program approach, we establish technological order in extensive egg production, which provides simple and durable mechanical control of *D. gallinae.* Farmers are able to efficiently control the most significant poultry ectoparasite without substantial expenses. Thanks to this adequate solution, consumers can consume eggs produced in optimal conditions, without the safety risk of residue created by uncritical *D. gallinae* control.

Predlog pristupa kontroli crvene kokošije grinje dermanyssus gallinae u ekstenzivnom živinarstvu

Aleksandar Pavlićević, Ivan Pavlović, Nemanja Zdravković , Luis Francisco Angeli Alves

Rezime

Predlog pristupa programske kontrole u ekstenzivnom živinarstvu zasniva se na uvođenju tehnološkog reda i uspostavljanju jednostavne mehaničke kontrole *D.gallinae.* Fizičkim položajem i barijerama, razdvaja se važan deo ambijenta od nevažnog i problematičnog dela ambijenta. Za barijere se koristi inernto ulje, sa dugotrajnim i efikasnim fizičkim dejstvom. Sedala i gnezda se izrađuju kao pomični predmeti, na kojima su isključena preklopljena mesta i pukotine. Izdvojena od ambijenta, sedala i gnezda omogućuju zaštitu od *D. gallinae* za vreme leženja jaja i spavanja. Kada živina ipak prenese *D. gallinae,* infestacija se lako elimiše mehaničkim putem sa sedala i gnezda, a zatim se osveže barijere kako bi sprečavale reinfestaciju. Po potrebi, postupak se ponavlja. Predlog pristupa je šansa da se jednostavno, trajno i efikasno kontroliše *D. gallinae* u ekstenzivnom živinarstvu, i ostvari dobrobit živine, farmera i potrošača jaja.

Ključne reči: ekstenzivno živinarstvo, gnezda, *Dermanyssus gallinae,* stres

References

ABBAS R.Z., COLWELL D.D., IQBAL Z., KHAN A. (2014): Acaricidal drug resistance in poultry red mite (Dermanyssus gallinae) and approaches to its management. Worlds Poultry Science Journal, 70, 113-124.

CAFIERO M.A., GALANTE D., RAELE D.A., NARDELLA M.C., LOMUTO M. (2017): Outbreaks of Dermanyssus gallinae (Acari, Mesostigmata) Related Dermatitis in Humans in Public and Private Residences, in Italy (2001-2017): An Expanding Skin Affliction. Journal of Clinical Case Reports, 7. https://doi.org/doi: 10.4172/2165- 7920.10001035.

DAVIDSON W.M. (1924): Results of experiments with miscellaneous substances against the chicken mite.Bulletin of the U.S. Department of Agriculture, 1228, 1-16.

VAN EMOUS R. (2005): Wage war against the red mite! Poultry International, 44 (11): 26-33.

VAN EMOUS R. (2017): Verwachtte schade bloedluis 21 miljoen euro. Pluimveeweb. nl. 2017.
https://www.pluimveeweb.nl/artikelen/2017/01/schade-bloedluis21-miljoen-euro/.
FLOCHLAY A.S., THOMAS E., SPARAGANO O. (2017): Poultry red mite (Dermanyssus gallinae)
infestation: a broad impact parasitological disease that still remains a significant challenge for the egg-laying industry in Europe. Parasites & Vectors 10:357.
NORDENFORS H. (2000): Epidemiology and Control of the Poultry Red Mite, Dermanyssus gallinae. Doctoral thesis, Swedish University of Agricultural Sciences.
PAVLIĆEVIĆ A., PAVLOVIĆ I., DOTLIĆ M. (2007a): A contribution to information on starvation survival capacity of poultry red mite Dermanyssus gallinae. Lucrari Stiintifice Medicina Veterinara, 50 (9), 485-491.
PAVLIĆEVIĆ A., PAVLOVIĆ I., STAJKOVIĆ N. (2007b): Metod za ranu detekciju crvenih kokošijih grinja Dermanyssus gallinae (De Geer,1778). Biotehnologija u stočarstvu, 23 (3-4), 119-127.
PAVLIĆEVIĆ A., PAVLOVIĆ I., RATAJAC R., POPOVIĆ D., DAVIDOVIĆ B., KRNJAJIĆ D. (2019): Poultry welfare in terms of poultry red mite (Dermanyssus gallinae).impact and control. Biotechnology in Animal Husbandry, 35 (1), 1-11.
PAVLOVIĆ I. (2004): Skin ectoparasites arachnida, order: Acarina, family Dermanyssidae Demanyssus Gallinae (De Geer). In: P. Karamelo (edit.): Atlas of Medical Parasitology, Carlo Denegri Fondation & Infectious Disease, Unit A, Tropical and Parasitology Service Amedeo di Savoia Hospital Turin, Italy (CD rom), http/www.cdfound. to.it/ HTML/ atlas.htm
PRITCHARD J., KÜSTERA T., GEORGEB D., SPARAGANOC O., TOMLEYA F. (2016): Impeding movement of the poultry red mite, Dermanyssus gallinae. Veterinary Parasitology, 225, 104-107.
SPARAGANO O., PAVLICEVIC A., MURANO T., CAMARDA A., SAHIBI H., KILPINEN O., MUL M., VAN EMOUS R., LE BOUQUIN S., HOEL K., CAFIERO M.A. (2009): Prevalence and key figures for the poultry red mite Dermanyssus gallinae infections in poultry farm systems. Exerimental and Apply Acarology, 48 (1-2), 3-10.
SPARAGANO O. A., GEORGE D. R., HARRINGTON D. W., GIANGASPERO A. (2014): Significance and control of the poultry red mite, Dermanyssus gallinae. Annual Review of Entomology, 59,447-466.
STANG V., WIRTH D. (1928): Tierheilkunde und Tierzucht.Eine Enzyklopädie der praktischen Natztiekunde. Urban & Schwarzenberg, Berlin-Wien.
Thorsten A. (2011): Milbenbekämpfung: Eine unendliche Geschichte? Landwirtschaftszentrum Haus Düsse. (Historisches). www.gefluegelspezialist.de.

WAGNER A. (1873): Ueber das vorkommen des Dermanyssus avium am menschen. Druck von F. Hache. Greifswald.
WOOD H.P. (1917): The chicken mite (Dermanyssus gallinae Redi): its life history and habits. Washington, D.C.: U.S. Dept. of Agriculture.

THE CHALLENGES OF JUMPING HORSES THROUGH THE TRAINING

Maja Gregić, Mirjana Baban, Pero Mijić, Vesna Gantner, Tina Bobić

Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia
Corresponding author: Maja Gregić, mgregic@fazos.hr

Abstract: The jumping sport represents a significant part of the equestrian industry that is largely focused on the realization of economic gain. In order to realise desired competition results, horses need to be adequately trained. Generally, training can be defined as 'a complex process of different activities with a planned and oriented goal of making progress in achieving fitness and oriented movements during work'. Through training, horses need to improve or maintain maximum performance, preparing the horse for competition, delay onset of fatigue, improve skills or work capacity, minimize the incidence of injuries or metabolic disorders, and maintain willingness and enthusiasm for exercise. Furthermore, warming-up represents the unavoidable preparatory step of each training session aiming to prepare the body for the training by increasing the heart rate, enhancing blood supply to tissues, and increasing the flexibility of joints, ligaments, and tendons. Also, horses' trainers and handlers should nurture natural curiosity enabling horses to suppress potential fear of new stimuli and lift the performances in training and breeding. Finally, an adequately trained and bred horse is the one with good results in equestrian sports

Key words: horse, jumping training, warm-up

Introduction

Jumping represents one of the most popular equestrian disciplines. Show jumping is one of three Olympic equestrian sports and represents the most popular equestrian discipline, with the highest number of registered equine and human competitors and competitions within the *Fédération Equestre Internationale* (*Fédération Equestre Internationale, 2021a*). *Ricard and Fournet-Hanocq (1997)* in their analysis of the factors influencing the sporting life of a show horse conclude that poor training will increase the likelihood of a faster termination of a horse's sporting career by 1.6 times. Furthermore, the goal of horse training is to achieve concentration and fitness for a particular equestrian sport, while preserving health, mental and physical condition and longevity in equestrian sports and further exploitation, for example in breeding. Furthermore, *Dyson and Pollard (2021)* determined that the performance and welfare may be improved by recognition and appropriate treatment of underlying problems. Based on research the Ridden Horse Pain Ethogram (RHPE) comprising 24 behaviours was developed in order to facilitate the identification of musculoskeletal discomfort, with scores ≥ of 8/24 indicating the presence of pain. Furthermore, the median RHPE score for 147 competitors at World Cup Grand Prix events from the year 2018 to the year 2020 was 3 (interquartile range 1–4; range 0–7). Horse training generally involves different combinations of physical effort and specific training requirements that depend on the future use of the horse. In jumping equestrian sport, the requirements for the horse are the best possible mastering of the given parkour in the jumping competition. Accordingly, the show horse requires constant concentration, fitness and motivation in training and competition. Still, the selection of individuals and training strategies for the equine jumping athlete are largely based on traditional methods. The aim of this paper was to present in detail the challenges of training a jumping horse.

Development of motoric abilities of the jumping horse

The use of the horse for athletic competition requires conditioning and training for specific varieties of performance. The motility of the jumping horse begins to develop at the earliest age of the foal. At this stage of the horse's life, it is necessary to ensure as many free motoric movements of the developing horse as possible (*Voswinkel, 2009*). Training of the jumping horse begins at the age of three years, i.e. when the horse is fully physically developed. At the age of three, a horse starts jumping its parkour in a free jump for the first time. In free jump, the horse is without equipment and riders, so it shows its exterior and interior in jumping, i.e. physical and mental predisposition for jumping equestrian sport. Furthermore, the use of the horse for athletic competition involves conditioning and training for specific types of performance. With proper training, the motility of the jumping horse gradually develops until the age of eight and then keeps that constant until the age of fifteen. According to *Trailović (2008),* there is a drastic difference between gallop and trotting races to long-distance riding when training with a jumping horse. The specificity lies in the fact that a horse in show jumping must be highly motivated, concentrated and precise. The strong jump strength of a horse alone is not enough. In any equestrian sport, the goal of training is always the same; the horse needs to develop and show its full form with all its genetic potential and show it off in competition. In addition, he must maintain good health and a desire to work without fears and anxiety. The specificity of show jumping lies in the fact that a horse needs many years of adequate training without injuries in order to fully develop its motoric skills and jump precision. Show jumping requires great physical endeavours of the equine athlete, since, at the highest levels, horses jump 10 to 13 obstacles up to 1.70 m high and 2.00 m wide and sometimes racing against the clock (*Fédération Equestre Internationale, 2021b*).

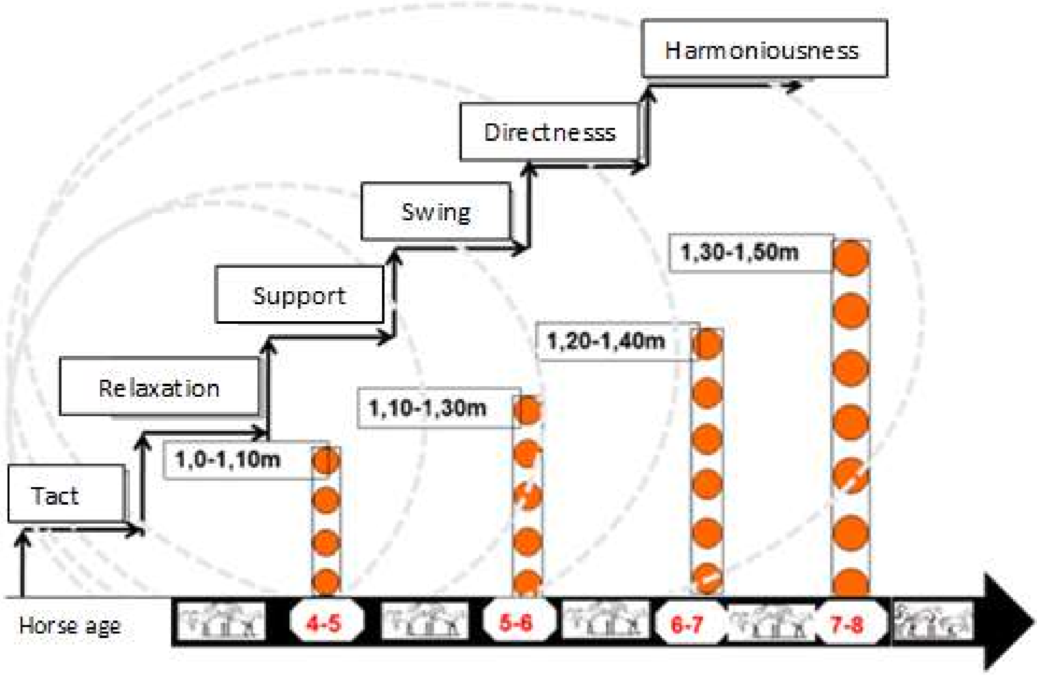


Figure 1. Motoric development of the jumping horse with adequate training, and use in competitions and breeding *(according to Pollmann-Schweckhorst, 2002*)

The above Figure 1 shows the training of horses over the years and makes it a logical sequence of the development of a sport jumping horse through adequate training. In order for a horse to develop accordingly, it is necessary to be consistent in the goals set in training. They are set as short-term and long-term and largely depend on the longevity of horses in sport and breeding. Therefore, several years of technical training and physiological conditioning are needed for a jumping horse to reach its full potential, which relates to significant expenditures of money, resources and time (*Santamaría et al., 2005*)

Development of sport condition of horses

Most of the conditioning programs used now are based on practical knowledge. For instance, different methods of strength training can be used when training horses depending on the set objective (*Castejon-Riber et al., 2017).* In general, training can be defined as 'a complex process of different activities (actions) with a planned and oriented goal of making progress in achieving fitness and oriented movements during work' (*Carl, 1989). Hick and Hick (2000)* defined training as the constant repetition of physical and mental actions with the aim of encouraging the development of adaptations to these actions that would lead to an increase in their effectiveness. Furthermore, *Hohmann (2003)* describes the training as the realization of systematic and planned measures (i.e. the implementation of training content through training methods) for the realization of the led goal of training with a particular sport. Training is also described as goal-oriented, with systematically set tasks and daily organized work to achieve "perfection", i.e. increase physical and motor performance in order to encourage and develop a mechanism of adaptation to higher body loads *(Schnabel et al., 2003). Castejon-Riber et al. (2017)* stated that generally training is organised in order to improve or maintain maximum performance, prepare the horse for competition, delay onset of tiredness, improve skills or work capability, minimize the occurrence of injuries or metabolic disorders and maintain readiness and enthusiasm for exercise. Furthermore, the goal of training is to achieve the best possible adaptation of the body to effort and at the same time improve motor skills. Training leads to the adaptation of the body to strength, speed, endurance and agility (mobility), which will lead to improved fitness and ability to work. In any form of training with a horse, a balance should be found between work and rest so that the health of the sport horse does not deteriorate *(Hintz et al., 2001*). Horse tiredness increases the possibility of mistakes in horse motor skills, which in parkour most often leads to breaking down an obstacle. Furthermore, *Castejon-Riber et al. (2017)* generated principles of training based on scientific literature and their own experience, aiming to avoid or minimize the occurrence of injuries, overcompensation, periodization, and progressive loading. Predictive indicators of future performance are especially important for the equine business, as early selection and/or training methods of jumping horses may facilitate improved competitive performance, career longevity and welfare, as well as decreases in time and economic expenses needed to produce prosperous athletes (*Santamaría et al., 2004*). Training pressure means a gradual increase in motor actions to the capacity of the horse in his sport. The success of a training program depends on the individual horse itself. Its outcomes are unpredictable at the beginning of entering training. Most of all, the success of a horse's training depends on the horse's adaptation to the training requirements in a physical and mental sense. Physical endeavour leads to significant deviations in the function of many organs and organ systems: cardiovascular, blood, bone, muscle, tendon, ligament, and thermoregulatory mechanism. Thanks to the specific adaptation of the organism during training, the organism gradually adapts to the efforts and the dynamics of training becomes more effective, which would gradually reduce the deviation of the individual from the equestrian discipline (*Trailović, 2008*). Most of the scientific papers are related to human training, which is divided into several components. Training of the equestrian horse represents the development of physical and mental genetic potential for achieving a sports result in the equestrian sport. Training improves the movements of horses, develops muscles, achieves better coordination of movements and desirable fitness. With the development of technology, the possibility of monitoring progress in intensive horse training is increasingly being achieved. By training, the horse develops the endurance of the organism for physical effort and its adaptation, which is the basis for the formation of any other physical ability (*Lindner, 1997*). Training, through aerobic exercise, aims to: increase certain motor movements over time without the horse developing fatigue; increase the horse's ability to maintain motor precision and concentration despite signs of fatigue at work; develop and improve the biomechanical qualities of horses; develop and maintain horse motivation in work; optimize the physiological potential of the horse; reduce the risk of injury; stabilize heart function due to exertion (*Rose and Evans, 1990; Clayton, 1991; Brings et al., 1998; Röthing and Prohl, 2003; Voswinkel, 2009*).

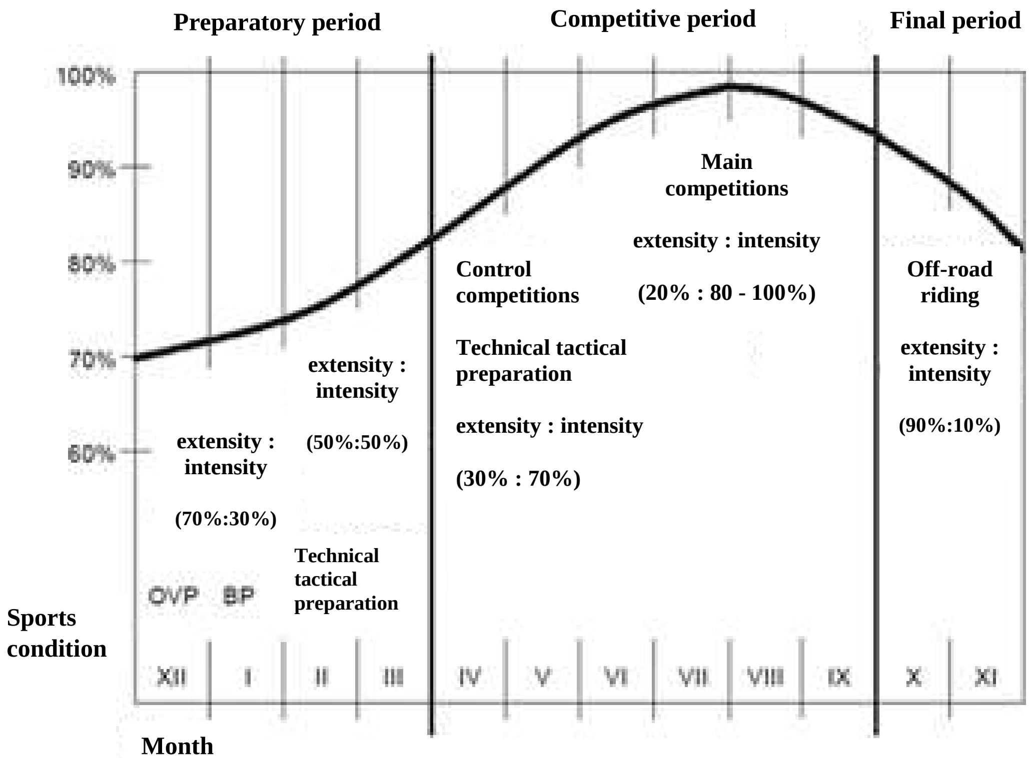


Figure 2. Example of the curve of sports form development in obstacle riding (jumping) in the annual training cycle (according to *Perinović and Milanović, 2013*)

When working with show horses, the daily, weekly and monthly rhythm of training differs according to the competitions in which the horse will participate. In the Republic of Croatia, the competition calendar is created by the Croatian Equestrian Federation (*HKS, 2021; Gregić et al., 2020*). During the year, horses in show jumping go through three periods: preparatory, competitive and final (*Perinović and Milanović, 2013*). Each period requires different fitness training. Horses are subjected to different training intensities to maintain fitness or raise it to the required level. The specificity of jumping requires a specific training plan and program. The preparation period should start in December, in January at the latest and last until the end of March. Equestrian competitions begin in early April and the competition period begins. It is necessary to conduct the first control competitions that serve to get to know more about the physical and mental readiness of horses for competition. The majority of competitions in equestrian jumping sport take place in June and July when the horse is expected to be at maximum fitness (*Perinović and Milanović, 2013*). Activity that adult horses handle seems to affect behavioural and physiological replies to different stimuli, thus influencing equine welfare (*Mendonça et al., 2019*).

Warming-up of horses

A warm-up is a fundamental element of each training session. It aims to prepare the body for the training by increasing the heart rate, enhancing blood supply to tissues, and increasing the flexibility of joints, ligaments, and tendons (*Young, 2007*). Warm-up is recognised as an unavoidable preparatory step of each training session. A warm-up improves body performance and also prevents injuries due to enhanced elasticity of the tendon tissues (*Soligard et al., 2008*). Forelimbs carry about 60% of the horse's body weight and their load increases during cantering and jumps (*Thorpe et al., 2010*). Injuries of hind limb tendons are reported less frequently, but their treatment and rehabilitation are as challenging as with the forelimbs (*Polly, 2019*). currently, there is very little published data on standards on how to warm-up the athletic horses (*Tranquille et al., 2021*). *Janczarek et al. (2021)* concluded that the warming-up effect is achieved earlier and lasts longer in heavily trained horses than in non-performance horses.

Curiosity in jumping horse training

Horses are naturally curious. Curiosity could be defined as s gift in helping a horse develop self-confidence. Curiosity creates confidence in a case when a horse learns and experiences new things, and he will gain more confidence in himself and life in general. Curiosity starts when they are young and discovering the world and continues with them the rest of their life. When any of their senses are stimulated, whether it is the sound of a ruffle in the bush or the sight of an object running towards them, it creates curiosity (*Dines, 2014*). Along with curiosity comes fear. The more you stretch a horse's comfort zone in a structured and controlled way, the more they will overcome their initial flight instincts. Natural curiosity can be suppressed by the way you handle and train your horse, and this can lead to stress and frustration, and also a horse that is unconfident or uninterested and unmotivated. It can even make a horse feel depressed. Horses trainers and handlers should be aware of this natural curiosity and nurture that curiosity instead of suppressing it. The inadequate training and environmental conditions may result in a high level of stress hormones because prolonged high levels of glucocorticoids can negatively affect neurons within the hippocampus. Furthermore, there is a balance between fear and curiosity. Fear is what drives the horse away from something and curiosity draws them back. The introduction of varied objects and obstacles in a structured and controlled way will stretch a horse's comfort zone so they will learn to overcome their initial fear instinct and become curious. Finally, natural curiosity should be nurtured in jumping horses' life and horses training.

Conclusion

The challenges of the jumping horse are extremely huge through training and competition. Jumping represents the most popular equestrian discipline. Through training, the horse needs to improve or maintain maximum performance, preparing the horse for competition, delay onset of fatigue, improve skills or work capacity, minimize the incidence of injuries or metabolic disorders, and maintain willingness and enthusiasm for exercise. The motor abilities of horses are realized through planned and consistent training from the earliest age of horses. The dynamics and complexity of training should be organised in accordance to the competition season and the horse's age. In highly trained horses, warming up is achieved earlier and lasts longer and also injuries are less frequent, than in inadequately trained horses. Finally, jumping sport is accompanied by an equestrian industry that requires economic gain which are higher in sport horses with longer lifespan.

Izazovi treninga preponskih konja

Maja Gregić, Mirjana Baban, Pero Mijić, Vesna Gantner, Tina Bobić

Rezime

Preponski sport predstavlja značajan deo konjičke industrije koji je u velikoj meri fokusiran na ostvarivanje ekonomske dobiti. Da bi se postigli željeni takmičarski rezultati, konji moraju biti adekvatno obučeni. Generalno, trening se može definisati kao „složen proces različitih aktivnosti sa planiranim i orijentisanim ciljem postizanja napretka u postizanju kondicije i orijentisanih pokreta tokom rada“. Obukom, konji moraju poboljšati ili održati maksimalne performanse, pripremajući konja za takmičenje, odgoditi početak umora, poboljšati veštine ili radnu sposobnost, smanjiti učestalost povreda ili metaboličkih poremećaja i zadržati volju i entuzijazam za vežbanje. Dalje, zagrevanje predstavlja neizbežni pripremni korak svakog treninga koji ima za cilj da pripremi telo za trening povećanjem otkucaja srca, poboljšanjem snabdevanja tkiva krvlju i povećanom fleksibilnošću zglobova, ligamenata i tetiva. Takođe, treneri i vodiči konja treba da

neguju prirodnu radoznalost koja omogućava konjima da suzbiju potencijalni strah od novih stimulusa i podignu performanse u obuci i uzgoju. Konačno, adekvatno obučen i uzgojen konj je onaj sa dobrim rezultatima u konjičkom sportu.

Ključne reči: konj, preponski sport, zagrevanje

References

CARL K. (1989): Trainingswissenschaft – Trainingslehre, Haag, H., Theorie- und Themenfelder der Sportwissenschaft, Verlag Hofmann, Schorndorf, 216-228.

CASTEJON-RIBER C., RIBER C., RUBIO M. D., AGÜERA E., MUÑOZ A. (2017): Objectives, principles, and methods of strength training for horses. Journal of Equine Veterinary Science, 56, 93-103.

DYSON S., POLLARD D. (2021): Application of the Ridden Horse Pain Ethogram to Horses Competing at the Hickstead-Rotterdam Grand Prix Challenge and the British Dressage Grand Prix National Championship 2020 and Comparison with World Cup Grand Prix Competitions. Animals, 11(6), 1820.

DINES L. (2014): Why Horses Do that: A Collection of Curious Equine Behaviors. Willow Creek Press.

FÉDÉRATION EQUESTRE INTERNATIONALE (2021a): Main Jumping. Available online: https://inside.fei.org/fei/disc/jumping (9.7.2021).

FÉDÉRATION EQUESTRE INTERNATIONALE (2021b): Jumping Rules. Available online: https://inside.fei.org/sites/default/files/Jumping_Rules_2020_clean.pdf (9.7.2021).

JANCZAREK I., KĘDZIERSKI W., TKACZYK E., KACZMAREK B., ŁUSZCZYŃSKI J., MUCHA K. (2021): Thermographic Analysis of the Metacarpal and Metatarsal Areas in Jumping Sport Horses and Leisure Horses in Response to Warm-Up Duration. Animals, 11(7), 2022.

GREGIĆ M. BABAN M., MIJIĆ P., BOBIĆ T., DOKIĆ D., BRKA M., GANTNER,V. (2020): The Effect of Horse Age and Competitive Season on Stress Indicators in Jumping Horses in Running Track. In 30th Scientific-Experts Conference of Agriculture and Food Industry: Answers for Forthcoming Challenges in Modern Agriculture, 78, 185. Springer Nature.

HINTZ, H. F., HINTZ, R. L., VAN VLECK, L. D. (2001): Growth rate of thoroughbreds, effects of age of dam, year and month of birth, and seks of foal. J. Anim. Sci. 48: 480-487.

HICK C., HICK A. (2000): Kurzlehrbuch Physiologie. Urban & Fischer Verlag, München, 41-77: 153-160.

HOHMANN L. L. (2003): Einführung in die Trainingswissenschaft. Limpert Verlag GmbH, Wiebelsheim.

HRVATSKI KONJIČKI SAVEZ (HKS) (2021.) www.konjicki-savez.hr (9.7.2021.)

YOUNG W.B. (2007): The use of static stretching in warm-up for training and competition. Int. J. Sport. Physio. l2, 212–216.

LINDNER A. (1997): Laktat und Leistung beim Pferd. ISBN 3-00-001346-6.

MENDONÇA T., BIENBOIRE-FROSINI C., KOWALCZYK I., LECLERCQ J., ARROUB S., PAGEAT P. (2019): Equine Activities Influence Horses' Responses to Different Stimuli: Could This Have an Impact on Equine Welfare?. Animals, 9 (6), 290.

PERINOVIĆ M., MILANOVIĆ D. (2013): Plan i program treninga u preponskom jahanju. Kondicijski trening, Zagreb 11 (1): 52-62.

POLLY S.S., NICHOLS A.E., DONNINI E., INMAN D.J., SCOTT T.J., APPLE S.M., WERE S.R., DAHLGREN L.A. (2019): Adipose-Derived Stromal Vascular Fraction and Cultured Stromal Cells as Trophic Mediators for Tendon Healing. J. Orthop. Res. 2019, 37, 1429–1439.

POLLMANN-SCHWECKHORST E. (2002): Springpferde-Ausbildung heute. Deutsche Reiterliche Vereinigung (FN), Njemačka Hardcover – Import.

PRUNIER A., MOUNIER A.M., HAY M. (2005): Effects of castration, tooth resection, or tail docking on plasma metabolites and stress hormones in young piglets. J. Anim. Sci. 83: 216-222.

RICARD A., FOURNET-HANOCQ F. (1997): Analysis of factors affecting length of competitive life of jumping horses. Genet Sel Evol 29: 251-267.

SANTAMARÍA S., BOBBERT M.F., BACK W., BARNEVELD A., VAN WEEREN P.R. (2004): Evaluation of consistency of jumping technique in horses between the ages of 6 months and 4 years. Am. J. Vet. Res. 2004, 65, 945–950.

SANTAMARÍA S., BOBBERT M.F., BACK W., BARNEVELD A., VAN WEEREN P.R. (2005): Effect of early training on the jumping technique of horses. Am. J. Vet. Res., 66, 418–424.

SCHNABEL G., HARRE D., BORDE A. (2003): „Trainingswissenschaft: Leistung- Training- Wettkampf": Funktionelle Mechanismen der Leistungsentwicklung: 56-89, Training der Ausdauer: 315-333, Leistungs- und Trainingssteuerung: 397-413, Sportverlag Berlin, Berlin.

SOLIGARD T., MYKLEBUST G., STEFFEN K., HOLME I., SILVERS H., BIZZINI M., ANDERSEN T.E. (2008): Comprehensive warm-up programme to prevent injuries in young female footballers: Cluster randomised controlled trial. BMJ 337

THORPE C.T., CLEGG P.D., BIRCH H.L. (2010): A review of tendon injury: Why is the equine superficial digital flexor tendon most at risk? Equine Vet. J., 42, 174–180.

TRANQUILLE C.A., CLARKE J., WALKER V.A., MURRAY R.C. (2021): A descriptive study quantifying warm-up patterns in elite and non-elite dressage horses in a field environment. Comp. Exerc. Physiol. 17, 35–41.

TRAILOVIĆ D. R. (2008): Sportska medicina konja: savremeni trendovi u praćenju treniranosti sportskih konja. Fakultet veterinarske medicine, Univerzitet u Beogradu i Veterinarska komora Srbije. Beograd, 2008.

VOSWINKEL L. (2009): Einfluss der bewegungsaktivität auf wachstums- und ausdauerparameter beim pferd. Aus dem Institut für Tierzucht und Tierhaltung der Agrar- und Ernährungswissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

ACTIVITY OF SOME PLANT ESSENTIAL OILS AGAINST COMMON ISOLATES IN VETERINARY BACTERIOLOGY - A PILOT STUDY

Nemanja Zdravković[1], Oliver Radanović[1], Milan Ninković[1], Radoslava Savić-Radovanović[2], Nataša Rajić Savić[3], Đordje Marjanović[2], Jovan Bojkovski[2]

[1]Veterinary Institute of Serbia Belgrade, Janisa Janulisa 14, Belgrade, Srbia
[2]University of Belgrade, Faculty of Veterinary Medicine, Bul.Oslobođenja 18, Belgrade, Serbia
[3]AlDahra, Ekolab, Industrijsko naselje bb, Padinska skela, Belgrade, Serbia
Corresponding author: Nemanja Zdravković, nemanja.zdravkovich@gmail.com

Abstract*:* Antimicrobial therapy is important tool in fighting infectious diseases. The significance of the role of antimicrobials in nature remains vastly uninvestigated. Plants produce secondary metabolites for, among other functions, natural protection against microbial infection. The aim of this research was to investigate antimicrobial effects of 5 different essential oils and 5 main constituents of plant essential oils toward some of the common veterinary microbial pathogens. Plant etheric oils (EO) of oregano (*Origanum vulgare* L.), black caraway (*Nigella sativa* L.), sandalwood (*Santalum album* L.), peppermint (*Mentha balsamea* Wild.) and eucalyptus (*Eucalyptus obliqua* L'Hér.) and active components of etheric oils: D-limonene, D-α pinene, thymol, carvacrol and cynamaldehyde were tested for antimicriobial activity against referent strains of: *Staphylococcus aureus*, Methicilin resistant *S. aureus* (MRSA), *Escherichia coli*; as well as against clinical isolates of: *Staphylococcus pseudintermedius*, *E. coli*, *Pseudomonas aeruginosa* and yeast *Candida* sp. For each etheric oil and active compound minimal inhibitory concentrations (MIC) are observed by method of broth microdilution. Results of these investigations have shown that active components of the EO have stronger antimicrobial effect than complete formulation of essential oils used in the study. Among tested EOs the most potent was the peppermint etheric oil, while carvacrol showed the strongest antimicrobial effect among active components of EO. Interesting finding is that there was almost no difference among MICs between referent *S. aureus* and MRSA.

Key words*:* carvacrol, MIC, pathogens, peppermint, Staphylococcus aureus

Introduction

A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment. A microorganism is defined as clinically resistant when the degree of resistance shown is associated with a high likelihood of therapeutic failure (*EFSA* and *ECDC, 2015*). The wide use of anti-infective agents has resulted in the development of bacterial resistance to particular antibiotics (*Howard et al., 2014; Huber, 1970; Krnjaic et al., 2005)*. Penicillin was first applied in clinical therapy in 1941 in the United Kingdom against *Staphylococcus aureus*. The first penicillin resistance was recorded at *S. aureus* in 1947, only four years after the start of industrial production of this antibiotic. In order to overcome the problem of resistance to penicillin, in 1959, meticillin, a synthetic penicillin resistant to penicillinase activity, was registered. In the 1960s, there was a report of resistance to methicillin (*Patric*ia, *1961*). In veterinary medicine, the first isolation of this Methicilin Resistant *S. aureus* (MRSA) was 1972 in Belgium from cow mastitis (*Devries*e et al., *1972*). Antibiotic resistance is one of the most pressing health problems worldwide due to the continuous appearance of antibiotic-resistant bacterial strains. Antibiotics decrease or attack bacterial pathogens by altering the functions of the bacterial cell wall, proteins, nucleic acids and metabolic pathways (*Lagha et al., 2019*). The elaboration of new antibiotics is expensive and time-consuming (*Garcia-Salinas et al., 2018*). In particular, it is necessary to identify new drugs that can serve as an alternative treatment of infections caused by microorganisms that are resistant to traditional therapies.

One approach is the study of local medicinal plants with possible antimicrobial properties (*Monzote et al., 2017)*. Essential oils, are natural products obtained from plants wich contain volatile organic compounds that can be obtained from various plant organs, such as fruits, seeds, flowers, stems or roots *(Bakkali et al., 2008)*. These oils as well as their respective constituencies have demonstrated various antimicrobial activities *(Monzote et al., 2017; Das et al., 2016; Yap et al., 2014*), as well as antiparastic properties (*Trailovic et al., 2015; Marjanović et al., 2018*). Interest in essential oils as potential therapeutics to eradicate antibiotic resistance has been increasing as well as the the rising concern whether the bacterial tolerance to the essential oil components would be induced. The extent of bacteria in acquiring resistance to essential oil components has yet to be systematically and extensively investigated. Limited studies have been carried out while much focus has been placed on identifying the novel compound expanding the phytopharmaceutical library (*Yap et al., 2014*).

Organic farming is the large potential consumer of plant-based antimicrobials. Organic farming in Serbia is regulated by Law on Organic production (*Official Gazette of the Republic of Serbia no 30/10*) and Order on Certification in the organic production (*Official Gazette of the Republic of Serbia no 48/11*) which are closely related to the European Union legislative in particular field. In the USA where the United States Department of Agriculture defines the standards for organic production in the United States, organic dairy farmers must follow strict healthcare guidelines: organic animals may not receive synthetic antibiotics or anti- inflammatory drugs unless the animal is very sick, in which case the farmer must not withhold conventional treatment to preserve the cow's organic status (*Mullen, 2013*).

Materials and Methods

Susceptibility of bacteria to active components and control antibiotics were tersted by the microdilution test prescribed by the M26-A standard (NCCLS, 1999). Essential oils of oregano (*Origanum vulgare* L.), black caraway (*Nigella sativa* L.), sandalwood (*Santalum album* L.), peppermint (*Mentha balsamea* Wild.) and eucalyptus (*Eucalyptus obliqua* L'Hér.) along with active components of etheric oils: D-limonene, D-α pinene, thymol, carvacrol and cynamaldehyde (Essentico, Kula, Serbia, active ingredient content 98.9%, density 1,098g / ml) were prepared by dissolving in DMSO (Serva, Heidelberg, Germany) and then in Cation Adjustem Mueller Hinton Broth (Becton, Dickinson and Company, Sparks, USA) to a concentration of 2560 μg/ml. Bacterial inoculum was obtained according to the standard M7-A7 (*CLSI, 2006*) in final concentration of 4-5 x 10^5 cfu/ml, i.e. 4-5 x 10^4 cfu/well. Exepction were made for *Candida* sp. as it were done in Sabouraud broth by the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts (*EUCAST, 2017*) Microtitration plates without active components and plates without bacterial inoculum were placed as controls, while streptomycin (Sigma, Germany) at initial concentration of 256 μg/ml were used as control antibiotics. The plates were incubated for 24 hours at a temperature of 37°C under aerobic conditions in the thermostat (Sutjeska, Belgrade, Serbia).

Bacterial strains of: *S. aureus* ATCC 25923, Methicillin resistant *S. aureus* (MRSA) ATCC 43300, *Escherichia coli* ATCC 25922 were used as well as clinical isolates of: *Staphylococcus pseudintermedius*, *E. coli*, *Pseudomonas aeruginosa* and yeast *Candida* sp. which were obtained from rutine microbiology diagnostics procedures in Scientific Veterinary Institute of Serbia, Belgrade.

Minimal inhibitory concentration is observed as the lowest concertation of active matter under which there are no observed growth by unaided eye (*CLSI, 2006*).

Results and Discussion

Table 1. Results of Minimal inhibitory concentration (MIC)

	D limonen	**D α pinene**	**Thymol**	**Carvacrol**	**Origano oil**	**Cynamaldehyde**	**Sandal wood oil**	**black caraway oil**	**Eucalyptus oil**	**Peppermint**
MR *S. aureus* ATCC 43300	>2560	2560	160	160	320	160	>2560	2560	>2560	1280
***S. aureus* ATCC 25923**	>2560	>2560	160	160	320	320	>2560	2560	>2560	1280
***E. coli* ATCC 25922**	>2560	>2560	320	80	160	640	>2560	>2560	>2560	>2560
***S. pseudintermedius* clinical isolate**	>2560	>2560	160	160	320	320	>2560	2560	>2560	1280
E. coli clinical isolate	>2560	>2560	320	160	320	640	>2560	>2560	>2560	>2560
P. aeruginosa clinical isolate	2560	2560	320	640	2560	640	2560	2560	2560	2560
Candida sp. clinical isolate	320	160	80	80	80	20	160	1280	2560	2560

The plant essential oils of generally exhibited high MIC values (> 1280µg/ml) toweard the tested bacteria, although oregano oil is showed a stronger antimicrobial effect (Table 1.). Among the active components of essential oils, carvacrol and cinnamaldehyde exhibited the most potent antimicrobial effect. Clinical isolate of *Candida* sp. Showed the highest sensitivity to the tested substances, while the least sensitive is the *P. aeruginosa* isolate. Essential oils exhibited a fairly uniform MIC according to pairs of sensitive and resistant bacteria such as MRSA ATCC 43300 and *S. aureus* ATCC 25923 or clinical isolate and referent *E. coli* strain. This study confirms that selected essential oils have antibacterial and antifungal activity and as such may be used in the treatment of infections with pathogenic bacteria (*Thormar et al., 2011*). This proven quality might be used in the treatment of infections. In the future, the authors see extension in citotoxicity investigations and adequate regime of aplication. Preparations and adjuvans are not the same regarding of addmission strategies. Essential oils curative effect has been known since antiquity. It is based on a variety of pharmacological properties which are specific for each plant species. Results from in vitro studies in this work showed that the essential oils inhibited bacterial and fungal growth but their effectiveness varied (*Rusenova and Parvanov, 2009*). We have confirmed that certain essential oils have noticable antimicrobial activity. Also, we have verified that active components have grater antimicrobial potencial than the whole essential oil with the same principal active component (*Dal Pozzo et al., 2011; Bakkali et al., 2008)*. Antibacterial activity of several plant-derived molecules, including carvacrol, thymol, eugenol, and cynamaldehyde have been demonstrated against both gram-positive and gram-negative pathogens (*Burt, 2004; Bakkali et al., 2008)*. Our result imply better than *Dal Pozzo et al*. (2011) whom reported effect against *S. aureus* from the oregano oil MIC a range from 800 µg / ml to 3200 µg / ml with a mean value of 1600 µg / ml with an estimated MIC_{50} of 1600 µg / ml. In that same report carvacrol and thymol had values in the range form 200 to 1600 µg / ml. In the study of *Choi et al. (2012)* the reference strains of *S. aureus* from the ATCC collection showed a sensitivity to origano oil that could be considered as close to the our results: from 100 µg / ml to 500 µg / ml but milk isolates of the same bacteria showed a higher MIC of 1000 µg / ml to 4000 µg / ml, which is above cobtained in our study. The same authors showed a concentration range for antibacterial effect of the timol goes from 100 µg / ml up to 4000 µg / ml (*Choi et al., 201*2). In our study, carvacrol exhibited strong antimicrobial properties on *E. coli,* in concordance with literature description that the essential oils of *Origanum vulgare* and *Thymus vulgaris*, as well as their components, carvacrol and thymol are the most promising (*Soković et al., 2010*). Simililar results can be cited with other tested active components.

Conclusion

Experiments on essential oils are not yet fully standardized; therefore literature finding has variety of methods and approach strategies. There are numerous reports on these substances antimicrobial effect, but while some investigate essential oils, other focus on active components of essential oils. As a natural product essential oils may have significant variations in active components content, but still they can be standardized for veterinary use by dominant compound activity following current standards where some natural antibiotics are standardized to international units (such as penicillin and bacitracin). Different approach could be seen in works witch focus on synthetically derived active oils' components. Methods of research vary as well. Disc diffusion, agar diffusion and broth diffusion methods are usually reported, but these methods are only remotely interchangeable between themselves (*CLSI, 2006, EUCAST, 2017*). For the future investigations, this research group will work on the refinement on this topic heading to formulate adequate preparation for the specific pathogens.

Aktivnost nekih biljnih eteričnih ulja protiv čestih izolata u veterinarskoj bakteriologiji - pilot studija

Nemanja Zdravković, Oliver Radanović, Milan Ninković, Radoslava Savić-Radovanović, Nataša Rajić Savić, Jovan Bojkovski

REZIME

Antimikrobna terapija je važno sredstvo u borbi protiv zaraznih bolesti. Značaj uloge antimikrobnih sredstava u prirodi ostaje neistražen zbog prilagođavanja patogena različitim oblicima rezistencije. Biljke proizvode sekundarne metabolite, između ostalog i za prirodnu zaštitu od mikrobne infekcije. Cilj ovog istraživanja bio je istražiti antimikrobne efekte 5 različitih esencijalnih ulja i 5 glavnih sastojaka biljnih etarskih ulja na neke od uobičajenih veterinarskih mikrobnih patogena. Biljna eterična ulja (EO) origana (*Origanum vulgare* L.), crnog kima (*Nigella sativa* L.), sandalovine (*Santalum album* L.), nane (*Mentha balsamea* Wild.) i eukaliptusa (*Eucalyptus obliqua* L'Hér.) kao i aktivnih komponenti: D-limonen, D-α pinen, timol, karvakrol i cinamaldehid testirani su na antimikrobnu aktivnost protiv referentnih sojeva: *Staphylococcus aureus*, *S. aureus* rezistentne na meticilin (MRSA), *Escherichia coli* kao i protiv kliničkih izolata: *Staphylococcus pseudintermedius*, *E. coli*, *Pseudomonas aeruginosa* i kvasca *Candida* sp. Za svako eterično ulje i aktivno jedinjenje dobijene su minimalne inhibitorne koncentracije (MIK) mikrodilucionom metodom u bujonu. Rezultati su pokazali da aktivne komponente EO imaju jači antimikrobni efekat od EO sa istom odgovarajućom dominantnom komponentom. Među testiranim EO najmoćnije je bilo eterično ulje nane, dok je karvakrol pokazao najjači antimikrobni efekat među aktivnim komponentama EO. Zanimljiv nalaz je da gotovo nije bilo razlike među MIK između referentne S. aureus i MRSA.

Ključne reči: karvakrol, MIK, nana, *Staphyloccocus aureus*

Refences

BAKKALI F., AVERBECK S., AVERBECK D. IDAOMAR M. (2008): Biological effects of essential oils--a review. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 46**,** 446-475.

BURT S. (2004): Essential oils: Their antibacterial properties and potential applications in foods--a review. International Journal of Food Microbiology, 94, 223-253.

CHOI J. Y., DAMTE D., LEE S. J., KI JC., PARK S.C. (2012): Antimicrobial activity of lemongrass and oregano essential oil against standard antibiotic resistant and field isolates from chronic mastitis cow. International Journal of Phytomedicine, 134-139.

CLSI C. a. L. S. I. (2006): M7-a7 methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—seventh edition. Clinical and Laboratory Standards Institute (CLSI) 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2006.

DAL POZZO M., SANTURIO D. F., ROSSATO L., VARGAS A. C., ALVES S. H., LORETO E. S., VIEGAS J. (2011): Activity of essential oils from spices against staphylococcus spp. Isolated from bovine mastitis. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 63, 1229-1232.

DAS B., MANDAL D., DASH S. K., CHATTOPADHYAY S., TRIPATHY S., DOLAI D. P., DEY S. K., ROY S. (2016): Eugenol provokes ros-mediated membrane damage-associated antibacterial activity against clinically isolated multidrug- resistant staphylococcus aureus strains. Infectious Diseases: Reserch and treatment, 16, 9:11-9.

DEVRIESE L. A., VAN DAMME L. R., FAMEREE L. (1972): Methicillin (cloxacillin)-resistant staphylococcus aureus strains isolated from bovine mastitis cases. Journal of veterinary medicine. Series B, 19, 598-605.

EFSA E. F. S. A., Ecdc E. C. F. D. P. A. C. (2015): Eu summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. EFSA Journal, 2, 178

EUCAST (2017): Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. *EUCAST DEFINITIVE DOCUMENT E.DEF 7.3.1*. The European Committee on Antimicrobial Susceptibility Testing – EUCAST.

GARSIA-SALINAS S., ELIZONDO-CASTILLO H., ARRUEBO M., MENDOZA G., IRUSTA S. (2018): Evaluation of the antimicrobial activity and cytotoxicity of different components of natural origin present in essential oils. Molecules, 23, 6, 1399.

HOWARD P., PULCINI C., LEVY HARA G., WEST R. M., GOULD I. M., HARBARTH S., NATHWANI D. (2014): Study Group for Antimicrobial Policies (ESGAP); ISC Group on Antimicrobial Stewardship An international cross-sectional survey of antimicrobial stewardship programmes in hospitals. The Journal of antimicrobial chemotherapy.

HUBER W. G. (1970): The public health hazards associated with the non-medical and animal health usage of antimicrobial drugs. Pure and applied chemistry, 21, 377-388.

KRNJAIĆ D., MIŠIĆ D., AŠANIN R. (2005): Investigation of sensitivity and resistance to antibiotics and chemotherapeutics in e. Coli strains isolated from animals bred inintensive farming conditions Acta Veterinaria, 55, 501-509.

LAGHA R., BEN ABDALLAH F., AL-SARHAN B. O., AL-SODANY Y. (2019): Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against escherichia coli isolated from uti patients. Molecules, 24, (6), 1161.

MARJANOVIĆ S. Đ., BOGUNOVIĆ D., MILOVANOVIĆ M., MARINKOVIĆ D., ZDRAVKOVIĆ N., MAGAŠ V., TRAILOVIĆ M. S. (2018): Antihelminic activity of carvacrol, thymol, cinnamaldehyde and p-cymen against the free-living nematode caenorhabditis elegans and rat pinworm syphacia muris. Acta Veterinaria-Beograd, 68, 445-456.

MONZOTE L., SCULL R., COS P., SETZER W. N. (2017): Essential oil from piper aduncum: Chemical analysis, antimicrobial assessment, and literature review. Medicines (Basel), 4, 3, 49.

MULLEN K. (2013): Research from the united states on alternative treatments for mastitis in dairy cattle: Efficacy of phyto-mast. Ph.D. Dissertation, Faculty of North Carolina State University, North Carolina.

CLSI. (1999): M26-a, methods for determining bactericidal activity of antimicrobial activitys; approved guideline [Online]. NCCLS 940 West Valley Road, Suite 1400 Wayne, Pennsylvania 19087 USA

PATRICIA J. M. (1961): “Celbenin” - resistant staphylococci. British medical journal, 14**,** b124–125.

RUSENOVA N., PARVANOV P. (2009): Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. Trakia Journal of Sciences, 7, 37-43.

OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA no 30/10 Zakon o organskoj proizvodnji. Beograd.

OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA no 48/11 Pravilnik o kontroli i sertifikaciji u organskoj proizvodnji i metodama organske proizvodnje. Beograd

SOKOVIĆ M., GLAMOČLIJA J., MARIN P. D., BRKIĆ D., VAN GRIENSVEN L. J. (2010): Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules, 15, 7532-7546.

THORMAR H., ASTANI A., BERGSSON G., BRATT C. L., BROGDEN K. A., CARSON C. F., DAWSON D. V., DRAKE D., HAMMER K. A., HILMARSSON H., ISAACS C. E., KRISTMUNDSDOTTIR T., QUINN P. J., REICHLING J., SCHNITZLER P., SKULASON S.,WERTZ P. (2011): Lipids and Essential Oils as Antimicrobial Agents, New Delhi, India., John Wiley & Sons, Ltd.

TRAILOVIĆ S. M., MARJANOVIĆ D. S., NEDELJKOVIĆ TRAILOVIĆ J., ROBERTSON A. P., MARTIN R. J. (2015). Interaction of carvacrol with the ascaris suum nicotinic acetylcholine receptors and gamma-aminobutyric acid receptors, potential mechanism of antinematodal action. Parasitology research, 114, 3059-3068.

YAP P. S., YIAP B. C., PING H. C., LIM S. H. (2014): Essential oils, a new horizon in combating bacterial antibiotic resistance. The Open Microbiology Journal, 8, 6-14.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

THE EFFECT OF *RYR1* GENE ON MEAT QUALITY IN AUTOCHTHONOUS BREED KRŠKOPOLJE PIG

Urška Tomažin[1], Klavdija Poklukar[1], Martin Škrlep[1], Nina Batorek Lukač[1], Marjeta Čandek-Potokar[1,2]

[1]Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia
[2]University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče, Slovenia
Corresponding author: Marjeta Čandek-Potokar, meta.candek-potokar@kis.si

Abstract: The study investigated the incidence of the mutation on the *RYR1* gene and its influence on meat quality in the only Slovenian local pig breed - Krškopolje. Samples of 201 fattening pigs from 25 farms were collected for genotyping and meat quality analysis. The frequency of the mutant allele was 19.9% (3, 74 and 174 pigs with n/n, N/n and N/N genotype, respectively). Pigs of the N/n genotype had lower daily gain and carcass fatness than N/N pigs. The rate of pH decline in the *longissimus lumborum* muscle (LL) was faster in heterozygous pigs, as evidenced by lower pH 45 min post-mortem, but no effect on ultimate pH was observed. Consistent with post-mortem pH decline, a higher drip loss of LL muscle was observed for N/n pigs. The fatty acid composition of backfat was also influenced by the *RYR1* genotype. Consistent with the relationship between fatness and fatty acid composition, higher levels of saturated and monounsaturated fatty acids and lower levels of polyunsaturated fatty acids were observed in fattier N/N pigs.

Key words: Krškopolje pig; *RYR1* gene; meat quality

Introduction

Krškopolje pig is the only Slovenian local (autochthonous) pig breed. As it has not been subjected to genetic selection, it is characterised by lower performance and higher fatness compared with modern breeds, but recognised for having better meat quality (*Batorek et al., 2019*). To ensure their sustainability, they are, similar like other European local breeds, particularly suitable for the production of value-added dry-cured products. Only meat of the highest quality is suitable for the production of high quality products, however, studies conducted in recent years have shown that the frequency of the recessive allele on the *RYR1* gene, which is responsible for the malignant hyperthermia and stress susceptibility and known to cause pale, soft and exudative meat, is quite high in Krškopolje pigs.

For example, *Muñoz et al. (2018)* reported that the frequency of the recessive allele in Krškopolje pigs was 0.21, which was the highest among the twenty studied European local breeds. The reason for that can be searched in many years of exclusion from the breeding programme, loss of interest for the breed and no professional work resulting in uncontrolled introgression of genes from the modern breeds (*Kastelic and Čandek-Potokar, 2013*). However, as the breed is reputed for excellent meat quality, it is important to show the breeders how *RYR1* gene affects the quality of meat and why it is important to eliminate the presence of damaging allele. In order to investigate the relationship between the presence of mutated allele and meat quality in the local pig breed, a large number of pigs from different farms from all over Slovenia were included in the study to screen the whole population and to investigate the possibility of including the result of the genomic analysis in the breeding programme.

Materials and Methods

The samples of Krškopolje pigs (n=201) originating from 25 Slovenian farms were collected for DNA extraction and meat quality analysis. Samples of ears for the purpose of DNA extraction were taken at slaughter line and the pigs were identified by their individual ear numbers. The carcasses were weighed and measurements of fat and muscle thickness were taken for the estimation of lean meat content according to the method approved for Slovenia (OJ EU L56/28, 2008). Back fat thickness at the level of last rib and at withers was additionally measured. When possible, measurement of pH was taken in *longissimus lumborum* muscle (LL) at the level of last rib 45 minutes (pH45) post mortem using MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland). A day after slaughter the carcasses were cut at the level of last rib and samples of *longissimus lumborum* (LL) muscles with corresponding fat and skin were taken for further analysis. On LL muscle, marbling (on scale from 1 to 7) and subjective colour (on scale from 1 to 6) were visually assessed, and surfaces of muscle and corresponding fat area were measured. Measurement of ultimate pH (pH24) was also taken. Objective colour (CIE L*, a*, and b* colour parameters) was measured with Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) on freshly cut surface of the muscle. In addition, chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h° = \tan^{-1}(b^*/a^*)$) were calculated. Protein, water and intramuscular fat (IMF) content was determined in minced samples by near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using internal calibrations with the predictive ability based on R^2 (0.82, 0.97, 0.81, for moisture, IMF and protein content, respectively) and Sy.x (0.65%, 0.30%, 0.73%, for moisture, IMF and protein content, respectively).

Water holding capacity was determined by means of drip loss (after 24 hours of storage) according to the EZ method (*Christensen, 2003*), thawing and cooking losses. For thawing loss, a chop of LL (8×5×4 cm) was weighed, vacuum packed and frozen at -20°C. After thawing (overnight at 4°C), the samples were gently drained with a paper towel and reweighed. The same sample was used for determination of cooking loss and shear force. For cooking loss, the samples were cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C, cooled and reweighed. Three to four 1.27-cm-diameter cores were excised from the cooked muscle parallel to the muscle fibres and shear force was measured perpendicular to muscle fibres using a TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s. Fatty acid composition was estimated in subcutaneous fat tissue by near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA). The percentages of fatty acid groups (saturated fatty acids – SFA, monounsaturated fatty acids – MUFA, polyunsaturated fatty acids – PUFA, n-3 PUFA and n-6 PUFA) were predicted using internal calibrations with the predictive ability based on R^2 (0.83, 0.91, 0.89, 0.83, 0.89 for SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA, respectively) and Sy.x (0.79 %, 0.70%, 0.57%, 0.06%, and 0.51 % for SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA, respectively).

Genomic DNA was extracted from pig ear tissue using QIAamp DNA Mini kit (Qiagen, Germantown, MD, USA), according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed to screen for C/T SNP (C1843T) in the amplified 134 bp fragment of RYR1 gene, using the forward: 5'-GTGCTGGATGTCCTGTGTTCCCT-3' and reverse: 5'-CTGGTGACATAGTTGATGAGGTTTG-3' primer pairs as described by Brenig & Brem (1992). Thermocycler programme was set as follows: 3 min at 95 °C, 30 cycles at 95 °C for 30 s, at 59 °C for 40 s, and at 72 °C for 60s, followed by final elongation step at 72 °C for 5 minutes. The reaction consisted of 5 µl of 2x DreamTaq buffer, 1 µM primers, 3.3 µl dH_2O and 1 µl of DNA isolate. The fragment was digested with restriction endonuclease HhaI (ER1851, Thermo Fisher Scientific) to obtain fragments of 50 and 84 bp in case of the wild type allele. The restriction reaction consisted of 10 µl PCR product, 1.5 µl TANGA buffer, 4.2 µl H_2O and 0.3 µl (3U) of restriction enzyme. The samples were incubated overnight at 37 °C. Fragments after restriction were separated by electrophoresis under 100 V for 2 h on 2 % agarose gel stained with ethidium bromide. The GeneGenius gel imaging system (Syngene, Cambridge, UK) was used to observe the results.

The analysis of variance was performed using the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA). The model included the fixed effect of *RYR1* genotype, however, due to low number of pigs with the n/n genotype, they were excluded from the analysis. In case of carcass traits, final live weight was included as a covariate in the model. Differences between genotypes were considered significant if $P<0.05$. The results are presented as least square means (LS-means) with root-mean-square errors (RMSE). Effect size, presented as Hedges' *g* (difference between means of N/N and N/n divided by pooled SD), was also calculated. Effect sizes of 0.20, 0.50, and 0.80 are considered small, medium, and large, respectively.

Results and Discussion

All three genotypes of the *RYR1* gene were detected in the investigated Krškopolje pigs; there were 3, 74 and 174 pigs with n/n, N/n and N/N genotype, respectively. Indicative frequency of the recessive "n" allele in Krškopolje pig population is thus 19.9% which is similar as reported in recent years (*Tomažin et al., 2017; Muñoz et al., 2018*).

In the investigated Krškopolje pigs, the age at slaughter and their final body weight were 366 ± 138 days and 159 ± 41 kg, respectively. There was no difference in final weight between N/N and N/n genotypes (Table 1); however, average daily gain (from birth to slaughter) was higher in N/N pigs, indicating an effect of *RYR1* gene on growth rate. Better growth rate of N/N pigs confirms the results obtained in our recent study on Krškopolje pig (*Tomažin et al., 2017*). Contrary to our results on local pig breed, most of the studies performed on modern pig breeds reported no significant differences in growth performance between N/N and N/n genotypes (*Sather et al., 1991; Leach et al., 1996; Larzul et al., 1997; Tor et al., 2001*) except the study of *McPhee et al. (1992)* which also reported lower daily gains for pigs of N/n genotype and lower feed intake (which was not measured in our study).

As expected, carcass traits (indicating body composition) were significantly influenced by *RYR1* genotype. The heterozygous (N/n) pigs had thicker muscle and thinner fat measured above the *gluteus medius* muscle which led to their higher meat percentage (Table 1). Meat percentage of both genotypes was very low, as known for local pig breeds (*Čandek-Potokar et al., 2019*) not subjected to selective breeding for improved meatiness. Meta-analysis performed on modern pig breeds (*Salmi et al., 2010*) showed similar results i.e. higher lean percentage in N/n compared to N/N pigs. Consistent with their higher lean meat percentage, N/n pigs exhibited bigger loin muscle thickness and area (Table 1). Similar results were also observed in our previous study (*Tomažin et al., 2017*). In modern pig breeds, only one study (*Fisher et al., 2000*) reported larger *longissimus dorsi* muscle thickness and area in N/n than N/N pigs, while other studies reported no significant differences in muscle size between the two genotypes (*De Smet et al., 1996; Leach et al., 1996; Hamilton et al., 2000*).

Table 1. Effect of *RYR1* genotype on productive performance and carcass traits of Krškopolje pigs

	N/N (n=124)	N/n (n=74)	RMSE	P value	Effect size[1]
Age at slaughter, days	353	390	137.0	0.0699	-0.27
Final body weight, kg	160.1	157.3	41.5	0.6548	0.07
Average daily gain, g/day	473	433	91.4	0.0039	0.45
Muscle thickness, mm	69.2	73.4	8.19	0.0010	-0.43
Fat thickness over *gluteus medius*, mm	44.3	40.3	8.08	0.0014	0.41
Fat thickness at last rib, mm	45.3	43.2	7.49	0.0542	0.27
Fat thickness at withers, mm	67.2	64.7	10.47	0.1126	0.20
Meat percentage, %	36.7	40.3	6.00	0.0001	-0.56
Loin eye area, cm^2	41.2	46.9	13.03	0.0047	-0.37
Loin eye fat area, cm^2	43.8	44.9	15.67	0.6446	-0.07

[1]Hedge's *g* value
RMSE – root-mean-square error

In agreement with literature reports (*De Smet et al., 1996; Larzul et al., 1997; Monin et al., 1999; Fisher et al., 2000; Jankowiak et al., 2010; Tomažin et al., 2017; Ólivan et al., 2018*) regarding the effect of *RYR1* on muscle pH decline, pH value at 45 min post-mortem was higher in N/N than N/n pigs, while no differences were observed for pH 24 h post-mortem (Table 2). There were no differences between N/N and N/n pigs in CIE colour parameters which agrees with the results of our previous study on Krškopolje pigs (*Tomažin et al., 2017*), while on the contrary, in modern pig breeds, meta-analysis of *Salmi et al. (2010)* reported the differences between N/N and N/n pigs in colour parameters lightness (L*) and yellowness (b*). Even though the differences in CIE colour parameters were insignificant in the present study, a trend towards more intense colour of N/N than N/n pigs was noted (P=0.096 and 0.117, Hedge's *g*=0.25 and -0.23, for subjective colour and L*, respectively). It is known that recessive allele of *RYR1* gene influences the dynamics of post-mortem glycolysis by favouring calcium release in muscle cells thus accelerating the pH decline (*Monin, 2004*) which results in reduced water retention capability. In accordance with lower pH 45 min post-mortem, higher drip loss of LL muscle was observed in N/n than N/N pigs which corroborates with numerous studies (see meta-analysis of *Salmi et al., 2010*). Contrary to our previous study on Krškopolje pigs (*Tomažin et al., 2017*), no differences in thawing and cooking losses were observed in the present one. In modern pig breeds, many studies also failed to observe the difference in thawing or cooking losses between N/N and N/n pigs (*Boles et al., 1991; Leach et al., 1996; Monin et al., 1999; Fisher et al., 2000; Hamilton et al., 2000; Van der*

Maagdenberg et al., 2008). Contrary to most of the literature reports on modern pig breeds showing lower tenderness or higher shear force in N/n than N/N genotype (*Boles et al., 1991; McPhee and Trout, 1995; Van der Maagdenberg et al., 2008*), shear force value did not differ between the two genotypes in the present study, which is inconsistent also with our previous study on Krškopolje pig (*Tomažin et al., 2017*). A comparison with our results on modern pig breeds slaughtered at similar weight and assessed with the same methodology in the same laboratory (unpublished results), Krškopolje pigs do not seem to differ from pigs of modern breeds (54.7 N vs. 54.6 N for Krškopolje and modern breeds, respectively; Hedge's g=0.01).

With regard to chemical composition of LL muscle, no differences between N/N and N/n pigs were determined (Table 2), which corroborates with the results reported for local breeds Zlotnicka Spotted breed (*Jankowiak et al., 2010*) and Krškopolje pig (*Tomažin et al., 2017*) or modern pig breeds (*Leach et al., 1996; Hamilton et al., 2000; Álvarez-Rodríguez et al., 2017*).

Table 2. Effect of *RYR1* genotype on meat quality (*longissimus lumborum* muscle)

	N/N (n=124)	N/n (n=74)	RMSE	P value	Effect size[1]
pH45	6.55	6.37	0.220	0.0120	1.02
pH24	5.52	5.52	0.228	0.8334	0.03
Subjective colour (1-6)	3.5	3.2	1.08	0.0959	0.25
Marbling (1-7)	2.78	2.66	1.27	0.5325	0.09
Objective colour parameters:					
CIE L*	52.1	53.3	5.30	0.1168	-0.23
CIE a*	9.92	10.37	2.40	0.2063	-0.19
CIE b*	4.46	3.96	3.05	0.2612	0.17
C*	11.2	11.4	2.85	0.5351	-0.09
h°	22.4	20.2	13.66	0.2737	0.16
Chemical analysis:					
Intramuscular fat, %	3.9	3.7	1.46	0.4057	0.12
Protein, %	23.5	23.3	0.98	0.3339	0.14
Water, %	71.9	72.1	1.22	0.2912	-0.16
Water holding capacity:					
Drip loss after 24 h, %	4.3	5.8	2.30	<0.0001	-0.64
Thawing loss, %	12.2	13.1	3.83	0.1373	-0.26
Cooking loss, %	26.8	27.0	4.37	0.7474	-0.06
Hardness, WBSF, N	53.6	56.0	11.97	0.2233	-0.21

[1]Hedge's g value; RMSE – root-mean-square error, C* - chroma, h° - Hue angle, WBSF – Warner-Bratzler shear force.

Fatty acids composition of backfat tissue differed between the two genotypes (Table 3). The percentages of SFA and MUFA were higher and the percentages of n-3, n-6 and total PUFA were lower in N/N than N/n pigs. There are few available studies that compared *RYR1* genotypes with respect to fatty acid composition and no major differences between N/N and N/n genotypes were reported (*García-Macías et al., 1996; Biedermann et al., 2000; Álvarez-Rodríguez et al., 2017*). In addition to the diet, which we were unable to control in the present study, fatty acid composition depends largely on the fat content of the pigs. The SFA and MUFA content increases, while the PUFA content decreases with increasing backfat thickness (*Wood et al., 2008*). Therefore, we can hypothesise that the difference between the two genotypes can be explained by the difference in body fatness, however, further research is needed in this regard.

Table 3. Effect of *RYR1* genotype on fatty acid composition (wt % of all fatty acids) of subcutaneous back fat tissue

	N/N (n=124)	N/n (n=74)	RMSE	P value	Effect size[1]
SFA	41.2	40.3	1.98	0.0193	0.54
MUFA	47.7	46.4	2.86	0.0182	0.54
PUFA	11.7	13.8	2.70	<0.0001	-0.95
n-3 PUFA	1.11	1.42	0.412	<0.0001	-0.91
n-6 PUFA	10.6	12.4	2.42	<0.0001	-0.92
n-6 PUFA / n-3 PUFA	10.1	9.4	2.62	0.1558	0.32

[1]Hedge's *g* value; RMSE – root-mean-square error, MUFA – monousaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids.

Conclusion

The incidence of the mutant *RYR1* allele in the population of Krškopolje pigs is relatively high so it was of interest to evaluate its effect on productive performance and meat quality and to compare with what is known for modern breeds. Results confirm a significant difference between N/n and N/N genotypes on performance and meat quality also in local breed Krškopolje pig, however, the effects are not always equivalent to what has been reported for modern pig breeds and this might be due to different (less intensive) production systems (diet, higher age and weight, outdoor access).

Uticaj mutacije *RYR1* gena na kvalitet mesa autohtone „krškopoljske“ rase svinja

Urška Tomažin, Klavdija Poklukar, Martin Škrlep, Nina Batorek Lukač, Marjeta Čandek-Potokar

Rezime

Studija je istraživala učestalost mutacije na genu *RYR1* i njen uticaj na kvalitet mesa jedine slovenačke lokalne/autohtone rase svinja – krškopoljska svinja. Uzorci 201 tovljenika sa 25 farmi prikupljeni su za genotipizaciju i analizu kvaliteta mesa. Učestalost mutiranog alela bila je 19,9% (3, 74 i 174 svinje sa n/n, N/n i N/N genotipom, respektivno). Svinje n/n genotipa imale su manji dnevni prirast i sadržaj masti u trupu od n/n svinja. Stopa pada pH u mišiću *longissimus lumborum* (LL) bila je brža kod heterozigotnih svinja, o čemu svedoči niži pH 45 minuta post mortem, ali nije primećen nikakav efekat na krajnji pH. U skladu sa post-mortem padom pH, primećen je veći kalo mišića LL kod N/n svinja. Na sastav masnih kiselina leđnog masnog tkiva takođe je uticao genotip *RYR1*. U skladu sa odnosom između masnog tkiva i sastava masnih kiselina, viši nivoi zasićenih i mononezasićenih masnih kiselina i niži nivoi polinezasićenih masnih kiselina zabeleženi su kod masnijih N/N svinja.

Ključne reči: krškopoljska svinja; gen RYR1; kvalitet mesa

Acknowledgment

The study is part of the project “Introduction of meat quality into the breeding programme of Krškopolje pigs” financed by the European Commission in the frame of Rural Development Programme 2014–2020 for Slovenia, sub-measure M16.2 (operational groups). Authors would also like to acknowledge the core financing of Slovenian Agency of Research (grant P4-0133) and Ministry of Agriculture, Food and Forestry (breeding programme of Krškopolje pig). Breeders of Krškopolje pigs are acknowledged for their cooperation and support.

References

ÁLVAREZ-RODRÍGUEZ J., GARCÍA-HERNÁNDEZ E., TORRÀS O., TOR M., VILLALBA D., PENA R. (2017): Nutrigenetic response of dietary linseed inclusion on pork fatty acid composition in halothane gene carriers. Proceedings of the 63rd International congress on meat science and technology (Cork, Ireland). Wageningen Academic Publishers. The Netherlands, 39–40.

BATOREK LUKAČ N., TOMAŽIN U., ŠKRLEP M., KASTELIC A., POKLUKAR K., ČANDEK-POTOKAR M. (2019): Krškopoljski prašič (Krškopolje pig). In: European local pig breeds - Diversity and performance. Eds. Čandek-Potokar M. and Nieto Linan R.M. IntechOpen, London, UK, 141–154.

BIEDERMANN G., JATSCH C., PESCHKE W., LINDNER J.-P., WITTMANN W. (2000): Mast- und Schlachtleistung sowie Fleisch- und Fettqualität von Pietrain-Schweinen unterschiedlichen MHS-Genotyps und Geschlechts - 2. Mitteilung: Fettsäurenmuster der Depotfette Rückenspeck, Flomen und intermuskuläres Fett sowie der Gesamt- und Phospholipide des *M. long. dorsi.* Archiv für Tierzucht, 43 (2), 165–178.

BOLES J.A., PARRISH Jr. F.C., SKAGGS C.L., CHRISTIAN L.L. (1991): Effect of porcine somatotropin. stress susceptibility, and final end point of cooking on the sensory, physical, and chemical properties of pork loin chops. Journal of Animal Science, 69, 2865–2870.

BRENIG B., BREM G. (1992): Molecular cloning and analysis of the porcine halothane gene. Archiv für Tierzucht, 35, 129–135.

CHRISTENSEN L.B. (2003): Drip loss sampling in porcine m. *longissimus dorsi*. Meat Science, 63, 469–477.

ČANDEK-POTOKAR M., BATOREK LUKAČ N., TOMAŽIN U., ŠKRLEP M., NIETO R. (2019): Analytical review of productive performance of local pig breeds. In: European local pig breeds - Diversity and performance. Eds. Čandek-Potokar M. and Nieto Linan R.M. IntechOpen, London, UK, 281–303.

DE SMET S.M., PAUVELS H., DE BIE S., DEMEYER D.I., CALLEWIER J., EECKHOUT W. (1996): Effect of halothane genotype, breed, feed withdrawal and lairage on pork quality of Belgian slaughter pigs. Journal of Animal Science, 74, 1854–1863.

GARCÍA-MACÍAS J.A., GISPERT M., OLIVER M.A., DIESTRE A., ALONSO P., MUÑOZ-LUNA A., SIGGENS K., CUTHBERT-HEAVENS D. (1996): The effects of cross, slaughter weight and halothane genotype on leanness and meat and fat quality in pig carcasses. Animal Science, 63, 487–496.

HAMILTON D.N., ELLIS M., MILLER K.D., McKEITH F.K., PARRETT D.F. (2000): The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. Journal of Animal Science, 78, 2862–2867.

FISHER P., MELLETT F.D., HOFFMAN L.C. (2000): Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. Meat Science, 54, 97–105.

JANKOWIAK H., KAPELAŃSKI W., WILKANOWSKA A., CEBULSKA A., BIEGNIEWSKA M. (2010): The effect of *CLPS* and *RYR1* gene polymorphism on meat quality of Złotnicka spotted pigs. Journal of Central European Agriculture, 11, 93–98.

KASTELIC A., ČANDEK-POTOKAR M. (2013): Application of quality labels in support of conservation of local breeds – a challenge for Slovenian Krškopolje pig. In Proceedings of the 8th International Symposium on the Mediterranean Pig, Ljubljana, Slovenia. Acta Agriculturae Slovenica, Supplement 4, 205–209.

LARZUL C., LE ROY P., GUEBLEZ R., TALMANT A., GOUGE J., SELLIER P., MONIN G. (1997): Effect of halothane (NN, Nn, nn) on growth, carcass and meat quality traits of pigs slaughtered at 95 kg or 125 kg live weight. Journal of Animal Breeding and Genetics, 114, 309–320.

LEACH L.M., ELLIS M., SUTTON D.S., MCKEITH F.K., WILSON E.R. (1996): The growth performance. carcass characteristics, and meat quality of halothane carrier and negative pigs. Journal of Animal Science, 74, 934–943.

McPHEE C.P., DANIELS L.J., KRAMER H.L., NOBLE J.W., TROUT G.R. (1992): The effect of the Halothane gene on performance, carcass and meat quality in a fat and a lean line of pigs. Proceedings of the Advancement of Animal Breeding and Genetics, 10, 76–79.

McPHEE C.P., TROUT G.R. (1995): The effects of selection for lean growth and the halothane allele on carcass and meat quality of pigs transported long and short distances to slaughter. Livestock Production Science, 42, 55–62.

MONIN G., LARZUL C., LE ROY P., CULIOLI J., MOUROT J., ROUSSET-AKRIM S., TALMANT A., TOURAILLE C., SELLIER P. (1999): Effects of the halothane genotype and slaughter weight on texture of pork. Journal of Animal Science, 77, 408–415.

MONIN G. (2004): Conversion of muscle to meat/Colour and texture deviations. In: Encycopedia of meat sciences. Eds Jensen W. K., Devine C. and Dikeman M. Elsevier Ltd., Oxford, UK, 323–330.

MUÑOZ M., BOZZI R., GARCÍA F., NÚÑEZ Y., GERACI C., CROVETTI A., GARCÍA-CASCO J., ALVES E., ŠKRLEP M., CHARNECA R., MARTINS J.M., QUINTANILLA R., TIBAU J., KUŠEC G., DJURKIN-KUŠEC I., MERCAT M.J., RIQUET J., ESTELLÉ J., ZIMMER C., RAZMAITE V., ARAUJO J.P., RADOVIĆ Č., SAVIĆ R., KAROLYI D., GALLO M., ČANDEK-POTOKAR M., FONTANESI L., FERNÁNDEZ A.I., ÓVILO C. (2018): Diversity across major and candidate genes in European local pig breeds. PLOS ONE, 13(11): e0207475, 1–30.

OJ EU L56/28 (2008): Commission Decision of 8th December 2005 authorising methods for pig carcasses in Slovenia.

OLIVÁN M., GONZÁLEZ J., BASSOLS A., DÍAZ F., CARRERAS R., MAINAU E., ARROYO L., PEÑA R., POTES Y., COTO-MONTES A., HOLLUNG K., VELARDE A. (2018): Effect of sex and RYR1 gene mutation on the muscle proteomic profile and main physiological biomarkers in pigs at slaughter. Meat Science, 141, 81–90.

SATHER A.P., MURRAY A.C., ZADAWSKI S.M., JOHNSON P. (1991): The effect of halothane gene on pork production and meat quality of pigs reared under commercial conditions. Canadian Journal of Animal Science, 71, 956–967.

TOMAŽIN U., BATOREK LUKAČ N., ŠKRLEP M., PREVOLNIK POVŠE M., OGOREVC J., DOVČ P., ČANDEK-POTOKAR M. (2017): Meat quality of krškopolje pigs as affected by RYR1 genotype. Proceedings of the 11th International Symposium Modern Trends in Livestock Production. Belgrade, Serbia, 528–538.

TOR M., ESTANY J., VILLALBA D., CUBILÓ D., TIBAU J., SOLER J., SANCHEZ A., NOGUERA J.L. (2001): Within-breed comparison of RYR1 pig genotypes for performance, feeding behaviour, and carcass, meat and fat quality traits. Journal of Animal Breeding and Genetics, 118, 417–427.

VAN DEN MAAGDENBERG K., STINCKENS A., CLAEYS E., BUYS N., DE SMET S. (2008): Effect of the insulin-like growth factor-II and RYR1 genotype in pigs on carcass and meat quality traits. Meat Science, 80, 293–303.

WOOD J.D., ENSER M., FISHER A.V., NUTE G.R., SHEARD P.R., RICHARDSON R.I., HUGHES S.I., WHITTINGTON F.M. (2008): Fat deposition, fatty acid composition and meat quality: A review. Meat Science, 78, 343–358.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

EFFECTS OF ANDROGEN DEPRIVATION ON HISTOMORPHOLOGICAL PROPERTIES OF FAT TISSUE IN PIGS

Klavdija Poklukar[1], Marjeta Čandek-Potokar[1,2], Milka Vrecl Fazarinc[3], Nina Batorek Lukač[1], Gregor Fazarinc[3], Kevin Kress[4], Volker Stefanski[4], Martin Škrlep[1]

[1]Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia
[2]University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče, Slovenia
[3] Institute of Preclinical Sciences, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, SI-1000 Ljubljana, Slovenia
[4]Behavioural Physiology of Livestock, Institute of Animal Science, University of Hohenheim, Garbenstrasse 17, 70599 Stuttgart, Germany
Corresponding author: Martin Škrlep, martin.skrlep@kis.si

Abstract: The present study aimed to provide an in-depth histo-morphological characterization of subcutaneous adipose tissue as affected by conventional (i.e. surgical castration, performed early in life) or immunological castration (i.e. immunocastration, performed in the later fattening stages) as compared to uncastrated (entire) male pigs. A total of 36 male pigs were allocated to three groups of equal size, entire males (EM), surgically castrated (SC) or immunocastrated i.e. vaccinated against GnRH (IC) at 12 and 21 weeks. After slaughter at 26 weeks of age, the samples of subcutaneous fat (superficial and deep layer together with dermis) were collected, fixed, stained with haematoxylin and eosin and subjected to histo-morphometric analysis. A thinner dermis ($p<0.05$) was observed in SC than in EM. In the superficial backfat layer, both IC and SC had larger ($p<0.05$) adipocytes and greater surface area of fat fascicles than EM. In the deep backfat layer, both sexually neutered categories had greater ($p<0.05$) fascicle area along with greater ($P<0.05$) number of adipocytes per fascicle in IC and a trend ($P<0.10$) for greater adipocyte surface in SC compared to EM. Overall our results demonstrate the increase in adiposity at cellular level, regardless of castration method. In addition, it is shown that IC, despite being neutred later in life, rapidly adapt their adipogenic potential , achieving similar histomorphological characteristics to SC as early as five weeks after effective immunization.

Key words: immunocastration, surgical castration, adipose tissue, histology, pigs

Introduction

Surgical castration of male piglets in the first days of life is a common procedure in pig husbandry. The procedure prevents boar taint development and male-specific aggressive behaviour and is advantageous for the quality of meat and fat (*Bonneau and Weiler, 2019*), and consequently meat products (*Škrlep et al., 2020a*). On the other hand, castration leads to increased deposition of carcass fat, which reduces economic benefits (*de Roest et al., 2009*). Recently, the practice of surgical castration as practiced today (without pain relief) causes public concern (*Prunier et al., 2006; Bonneau and Weiler, 2019*). Many actors in pig production sector are thus considering other alternatives to replace surgical castrates (SC), like raising entire males (EM) or immunocastration. The alternatives have their pros and cons, but from meat quality perspective raising EM is critical, whereas immmunocastration faces acceptability challenge (*Čandek-Potokar et al., 2015*). Compared to classically performed surgical castration, immunocastration induces androgen deprivation later in life. The procedure consists of two vaccinations, the effect (i.e. immunisation against endogenous gonadotropin-releasing hormone) takes place after the second dose, which is usually applied 4-6 weeks before slaughter, at least when standard vaccination protocol is adopted. Thereafter, IC pigs rapidly shift their metabolism from EM-like to castrate-like, the change being mainly evident in enhanced fat deposition, triggered by the sudden drop of steroid hormones and increase in feed intake (*Claus et al., 2007; Batorek-Lukač et al, 2016*). There are numerous studies that characterize differences between the mentioned alternatives in fat deposition and composition (*Škrlep et al., 2020a*). On the other hand, the aetiology of the changes and detailed insights into the associated physiological processes are still generally lacking (*Škrlep et al., 2018*). To explore this issue further, the present study aimed to perform a detailed histo-morphological examination of the adipose tissue (including two subcutaneous tissue layers and the dermis) of the two sexually neutered categories (SC and IC) in comparison with EM.

Materials and Methods

For the present study, 36 male pigs, commercial crosses of Landrace and Pietrain were used. Early after birth, animals were assigned to three treatment groups of 12 pigs, being either left entire (i.e. uncastrated), surgically castrated within the first seven days of life, or immunocastrated (EM, SC, IC, respectively). The procedure of immunocastration was performed by applying two doses of anti-GnRH vaccine (Improvac, Zoetis Deutschland GmbH, Berlin, Germany) when pigs reached the age of 82 and 150 days. Animals were reared in equal living conditions, fed the same diet *ad libitum* (for details see Kress and Verhaagh, 2020). At the average age of 184 days all pigs were slaughtered in one slaughter batch according to abattoir routine procedure (CO_2 stunning, exsanguination, vapour scalding and dehairing). After evisceration and veterinary inspection, the carcasses were split apart, weighed and graded according to SEUROP. For histo-morphological analysis, small pieces of subcutaneous dorsal fat tissue (from upper and lower layers determined as lying above and below the connective tissue layer between the both) and the attached dermis were cut from the carcasses at the level of withers. The samples were stored in 10% buffered formalin solution to assure proper fixation, after which they were dehydrated and embedded in paraffin. After being cut into 5 μm thick histological sections, the samples were stained with haematoxylin and eosin. Examination was carried out on the digital images acquired with a light microscope (Nikon Ni/U, Nikon Instruments Europe B.V., Badhoevedrop, The Netherlands) equipped with a high resolution digital camera (DS-Fi1, Nikon Instruments Europe B.V.). The images were analysed by imaging software (NIS-Elements Basic Research, Nikon Instruments Europe B.V.); the analysis consisted of measurements taken for each of the layers, within three selected regions. Within each region, five random adipocyte cell fascicles were chosen and their surface measured, number of adipocytes per fascicle counted and their area measured. Additionally, dermis thickness was measured. Statistical analysis was carried out with R statistical software (version 3.6.1) using one-way analysis of variance, testing the effect of sex category. Significant differences ($p<0.05$) between the means were assessed using Tukey-Kramer test.

Results and Discussion

In the upper backfat layer (Figure 1), no significant differences ($P>0.10$) were observed for the number of adipocytes per fascicle, although it is worth mentioning that IC and SC had somewhat higher number than EM (28 and 15 % more adipocytes, respectively). Regarding the adipocyte surface area, both castrated categories (i.e. IC and SC) showed very similar values, being 20 and 23% higher ($P<0.05$) than in EM pigs. Similar was the situation for fascicle surface area, with almost identical values measured for IC and SC, both exhibiting nearly 40% higher ($P<0.05$) values than EM.

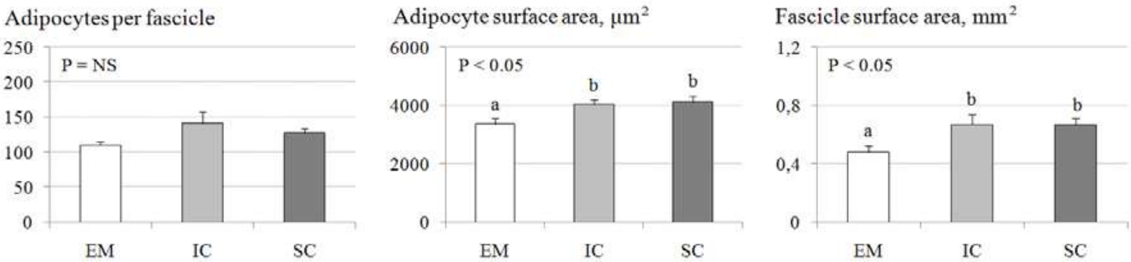


Figure 1. Histo-morphological traits of the upper backfat layer according to male sex category. EM = entire males; IC = immunocastrates, SC = surgical castrates

Higher number of adipocytes per fascicle in IC than EM was also observed at the lower backfat layer (Figure 2), although this time, the differences were statistically significant (P<0.05), and with IC having 36% more adipocytes per fascicle than EM. The SC exhibited intermediate values, not differing (P>0.10) from either EM or SC. Adipocyte surface area, tended to be greater in SC than EM (the difference being 32%; P<0.10). On the other hand, a significant effect (P<0.01) of the sex category was noted in the case of fascicle surface area, with both SC and IC exhibiting higher values than EM, the differences being relatively high, from 49% (SC vs. EM) to 58% (IC vs. EM).

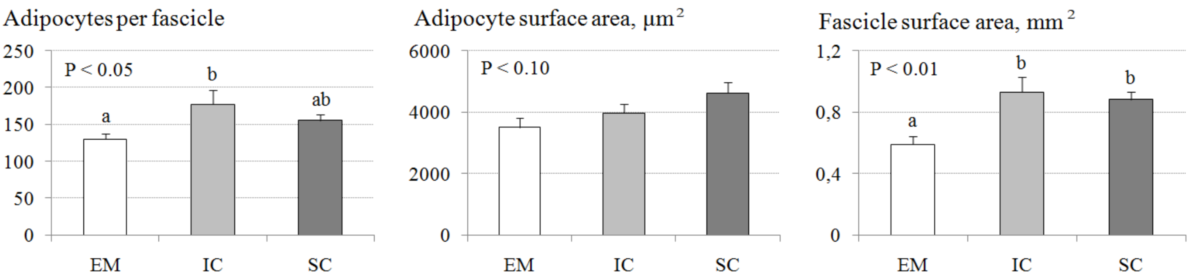


Figure 2. Histo-morphological traits of the lower backfat layer according to male sex category. EM = entire males; IC = immunocastrates, SC = surgical castrates

It is a well established fact that castration increases fat deposition in pigs, castrates being notably fatter than EM (*Kouba and Sellier, 2011*) while muscularity traits not being markedly affected (*Škrlep et al., 2020b*). Regarding the quantity and composition of fat depots, the majority of literature and meta-analytical studies (*Škrlep et al., 2020a; Batorek et al., 2012a*) positions IC intermediate between SC (the fattest) and the EM (the leanest). This is the case at least when a standard immunocastration protocol is applied, with two subsequent vaccinations and slaughter at 4 to 6 weeks after the second vaccination. Only the prolongation of the period between the second vaccination and slaughter to 8 or more weeks diminishes the differences between IC and SC in either quantity or composition of fat depots (*Tavárez et al., 2014; Harris et al., 2018*). The results of the present research are indicating a much faster response at histo-morphological level. As expected, the SC exhibited higher level of lipid cell hypertrophy than EM (either at the level of individual cells or entire adipocyte fascicles), confirming previous histological comparisons between EM and SC (*Mersman, 1984*). The IC (here vaccinated 5 weeks prior to slaughter) already reached the same level of cell hipertrophy as SC. Even more interesting is the case of the number of adipocytes per fascicle, a measure indicating cell hypertrophy. The number of adipocytes in IC not only exceeded that of EM, but was also greater than in SC (though not significantly). Rapid change in the adipose tissue histomorphological adaptation is most probably influenced by a fast drop in testosterone production (reaching minimal levels as fast as one week after second immunization (*Brunius et al., 2011*). This intensive hormonal change enables subsequent adipocyte proliferation and differentiation followed by increased deposition of the adipose tissue (*O'Reilly et al., 2014*). Additionally, reduced levels of testicular steroids are associated with a decreased level of insulin like growth factor (IGF1), which (among other functions) stimulates cell proliferation (*Hausmann et al., 2001*) and may be also related to increased adipocyte numbers in IC. Due to its substantially long half-life, the IGF1 has been reported to decrease only gradually after effective immunocastration (*Claus et al., 2007; Brunius et al., 2011*). Along with relatively low leptin levels, it positively affects growth and an increase in appetite (*Batorek et al., 2012b*), surpassing thus not only EM but also SC, which corroborates well also with the present results on histo-morphology.

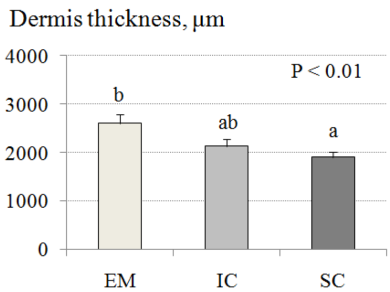


Figure 3. Dermis thickness according to male sex category. EM = entire males; IC = immunocastrates, SC = surgical castrates

In addition to subcutaneous fat, attached dermis was also examined. As presented in Figure 3, SC exhibited 27% lower ($p<0.05$) dermis thickness compared to EM, whereas IC took intermediate position, not differing from either of the two. These results corroborate with the reports of *Vold and Moen (1972)* indicating thinner dermis in castrated pigs compared to EM and also lower synthesis of collagen (presenting the main structural component of the skin). This may mainly be a consequence of changed hormonal regulation, caused by the absence of hormones like testosterone (*Petersen et al., 1997*), oestrogen and IGF1, exerting anti-catabolic or proliferative features (*Claus et al., 2007*). Reduction of collagen, as a response to castration, has also been shown in other tissues, like *longissimus dorsi* muscle (*Škrlep et al., 2019*) or backfat (*Wood et al., 1989*). As to the differences in backfat connective tissue, it should be noted here that similar influences of castration was observed also in the present study (results not shown), although these were not numerically quantified.

Conclusion

The study elucidated histo-morphological changes of pig adipose tissue as related to early (surgical) or late (immunological) castration. Both ways of castration lead to adipocyte hypertrophy, but the IC, even though effectively castrated only few weeks before slaughter are characterized by fast adoption of castrate-like features either in terms of increase in adipocyte proliferation and size, which denotes a rapid turnover of IC metabolism.

Uticaj androgene deprivacije na histomorfološka svojstva masnog tkiva muških svinja

Klavdija Poklukar, Marjeta Čandek-Potokar, Milka Vrecl Fazarinc, Nina Batorek Lukač, Gregor Fazarinc, Kevin Kress, Volker Stefanski, Martin Škrlep

Rezime

Cilj ovog istraživanja je bio da pruži dubinsku histomorfološku karakterizaciju potkožnog masnog tkiva na koje utiče konvencionalna (tj. hirurška kastracija, izvedena u ranoj mladosti) ili imunološka kastracija (tj. Imunokastracija, izvedena u kasnijim fazama tova) u poređenju sa nekastriranim mužjacima. Ukupno 36 nerastova raspoređeno je u tri grupe jednake veličine, nekastririani (EM), hirurški kastrirani (SC) ili imunokastrirani nerastovi, odnosno vakcinisane protiv GnRH (IC) u 12 i 21 nedelji. Nakon klanja u dobi od 26 nedelja, uzorci potkožne masti (površinski i duboki sloj zajedno sa dermisom) su prikupljeni, fiksirani, obojeni hematoksilinom i eozinom i podvrgnuti histomorfološkoj analizi. U SC grupi je primećen tanji dermis ($p<0,05$) od EM. U površinskom sloju pozadinske masti, i IC i SC su imali veće ($p<0,05$) adipocite i veću površinu masnih naslaga. U dubokom sloju leđne slanine, obe polno kastrirane kategorije imale su veću (p <0,05) površinu snopa zajedno sa većim (P<0,05) brojem adipocita po snopu mišića u IC grupi, i trend (<0,10) za veću površinu adipocita u SC u poređenju sa EM. Sve u svemu, naši rezultati pokazuju porast masnoće na ćelijskom nivou, bez obzira na metod kastracije. Osim toga, pokazano je da IC, uprkos tome što je neutraliziran kasnije u životu, brzo prilagođava svoj adipogeni potencijal, postižući slične histomorfološke karakteristike sa SC već pet nedelja nakon efikasne imunizacije.

Ključne reči: imunokastracija, hirurška kastracija, masno tkivo, histologija, svinje

Acknowledgment

The study was conducted within the project SuSI (ERA-NET SusAn, grant number 696231), co-financed by the European Union's Horizon 2020 research and Innovation Program, German Federal Office for Agriculture and Food and Slovenian Ministry of Agriculture, Forestry and Food. Core financing of Slovenian Research Agency (grants P4-0133, P4-0053, Z7-9461 and PhD scholarship for Klavdija Poklukar) are also acknowledged.

References

BATOREK-LUKAČ N., DUBOIS S., NOBLET J., ČANDEK-POTOKAR M., LABUSSIÉRE E. (2016): Effect of high dietary fat content on heat production and lipid and protein deposition in growing immunocastrated male pigs. Animal, 10: 1941-1948.

BATOREK N., ČANDEK-POTOKAR M., BONNEAU M., VAN MILGEN J. (2012a): Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal, 6: 1330-1338.

BATOREK N., ŠKRLEP M., PRUNIER A., LOUVEAU I., NOBLET J., BONNEAU M., ČANDEK-POTOKAR M. (2012b): Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. Journal of Animal Science, 90: 4593-4603.

BONNEAU M., WEILER U. (2019): Pros and cons of alternatives to piglet castration: welfare, boar taint and other meat quality traits. Animals, 9: 884.

BRUNIUS C., ZAMARATSKAIA G., ANDERSSON K., CHEN G., NORRBY M, MADEJ A., LUNDSTRÖM K. (2011): Early immunocastration of male pigs with Improvac® - effect on boar taint, hormones and reproductive organs. Vaccine, 29: 9541-9520.

CLAUS R., LACORN M., DANOWSKI K., PEARCE M.C., BAUER A. (2007): Short-term endoctire and metabolic reactions before and after second immunization against GnRH in boars. Vaccine, 25: 4689-4696.

ČANDEK-POTOKAR M., ŠKRLEP M., BATOREK LUKAČ N. (2015): Raising entire males or immunocastrates – outlook on meat quality. Procedia Food Science, 5: 30-31.

DE ROEST K., MONTANARI C., FOWLER T., BALTUSSEN W. (2009): Resource efficiency and economic implications of alternatives to surgical castration without anaesthesia. Animal, 3: 1522-1531.

HARRIS E.K., MELLENCAMP M.A., JOHNSTON L.J., COX R.B., SHURSON G.C. (2018): Effectiveness of different corn dried distillers grains with soluble feeding strategies and increasing the time intervals between the second Improvest dose and slaughter of immunologically castrated pigs on belly and pork quality. Meat Science, 135: 62-73.

HAUSMAN D.B., DI GIROLAMO M., HAUSMAN G.J., MARTIN R.J. (2001): The biology of white adipocyte proliferation. Obesity reviews, 2: 239-254.

KOUBA M., SELLIER P. (2011): A review of the factors influencing the development of intermuscular adipose tissue in the growing pig. Meat Science, 88: 213-220.

KRESS K., VERHAAGH M. (2020): The economic impact of German pig carcass pricing systems and risk scenarios for boar taint on the profitability of pork production with immunocastrates and boars. Agriculture, 9: 204.

MERSMAN H.J. (1984): Effect of sex on lipogenic activity in swine adipose tissue. Journal of Animal Science, 58: 600-604.

PETERSEN J.S., BERGE P., HENCKEL P., SOERENSEN M.T. (1997): Collagen characteristics and meat texture of pigs exposed to different levels of physical activity. Journal of Muscle Foods, 8: 47-61.

O’REILLY M.W., HOUSE P.J., TOMLINSON J.W. (2014): Understanding androgen action in adipose tissue. The Journal of Steroid Biochemistry and Molecular Biology, 143: 277-284.

PRUNIER A., BONNEAU M., VON BORELL E.H., CINOTTI S., GUNN M., FREDRIKSEN B., GIERSING M., MORTON D.B., TUYTTENS F.A.M., VELARDE A. (2006): A review of welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. Animal Welfare, 15: 277-289.

ŠKRLEP M., TOMAŠEVIĆ I., MÖRLEIN D., NOVAKOVIĆ S., EGEA M., GARRIDO M.D., LINARES M.B., PEÑARANDA I., ALUWÉ M., FONT-I-FURNOLS M. (2020a): The use of pork from entire male and immunocastrated pigs for meat products – An overview with recommendations. Animals, 10: 1754.

ŠKRLEP M., POKLUKAR K., KRESS K., VRECL M., FAZARINC G., BATOREK LUKAČ N., WEILER U., STEFANSKI V., ČANDEK-POTOKAR M.

(2020b): Effect of immunocastration and housing conditions on pig carcass and meat quality traits. Translational Animal Science, 4: 1224-1237.
ŠKRLEP M., TOMAŽIN U., BATOREK LUKAČ N., POKLUKAR K., ČANDEK-POTOKAR M. (2019): Proteomic profiles of the longissimus muscles of entire male and castrated pigs as related to meat quality. Animals, 9:74.
ŠKRLEP M., ČANDEK-POTOKAR M. (2018): Meat quality issues in entire male and immunocastrated pigs. Book of Abstracts of the 69th Annual Meeting of the European Federation of Animal Science, August 27 – 31, Dubrovnik, p. 231.
TAVÁREZ M.A. PULS C.L., SCHROEDER A.L., DILGER A.C. (2014): Effects of immunocastration and time after second improves dose on adipose tissue fatty acid profile of finishing barrows. Journal of Animal Science, 92: 3736-3744.
VOLD E., MOEN R.A. (1972): A note on the effect of castration upon the development of the skin in the pig. Animal Science, 14: 253-254.
WOOD J.D., ENSER M., WHITTINGTON F.M., MONCRIEFF C.B., KEMPSTER A.J. (1989): Backfat composition in pigs: differences between fat thickness groups and sexes. Livestock Production Science, 22: 351-362.

IN VITRO BOAR FERTILITY DURING SUMMER AND AUTUMN SEASON

Aleksandra Petrović[1], Dragan Radojković[1], Čedomir Radović[2], Marija Gogić[2], Nenad Stojiljković[2], Nenad Parunović[3], Radomir Savić[1]

[1] University of Belgrade, Faculty of Agriculture, 11080, Belgrade - Zemun, Republic of Serbia
[2] Institute for Animal Husbandry, 11080, Belgrade - Zemun, Republic of Serbia
[3] Institute of Meat Hygiene and Technology, 11000, Belgrade, Republic of Serbia
Corresponding author: Radomir Savić, savic@agrif.bg.ac.rs

Abstract: A primary objective of this study was to determine the effect of breed, frequency of utilisation, boar age and season on following sperm characteristics: volume of ejaculate (ml), sperm concentration ($x10^6$ spermatozoa/ml), total count and count of functional spermatozoa in ejaculate ($x10^9$ spermatozoa), motility of spermatozoa in native ejaculate and after dilution (%), number of produced doses, percent of dead and viable spermatozoa. Research included 4 boars of Landrace breed (n=40 ejaculates) and 13 boars of Large White pig (n=89 ejaculates). Ejaculates were analyzed during two seasons (summer and autumn), a dividing line being a calender start of autumn. Interval between two mounts was observed on two levels: ≤7 and ≥ 8 days. The assessment of the effect was done by means of a General Linear Model procedure. Breed and frequency of utilisation did not affect an average expression and variability of sperm characteristics. By increasing boar age by one day the volume of ejaculate increases by 0.142 ml. In ejaculates taken during autumn season a higher concentration of sperm per ml of ejaculate by $53.38x10^6$ spermatozoa ($p=0.033$) was determined. Higher motility ($p<0.001$) of native i.e. diluted semen of 5.7, i.e. 8.2% was determined in ejaculates taken during summer season. A higher percent (+13.47%; $p<0.001$) of dead spermatozoa was determined in ejaculates in autumn season.

Key words: boar, breed, sperm, frequency of utilisation, age, season

Introduction

An artificial insemination is a primary method of reproduction in an intensive pig production *(Lopez Rodruguez et al., 2017)*. Boar fertility exerts a considerably greater impact on reproductive efficiency of breeding stock in comparison with the fertility in sows *(Stančić, 2014).* In selection of breeding animals a great attention was payed to the traits of growth and food conversion. Thus, *Robinson and Buhr (2005)* suggest that major aims of selection of male animals are in the first place the traits that can have greater economic importance, primarily traits of growth.

The characteristics of sperm may vary under the impact of different genetic and non-genetic factors: breed, age, season, intensity of utilisation and other factors (*Kondracki et al., 2009; Smital, 2010; Kunowska-Slósarz and Makowska, 2011; Wilczyńska et al., 2013).*

There are differences between breeds regarding the size of testes, number of spermatozoa per ejaculate, volume of ejaculate, concentration and motility of sperm *(Caisin and Snitco, 2016). Wolf and Smital (2009)* determined in their research that there exists a difference in volume of ejaculates between boar breeds. Boar breed and season of collecting sperm have a significant effect on the volume of ejaculate, as well as on the share of motile spermatozoa *(Okere et al., 2005).*

Frequency of sperm collecting is an important factor in boar reproduction. It happens that some boars are more often used because of their easier manipulation or shorter preparation time for mount, while the intensity of using is ignored and the pauses between mounts are too short what leads to excessive exhaustion of boar and to obtaining ejaculates of poor fertile quality *(Savić, 2014).*

High summer temperatures have a direct negative impact on the process of spermatogenesis in testes. In their research *Savić et al. (2013)* determined that ejaculate quality parameters are significantly higher during a cooler season of the year in relation to a warm season. *Argenti et al. (2018)* state that although there are differences in microclimatic parameters between seasons which are manifested primarily through higher temperature-humidity index during summer in relation to other periods of the year, the impact of season on sperm characteristics is low.

Volume of ejaculate increases up to the age of about two years and remains more or less constant *(Wolf and Smital, 2009*). From a biological aspect one of the reasons of increase in the production of sperm with boar age can be explained by the increase in the number of Sertoli cells in testes (*Kanokwan, 2011).* The highest values of volume of ejaculates, total number and number of functional spermatozoa were determined in the age of 2.5-3 years *(Wang et al., 2017)*.

The aim of this research was to study the effect of the most important factors (breed, interval between two mounts, season and age of boar) on average manifestation and variability of sperm characteristics.

Materials and Methods

The research was conducted on a pig farm with its own reproductive and commercial breeding stock in the period from August to October. Boars were placed in a separate pen, in boxes of dimensions 2x4 m, with partially latticed and concrete flooring. Housing microclimatic conditions were manually regulated by a vertical and horizontal ventilation. Nutrition was based on balanced feed mixtures while fresh water was available ad libitum.

The trial included 4 boars of the Landrace breed (n=40 ejaculates) and 13 boars of Large White breed (n=89 ejaculates). In order to be included in the analysis a boar was to realize a minimum of three successful mounts during a trial period. The ejaculates were analysed in the course of the two seasons (summer and autumn), and the dividing line was a calendar beginning of autumn. The interval between two mounts was observed on two levels: ≤7 and ≥ 8 days.

The research included: volume of ejaculate (VOL, ml), concentration of sperm (CON, x10^6 spermatozoa/ml), total count of spermatozoa in ejaculate (NT, x10^9 spermatozoa), total count of functional spermatozoa (NF, x10^9 spermatozoa), percentage of motility of native semen (MOTN, %), percentage of motility of semen after dilution (MOTD, %), number of produced doses (NPD), percentage of dead (PM, %) and percentage of viable spermatozoa (PZ, %).

Boar sperm collection was done by a standard manual method, by bringing the boar into the box with phantom. Volume of ejaculate was expressed in millilitres with an accuracy of ±2 ml, and it was measured by a graduated cylinder. By means of a photo colorimeter concentration of native sperm was estimated. Total number of spermatozoa in ejaculate was obtained by multiplying VOL by CON. Number of functional spermatozoa was calculated by multiplying NT by MOTN. The estimation of motility of the mass of spermatozoa in native ejaculate and after dilution was carried out by a subjective assessment, by a microscopic examination. A percentage of dead and viable spermatozoa in sperm was determined on a permanent Eosin-Nigrosin stain preparation where living spermatozoa appear on a dark background non-stained while the dead ones are parly or completely stained (*Savić and Petrović, 2019*). Doses for insemination were standardized at the volume of 100 ml.

The estimation of the effect of factors on variation of the sperm characteristics was performed by means of a General Linear Model in SAS 9.1.3 statistical package *(SAS Inst. Inc., 2002-2003)*, by a following statistical model:

$$y_{ijkl} = \mu + B_i + I_j + S_k + BI_{ij} + BS_{ik} + IS_{jk} + BIS_{ijk} + b(x_{ijkl} - \bar{x}) + e_{ijkl},$$

where: $y_{ijk}l$ – is an analysed characteristic of ejaculate; μ – general population average; B_i – effect of boar breed (i= 1,2); Ij – effect of interval between two consecutive mounts (j= 1,2); S_k – effect of season (k= 1,2); BI_{ij}, BS_{ik}, IS_{jk}, BIS_{ijk} – interactions; $b(x_{ijkl}-\bar{x})$ - linear regression effect of boar age and e_{ijkl} – random error.

Comparing the Least Square Means (LSMeans) values of sperm characteristics was done by t-test.

Results and Discussion

The effect of studied factors on variability of sperm characteristics is shown in Table 1. The season of sperm collecting had an effect on qualitative traits of ejaculates (CON, MOTN, MOTD, PM, PZ). The low values of determination coefficients (<30%) obtained by the model applied suggest that studied effects can to a small degree explain variability of studied sperm characteristics. The age of boar during collecting the ejaculate only had an effect on volume, while determined value of regression coefficient shows that with increasing the age of boar by one day the volume of ejaculate increases by 0.142 ml.

The research conducted is parly consistent with the results of the research of *Smital (2010) and Wierzbicki et al. (2010)* who determined the effect of different genetic and non-genetic effects on mean expression and variability of sperm characteristics. *Petrocelli et al. (2015)* determined a significant effect of season on the vitality, total and primary abnormalities, volume and concentration of sperm. The research of *Tereszkiewicz and Pokrywka (2020)* showed a seasonal differences in physical characteristics of semen of studied boar breeds.

Table 1. Effect of factors included in the model on variability of sperm traits

Traits	B	I	S	b±SE	R^2
VOL (ml)	ns	ns	ns	0.142±0.046 **	0.140
CON ($x10^6$/ml)	ns	ns	*	-0.071 ±0.058 ns	0.134
NT ($x10^9$)	ns	ns	ns	0.021±0.025 ns	0.096
NF ($x10^9$)	ns	ns	ns	0.015±0.021 ns	0.128
MOTN (%)	ns	ns	***	-0.003±0.004 ns	0.231
MOTD (%)	ns	ns	***	0.002±0.005 ns	0.232
NPD	ns	ns	ns	0.002±0.002 ns	0.058
PM (%)	ns	ns	***	0.005±0.007 ns	0.294
PZ (%)	ns	ns	***	-0.005±0.007 ns	0.294

VOL – volume of ejaculate, CON – concentration of sperm, NT – total number of spermatozoa, NF – number of functional spermatozoa, MOTN – motility of native semen, MOTD – motility of semen after dilution, NPD – number of produced doses, PM – percentage of dead spermatozoa, PZ – percentage of viable spermatozoa, B – effect of breed, I – interval between two consecutive jumps, S – season; b – coefficient of regression (age of boar), R^2 – coeficient of determination; Statistical significance (p): ns=p>0.05; *=p<0.05; **=p<0.01; ***=p<0.001; ¶Model also included interactions of factors that were not statistically significant.

Sperm concentration varied under the effect of season (Table 2). In the ejaculates collected during autumn season a higher concentration ($p=0.033$) of sperm per ml of ejaculate by 53.38×10^6 spermatozoa was determined. Lower concentration of sperm during summer period can be a consequence of a negative effect of high summer temperatures. *Savić et al. (2015)* likewise determined that season had an effect on concentration of spermatozoa, the highest concentration (242.16×10^6 spermatozoa/ml) being recorded during autumn months. The effect of season on concentration of sperm was determined also in the research of *Chinchilla-Vargas et al. (2018)* where ejaculates taken during summer had higher concentration compared to the ejaculates collected during other seasons in the year. In our research higher concentrations of sperm were recorded in the ejaculates collected during autumn season what is in contrast to the research mentioned. In spite of decrease in concentration of sperm in spring and summer, majority of semen parameters was constant over an entire year *(Argenti et al., 2018).* These differences between the studies may be a consequence of different experimental designs but also of genetic structures of studied populations.

Contrary to our research, *Apić et al. (2015)* state that the season affected variability of total count of spermatozoa in ejaculate, a higher value being determined during a cold season. Different to our research, *Smital (2010)* determined that number of functional spermatozoa varied under the effect of season, the highest number being observed in winter period while in summer period the lowest number was detected.

A higher motility ($p<0.001$) of native, i.e. diluted semen of 5.70, i.e. 8.52% was determined in the ejaculates taken during summer season (Table 2). Our results are in contrast with the conclusions of *Savić et al. (2020)* whose research showed that motility of native semen did not vary under the effect of season.

Table 2. Effect of season on variability of sperm traits

Traits	LSMeans±SE		p
	Summer	Autumn	
VOL (ml)	311.13±12.93	303.04±11.90	0.646
CON ($\times 10^6$/ml)	329.86±18.59	383.24±16.05	0.033
NT ($\times 10^9$)	106.36±8.08	117.67±6.97	0.292
NF ($\times 10^9$)	101.11±5.89	85.34±6.83	0.085
MOTN (%)	85.81±0.98	80.11±1.08	<0.001
MOTD (%)	80.94±1.35	72.42±1.76	<0.001
NPD	15.61±0.61	15.51±0.77	0.922
PM (%)	18.61±1.80	32.08±2.27	<0.001
PZ (%)	81.38±1.80	67.92±2.27	<0.001

VOL – volume of ejaculate, CON – concentration of sperm, NT – total count of spermatozoa, NF – number of functional spermatozoa, MOTN – motility of native semen, MOTD – motility of semen after dilution, NPD – number of produced doses, PM – percent of dead spermatozoa, PZ – percent of living spermatozoa; p - statistical significance.

Contrary to the results of our research, *Savić et al. (2015)* determined that the number of doses produced varied under the impact of the interval between the two mounts and the season further stating that the highest number of doses was produced over autumn months. Also, *Chinchilla-Vargas et al. (2018)* determined that number of doses obtained per ejaculate was lower in the ejaculates collected during spring and winter in comparison with the ejaculates from summer period.

Percentage of dead and viable spermatozoa varied under the effect of season (Table 1). Higher percentage ($p<0.001$) of dead spermatozoa by 13.47% was determined in sperm taken in the autumn season (Table 2). *Knecht et al. (2014)* determined the highest percentage of viable spermatozoa in winter period. A higher count of dead and lower count of viable spermatozoa during the autumn season determined in this research could be a consequence of the occurrence of chronic thermal stress during the summer months. High day temperatures occur in our climatic region all up to early autumn and boars were in longer time period exposed to this stress effect. A period of resuming a physiological optimum was too short what might have affected the results of research.

Conclusion

Sperm characteristics did not vary under the effect of boar breed and frequency of utilization. The age of boar had an effect on the variability of the ejaculates volume. The season of collecting ejaculates affected sperm qualitative characteristics. Low values of determination coefficients indicate that studied effects can to a small degree explain variability of examined sperm characteristics. Taking into account that research period included the end of summer and beginning of autumn period the boars were in a longer time period exposed to the effect of high summer temperatures therefore a longer time was needed in order that production of sperm should resume its physiological optimum what probably affected the results obtained.

In vitro plodnost nerasta tokom letnje i jesenje sezone

Aleksandra Petrović, Dragan Radojković, Čedomir Radović, Marija Gogić, Nenad Stojiljković, Nenad Parunović, Radomir Savić

Rezime

Osnovni cilj ovog istraživanja bio je da se utvrdi uticaj rase, frekvence korišćenja, starosti nerasta i sezone na osobine sperme: volumen ejakulata (ml), koncentracija sperme ($x10^6$ spermatozoida/ml), ukupan broj i broj funkcionalnih spermatozoida u ejakulatu ($x10^9$ spermatozoida), pokretljivost spermatozoida u nativnom ejakulatu i nakon razređenja (%), broj proizvedenih doza, procenat mrtvih i živih spermatozoida. U istraživanje su bila uključena 4 nerasta rase landras (n=40 ejakulata) i 13 nerasta velikog jorkšira (n=89 ejakulata). Ejakulati su analizirani tokom dve sezone (letnje i jesenje), a granica razdvajanja bila je kalendarski početak jeseni. Interval između dva skoka posmatran je na dva nivoa: ≤7 i ≥ 8 dana. Procena uticaja izvršena je primenom procedure opšteg linearnog modela. Rasa i frekvenca korišćenja nisu uticale na prosečnu ispoljenost i varijabilnost osobina sperme. Povećanjem starosti nerasta za jedan dan, volumen ejakulata se povećava za 0,142 ml. U ejakulatima uzetim tokom jesenje sezone utvrđena je veća koncentracija sperme po ml ejakulata za $53{,}38x10^6$ spermatozoida (p=0,033). Veća pokretljivost ($p<0{,}001$) nativnog, odnosno razređenog semena od 5,7 odnosno 8,2% je utvrđena u ejakulatima uzetim tokom letnje sezone. Veći procenat (+13,47%; $p<0{,}001$) mrtvih spermatozoida utvrđen je kod ejakulata u jesenjoj sezoni.

Ključne reči: nerast, rasa, sperma, frekvenca korišćenja, starost, sezona

Acknowledgements

The results of the research presented in this paper were financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, on the basis of the Agreement on the realization and financing of scientific research work of SRO in 2021 no. 451-03-9/2021-14/200116, 451-03-9/2021-14/200022 and 451-03-9/2021-14/200050.

References

APIĆ J., VAKANJAC S., RADOVIĆ I., JOTANOVIĆ S., STANKOVIĆ B., KANAČKI Z. (2015): Effect of season on boar semen quality. Contemporary Agriculture, 64, 1-2, 9-13.

ARGENTI L.E., PARMEGGIANI B.S., LEIPNITZ G., WEBER A., PEREIRA G.R., BUSTAMANTE-FILHO I.C. (2018): Effects of season on boar semen parameters and antioxidant enzymes in the south subtropical region in Brazil. Andrologia. 50, 4,e12951. doi: 10.1111/and.12951

CAISIN L., SNITCO T. (2016): The influence of the seasonality on boars sperm production - breeder of different species. Bulg. J. Agric. Sci., 22, Suppl. 1, 118–122.

CHINCHILLA-VARGAS J., KERNS K., ROTHSCHILD F. M. (2018): Lunar and climatic effect on boar ejaculate traits. Animal Reproduction Science, 193, 117-125.

KANOKWAN K. (2011): Association and expression study of CD9, PLCz and COX-2 as candidate genes to improve boar sperm quality and fertility traits. Institut für Tierwissensechaften, Abt. Tierzucht und Tierhaltung der Rheinischen Friedrich-Wilhelms- Universität Bonn. Inaugural-Dissertation.

KNECHT D., ŚRODOŃ S., DUZIŃSKI K. (2014): The influence of boar breed and season on semen parameters. South African Journal of Animal Science, 44, 1, 1-9.

KONDRACKI S., WYSOKIŃSKA A., KOWALEWSKI D., MUCZYŃSKA E., ADAMIAK A. (2009): Season's influence on the properties of male domestic pig semen. Rozprawy naukowe Pope John Paul II State School of Higher Vocational Education in Biała Podlaska, III**,** 177-187.

KUNOWSKA-SLÓSARZ M., MAKOWSKA A. (2011): Effect of breed and season on the boar's semen characteristics. Annals of Warsaw University of life sciences- SGGW. Animal Science, 49, 77-86.

LOPEZ RODRIGUEZ A., VAN SOOM A., ARSENAKIS I., MAES D. (2017): Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Management, 3, 1, 1-12.

OKERE C., JOSEPH A., EZEKWE M. (2005): Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. Journal of Animal and Veterinary Advances, 4, 10, 885-888.

PETROCELLI H., BATISTA C., GOSÁLVEZ J. (2015): Seasonal variation in sperm characteristics of boars in southern Uruguay. Revista Brasileira de Zootecnia, 44, 1, 1–7.

ROBINSON J.A.B., BUHR M.M. (2005): Impact of genetic selection on managment of boar replacement. Theriogenology, 63, 668-678.

SAS INST. INC. (2002-2003): The SAS System for Windows. Cary. NC.

SAVIĆ R. (2014): Fenotipska i genetska varijabilnost plodnosti nerasta. Doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu.

SAVIĆ R., PETROVIĆ M. (2019): Praktikum iz svinjarstva. Univerzitet u Beogradu, Poljoprivredni fakultet, 1-182.

SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N. (2013): The effect of breed, boar and season on some properties of sperm. Biotechnology in Animal Husbandry 29, 2, 299-310.

SAVIĆ R., PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., PARUNOVIĆ N., POPOVAC M., GOGIĆ M. (2015): Ejaculate properties and reproductive efficiency of large white boars during exploitation. Biotechnology in Animal Husbandry 31, 3, 397-405.

SAVIĆ R., RADOJKOVIĆ D., STOJILJKOVIĆ N., PARUNOVIĆ N., GOGIĆ M., RADOVIĆ Č. (2020): Effect of breed of performance tested boars on ejaculate traits. Biotechnology in Animal Husbandry 36, 3, 309-316.

SMITAL J. (2010): Comparasion of environmental variations in boar semen characteristics of six breeds and their crossbreds over an eight-year period. Research in Pig Breeding, 4, 1, 26-32.

STANČIĆ I. (2014): Reprodukcija domaćih životinja. Poljoprivredni fakultet, Novi Sad.

TERESZKIEWICZ K., POKRYWKA K. (2020): Results of breeding use of insemination boars of selected breeds in the seasons of the reproductive cycle of the European wild boar (*Sus scrofa* L.). Acta Sci. Pol. Zootechnica, 19, 2, 37–46.

WANG C., LI J.L., WEI H.K., ZHOU Y.F., TAN J.J., SUN H.Q., JIANG S.W., PENG J. (2017): Linear growth model analysis of factors affecting boar semen characteristics in Southern China1. Journal of Animal Science, 95, 12, 5339–5346.

WIERZBICKI H., GÓRSKA I., MACIERZYŃSKA A., KMIEĆ M. (2010): Variability of semen traits of boars used in artificial insemination. Medycyna Weterynaryjna, 66, 11, 765-769.

WILCZYŃSKA E., KONDRACKI S., WYSOKIŃSKA A., KOWALEWSKI D., GAJOWNIK K. (2013): The quality of boar semen of Polish Large White, Polish Landrace, Duroc and Pietrain breeds in different months of the year. Scientific Annals of Polish Society of Animal Production, 9, 1, 49-56.

WOLF J., SMITAL J. (2009): Effect in genetic evaluation for semen traits in Czech Large White and Czech Landrace boars. Czech J. Anim. Sci., 54, 8, 349-358.

VARIABILITY OF THE NUMBER OF LIVE-BORN PIGLETS UNDER THE INFLUENCE OF FEMALE GENOTYPE, YEAR OF FARROWING AND PARITY

Nenad Stojiljković[1], Dragan Radojković[2], Čedomir Radović[1], Marija Gogić[1], Vladimir Živković[1], Zoran Luković[3], Dubravko Škorput[3]

[1]Institute for Animal Husbandry, Autoput 16, P.Fax 23, 11080, Belgrade, Serbia
[2] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Serbia
3 University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb
Corresponding author: Nenad Stojiljković, zake9103@gmail.com

Abstract: The aim of this study was to determine the influence of sow genotype, year of farrowing and parity on the number of live-born piglets. Fertility traits of sows were tested during fourteen years (from 2007 to 2020) in one pig herd/population. The study included 10159 sows, 4 genotypes: Landrace, large White, Landrace x large White and large White Yorkshire x Landrace. The sow fertility data set contained data on 36189 parities. Based on the obtained results, it was determined that the genotype of sows, year of farrowing and parity had a statistically highly significant (P <0.01) influence on the number of live - born piglets.

Key words: fertility, sows, genotype, parity, year.

Introduction

The achieved production results in pig breeding directly depend on a large number of economically important groups of traits. Two important traits, which affect the economic efficiency of pig production, are the reproductive and productive capacity of sows (*Wähner and Brüssow, 2009; Škorput et al. 2020*). The annual productivity of sows is determined by the size of the litter and the number of parities per sow per year. Increasing one parameter affects the increase in sow productivity (*Kosovac et al., 2005; Radojković et al., 2005; Popovac et al., 2012; Živković et al., 2018*).

The size of a sow litter can be described as the number of live-born, stillborn, total-born and weaned piglets. The number of weaned piglets is even more commercially important than the size of the litter at birth. However, due to the widespread technological procedure of uniforming-equalization of litters of different sows that were farrowed at approximately the same time, this trait is second in importance, right after the number of live-born piglets. (*Luković, 2006; Radojković, 2007)*.

The number of live-born piglets is influenced by numerous external and genetic factors, as well as their interactions. There are reported data on farms on many effects that can be included in the models. Data on pig fertility, which are recorded on modern industrial farms, give a satisfactory description of the effects on the number of live-born piglets. The effects that can be included in the models are: parity, year of farrowing, mating or farrowing season, genotype, sire of the litter or sire breed, age at farrowing and different reproductive cycle intervals that affect the number of live piglets, and therefore the overall efficiency of pig production. Research in the direction of analysis of variability of fertility traits of sows was performed by: *Luković (2006); Luković et al. (2006, 2007); Radojković (2007); Radojković et al. (2005, 2014, 2018); Popovac et al. (2012); Luković and Radojković (2013); Škorput et al. (2016); Živković et al. (2018); Freyer (2018).*

Given the above, the aim of this study was to determine the influence of sow genotype, age and parity on the variation of fertility traits.

Material and Methods

Fertility traits of sows were tested during fourteen years (from 2007 to 2020) in one pig herd/population. The study included 10159 sows, 4 genotypes of Landrace, Large White and F1 crossbreeds of these breeds. The data set contained 36189 records on sow fertility and the following variables: animal identification number, genotype of the litter sire and dam, date of mating, date of farrowing, parity, and number of live-born piglets.

The values of statistical indicators for phenotypic expression and variability of the tested trait were calculated by the method of least squares using the GLM procedure of the software package SAS (*SAS Inst., Inc., Cary, NC*), using the following model:

Model:

$$Y_{ijkl}=\mu+S_i+P_k+G_l+e_{ijkl}$$

where:
Y_{ijkl} - observation vector for litter size,
S_i - fixed influence of the year of farrowing,
P_k - fixed influence of parity,
G_l - fixed influence of litter genotype,
e_{ijkl} - random error.

The choice of systemic influences in the model is based on the significance of the influences, the coefficient of determination and the degrees of freedom. The results are presented as mean values obtained by the method of least squares (LSMEAN) or as deviations of LSMEAN values from the population average in the form of a graph.

Results and Discussion

Based on the obtained research results (Table 1), a high number of live-born piglets is observed, which indicates a high average fertility of sows in the analysed population. The high coefficient of variation for the analysed trait is a consequence of large differences in litter size of different sows. The presented result of the average value of the number of live-born piglets (16.16) is higher compared to the results presented by *Luković (2006) and Radojković (2007).*

Table 1. Descriptive statistics of the number of liveborn piglets in the analysed population

N	$\bar{x}$	SD	Min	Max	CV
36189	16.16	3.59	0.00	31.00	22.20

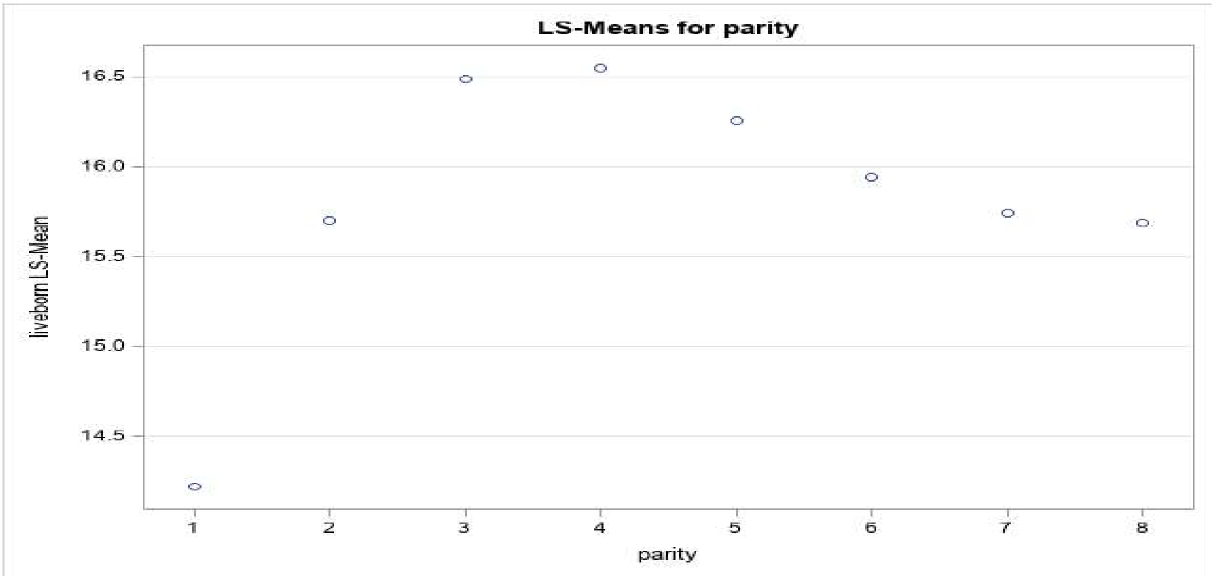
N- Number of litters, $\bar{x}$- mean value, ***SD***-standard deviation, ***Min***- minimum, ***Max***-maximum, ***CV***-coefficient of variation

The influence of genotype on the number of live-born piglets was highly statistically significant (Graph 2 and Table 2). Also, the parity and the year of farrowing had a highly statistically significant ($P<0.01$) influence on the size of the sow litter.

Table 2. Analysis of the significance of the influence in the model for the number of liveborn piglets in the analysed population

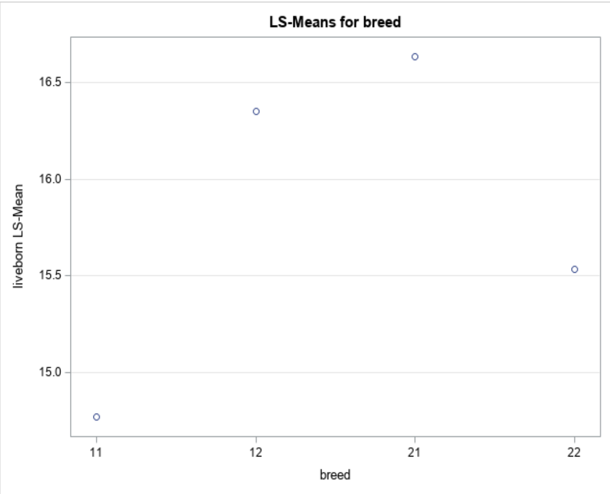
Source of variation	d.f.	MS	F	Pr>F
Genotype	3	778.23	79.89	<0.0001
Year	13	4905.91	503.60	<0.0001
Parity	7	3945.29	404.99	<0.0001

The highest number of live-born piglets in sow litters is observed in the third and fourth parity, after which a slight decline is observed (Graph 1). This is in accordance with reports by number of authors (*Kosovac et al., 2005; Luković and Radojković, 2013; Popovac et al., 2012; Škorput et al., 2016; Freyer, 2018)* who state that the highest numbers live-born piglets are achieved in the interval between the third and sixth farrowing, and after reaching maximum fertility in subsequent parities the value of this parameter slowly decreases. Such a result is based on the influence of sow age on litter size. Females grow until the end of the second year, when they reach their final size, and the size of their reproductive organs increases in that period, which leads to an increase in fertility.



Graph 1. Influence of parity on the number of live-born piglets

In the examined litter, the average number of live - born piglets varied between sow genotypes from 14.77 to 16.63 (Table 3). Differences in the average number of live-born piglets were highly statistically significant between all genotypes included in the analysis. The number of live-born piglets in a sow's litter can also be affected by whether the sows are purebred or are the result of crossbreeding (*Radojković, 2007*). When crossing different breeds, a heterosis effect can be manifested in a larger number of live-born piglets, which is confirmed in this analysis. Purebred sows had a lower number of live-born piglets compared to crossbreeds (Graph 2).



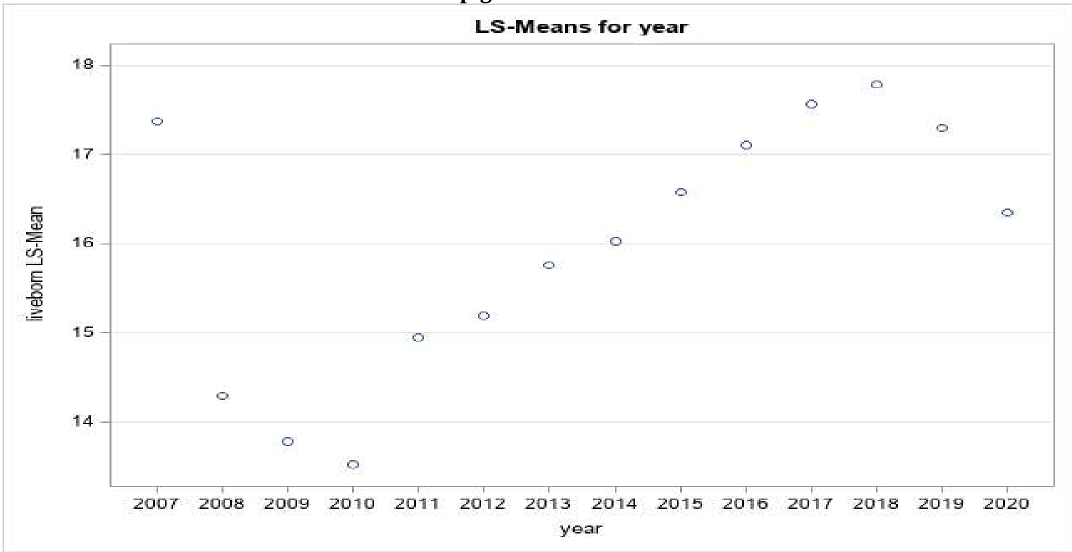
Breed	Live born	Pr > |t|
11	14.77±0.14	<0.0001
12	16.35±0.04	<0.0001
21	16.63±0.05	<0.0001
22	15.53±0.10	<0.0001

Graph 2. Influence of genotype on the number of live born piglets for the number of liveborn
11-Large White, **22**-Landrace, **12**- Large White x Landrace, **21**- Large White x Landrace

Table 3. LSM ± S.E.value piglets

The influence of the year on the number of live-born piglets of sows and gilts is shown in Graph 2. Great variability in the number of live-born piglets between years was observed, which is contrary to *Savić et al. (2011*). The mentioned authors have not determined a statistically significant influence of the year of farrowing on the number of liveborn piglets. The influence of the year of farming usually explains the effect of environmental factors that prevailed in certain years, such as nutrition and various technological procedures during the rearing of females (*Radojković, 2007; Popovac, 2016)*. Also, this influence can include the effects of selection in successive overlapping generations, producing at the same time. This usually happens when the study is conducted for many years.

Graph 3. Influence of the year on the number of liveborn piglets



Conclusion

Based on the results obtained in this study, we can conclude that the genotype of sows, age and parity have a statistically highly significant ($P<0.01$) influence on the number of live-born piglets.

In the analysed population, the number of live-born piglets of large White sows was 14.77, Landrace 15.53, Landrace x Large White 16.63 and Large White x Landrace 16.35. Purebred sows had a smaller number of live-born piglets compared to crossbreeds. The highest number of live-born piglets in sow litters is observed in the third and fourth parity, after which a slight decline is observed. The variability in the number of live-born piglets between years is a consequence of the action of external factors and partly of the selection effect that is carried out over many years.

Varijabilnost broja živorođene prasadi pod uticajem genotpa plotkinja, godine prašenja i pariteta

Nenad Stojiljković, Dragan Radojković, Čedomir Radović, Marija Gogić, Vladimir Živković, Zoran Luković, *Dubravko Škorput*

Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj genotipa krmače, godine prašenja i pariteta - rednog broja prašenja na broj živorođene prasadi. Osobine plodnosti krmača ispitivane su tokom četrnaest godina (od 2007. do 2020. godine) u jednom zapatu svinja. Istraživanjem je obuhvaćeno 10159 krmača, 4 genotipa: Landras, Veliki Jorkšir, Landras x Veliki Jorkšir i Veliki Jorkšir x Landras. Set podataka o plodnosti krmača sadržao je podatke o 36189 prašenja. Na osnovu dobijenih rezultata utvrđeno je da genotip krmača, godina prašenja i paritet statistički visoko značajno ($P<0,01$) utiču na broj živorođene prasadi.

Ključne reči: plodnost , krmače, genotip, paritet, godina.

Acknowledgment

Research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia**,** Agreement on the realization and financing of scientific research work of SRO no. 451-03-9/2021-14/200022**.**

References

FRAJER G. (2018): Maximum number of total born piglets in a parity and individual ranges in litter size expressed as specific characteristics of sows. Journal of Animal Science and Technology 60:13.
KOSOVAC O., PETROVIĆ M., ŽIVKOVIĆ B., FABJAN M., RADOVIĆ Č. (2005): Uticaj genotipa i prašenja po redu na variranje osobina plodnosti svinja. Biotechnology in Animal Husbandry, Institut za stočarstvo, Zemun 21 (3-4): 61-68.

LUKOVIĆ Z., RADOJKOVIĆ D. (2013): Dispersion parameters for litter size in pigs. International Symposium Modern Trends in Livestock Production, October 2013., 129-139.
LUKOVIĆ Z. (2006): Covariance functions for litter size in pigs using a random regression model. Doctoral Dissertation. University of Ljubljana, Biotechnical Faculty Ljubljana, Zootechnical Department, pp 1–93.
POPOVAC M. (2016): Fenotipska i genetska varijabilnost proizvodnih i osobina dugovečnosti krmača. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet Beograd, 1-178.
POPOVAC M., RADOJKOVIĆ D., PETROVIĆ M., MIJATOVIĆ M., GOGIĆ M., STANOJEVIĆ D., STANIŠIĆ N. (2012): Heritability and connections of sow fertility traits. Biotechnology in Animal Husbandry, 28 (3): 469-476.
RADOJKOVIĆ D. (2007): Varijabilnost osobina plodnosti i procena priplodne vrednosti svinja. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet Beograd, 1-208.
RADOJKOVIĆ D., PETROVIĆ M., MIJATOVIĆ M. (2005): Estimation of genetic variability of fertility traits of pigs. Biotechnology in Animal Husbandry, 21 (3-4): 93-97.
SAS Inst. Inc. (2011): The SAS System for Windows, Release 9.4. Cary, NC.
SAVIĆ R., PETROVIĆ M., RADOVI Č. (2011): Estimation of heritability coefficients of number of born alive piglets in the first three farrowings Swedish Landrace sows. Biotechnology in Animal Husbandry, 7 (1): 85- 92.
ŠKORPUT D., TORČEK I., MENČIK S., MAHNET Ž., KLIŠANIĆ V., KAROLYI D., LUKOVIĆ Z., SALAJPAL K. (2020): Čimbenici plodnosti krmača banijske šare svinje. 55th Croatian & 15th International Symposium on Agriculture, February 16-21, 2020, Vodice, Croatia, 389-393.
ŠKORPUT D., SMETKO A., KLIŠANIĆ V., ŠPEHAR M., MAHNET Ž., LUKOVIĆ Z. (2016): Mogućnosti selekcije na veličinu legla u crne slavonske svinje. Proceedings of 51st Croatian and 11th International Symposium on Agriculture, Opatija, Croatia, February 2016, 368-370.
WÄHNER M., BRÜSSOW K. P. (2009): Biological potential of fecundity of sows. Biotechnology in Animal Husbandry, 25 (5-6): 523-533.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

EFFECTS OF INVESTMENTS IN CAPITAL CROP PRODUCTION - A COMPARATIVE ANALYSIS OF THE REPUBLIC OF CROATIA AND THE EUROPEAN UNION

Dragan Dokić[1], Maja Gregić[2], Mirna Gavran[2], Vesna Gantner[2]

[1] Municipality of Erdut, Bana Josipa Jelačića 4, Dalj, Croatia
[2] Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek, Vladimira Preloga 1, Osijek, Croatia
Corresponding author: Dragan Dokić, dragan.dokic79@gmail.com

Abstract: Creating preconditions for economic development nowadays is unthinkable without investments. From the economic aspect, investments affect the turnover of capital, which creates material and technical preconditions for local development. It is noticeable that in practice, investments are given importance only in the theoretical sense, in other words, they are talked about a lot and speculated in the media. Therefore, it is necessary to improve business in terms of modernization of tangible assets, all to create new values, as a necessary prerequisite for further survival. The paper is thematically focused on the importance of investments and their direction in the improvement of the production process, particularly the introduction of innovations and the importance of modernization of production. The increase in the amount of investment on the one hand is determined by the increase in capital equipment, but on the other hand it must be accompanied by investment spending. That is why the paper aims to show the economic importance of investing in technologies that modernize the production process, which in the long run contributes to a stronger competitive position.

Key words: investments, crop production, capital intensive production, tangible assets, production volume

Introduction

The production process is the basis of any industrial production and includes all activities and actions that result in the conversion of input materials (raw materials, semi-finished products) into a finished product. It also includes all assets and personnel on which and with which activities are performed from the warehouse of input material to the warehouse of finished products. It consists of: technological process, transport process, organization process, and information process, so it is an indivisible whole of technology, technology, organization, and economy *(Mikac and Blažević, 2007)*. The concept of capital-intensive agricultural production implies the elaboration of the following elements *(Selaković, 1994)*:

• Production is a spatially and temporally determined process of conscious action of several factors, the result of which is the creation of material goods and services.

• The production process is the basis of any production and implies the process of converting input materials (raw materials, semi-finished products) into a finished product.

• Production goals imply the realization of the planned quantities of products from the production program.

• Technological process refers to the change of appearance, shape, dimensions, and properties of materials, change of physical and chemical properties.

• Production system is defined as an organizational form that integrates a group of different functions as subsystems necessary for the realization of the production process.

• The business system includes all the resources and activities necessary to achieve a particular business goal. Business success is reflected in the ratio of income and expenses.

• Information is a unit of measure of an organization, it represents everything that acts as an entrance to an organizational node, subsystem, or system, and its manifestations are always related to the material or energy that are its carriers, but not its content.

• The information system aims to collect and process information that is put into the function of production.

• Production organization is any connection and harmonization of an activity and solving the problems that arise from it, with the purpose of achieving common goals, for example raising performance. Organization implies both the process of organization and the formal structure that results from that process. The task of the organization of production is the temporal and spatial harmonization of the elements of production: human labour, available resources and means of labour.

Thus, the process of capital-intensive agricultural production does not consist only of simple physical activities, but also involves the application of teamwork and modern systems *(Bell and Senge, 1980)*. Capital-intensive production is inconceivable without investment. Investments have an important place in the application of this concept. The importance of investment is reflected primarily in the following *(Jones, 2007)*:

• ensure the continuity of production, and thus economic growth,

• provide extended reproduction,

• create material and technical conditions for the improvement of living standards,

• contribute to maintaining economic stability at the local level.

Investment management includes a set of all activities undertaken to effectively bring the investment process to a final conclusion. Investing as a process contains two very important and interrelated dimensions. These are time and uncertainty *(Hirt and Block, 2005)*. The time period that elapses from the appearance of the idea of need for investment to the realization of the investment, such as putting the investment into operation is usually very long. The time period of investment and uncertainty are directly correlated *(Pike, 2009)*. Longer investment times cause more uncertainty. Given its complexity, investing is a process that can cause negative effects due to wrong decisions *(Litterman, 2003)*. Therefore, investing cannot and must not be a spontaneous process, because it necessarily produces a situation in which wrong decisions are completely certain *(Sisek, 2005)*. The damage that can occur due to a spontaneous investment process or even mismanagement of investments is not limited to the company that leads the investment process, but can be extended to partners and the wider community.

In capital-intensive crop production, there is no need to plan to increase the area and intensity of resource use. On the contrary, we should insist on development in terms of increasing sustainability, productivity, and quality to optimally use and protect available resources *(Vukadinović and Jović, 2012)*. In the continuation of the research, the volume of investments into tangible assets will be considered. Investments into tangible assets can include, among other things, investments in the regulation of irrigation and land reclamation systems of agricultural land as an important factor in crop production. In addition to irrigation itself, it is necessary to take into account agro-technical measures which significantly increase the amount of humus in depleted soils, and thus the absorption capacity of the soil. Furthermore, energy efficiency and long-term competitiveness of agricultural producers can significantly support the diversification of energy sources, i.e. the use of biodiesel from their production capacities. And finally, investing in fixed assets, facilities, work equipment, and sophisticated equipment is very important because in this way a better production performance is achieved. All listed investments are statistically determined as tangible assets.

The aim of the paper was to show the economic importance of investing in technologies that modernize the production process, which in the long run contributes to higher production with a tendency to reduce total costs, while creating the preconditions for more competitive production.

Materials and Methods

The following table 1 shows the values of total production and investment into tangible assets. The data refer to the Republic of Croatia and the European Union (EU) and are expressed in millions of euros.

Table 1. Volume of production and investments into tangible assets in the Republic of Croatia and the EU (Prepared by the author based on data from the Central Bureau of Statistics of the Republic of Croatia; *DZS, 2020, Eurostat, 2020*)

Year	Republic of Croatia		European Union	
	Production volume	Investments	Production volume	Investments
2016	2.165.997.833	371.343.521	55.448.697.787	10.189.096.313
2017	2.311.802.514	299.566.871	57.862.355.002	11.996.353.245
2018	2.356.531.661	314.387.844	58.402.996.404	12.105.347.012
2019	2.278.756.551	347.554.226	59.640.428.902	12.401.994.338
2020	2.543.046.357	336.887.417	59.814.695.714	12.922.600.004

The data from the table above show the realized volume of field (plant) production in the Republic of Croatia and the EU, and investments directed to field production. In the Republic of Croatia, on average, 14.32% of the realized production volume is invested in new fixed assets (working stands), construction facilities, irrigation systems, agro-technical measures and renewable energy sources, while the value of investments for this purpose in the EU is 20, 87%.

Results and Discussion

The values expressed in Table 1 show that in the EU the volume of crop production and investments have a continuous growth, i.e. a higher volume of production for each subsequent year, is encouraged by a larger volume of investments, while in the Republic of Croatia the situation is diverse. On average, EU farmers invest 6.15% more of their earnings in new capital values compared to agricultural producers from the Republic of Croatia, which results in an increase in the total mass produced. Based on the correlation coefficient, it is possible to express the strength of the connection between the production concept in the Republic of Croatia and the EU. The ratio of production and investment in the Republic of Croatia is negative, i.e. the correlation is -0.39, which indicates a weak and negative relationship. In the Republic of Croatia, the concept of labour-intensive production is more represented, i.e. the labour force is significantly represented in the process of field production. The situation is quite the opposite in the EU, the data from Table 1 show a moderate positive relationship of 0.97, which indicates the conclusion that new capital is continuously invested, which creates new production values in the following cycles of reproduction. For large economic organizations in which capital-intensive production dominates, cost reimbursement is of great importance due to the structure of their total costs, because in such companies fixed costs have a significant share in total costs. "Fixed costs increase with increasing depreciation, i.e. increasing the capital intensity of the company, while variable costs decrease. Hence the division into labour-intensive companies and capital-intensive companies." *(Belak, 1995).* The dependence of investments on the volume of production is calculated by the linear trend equation:

$$y = a + bx$$

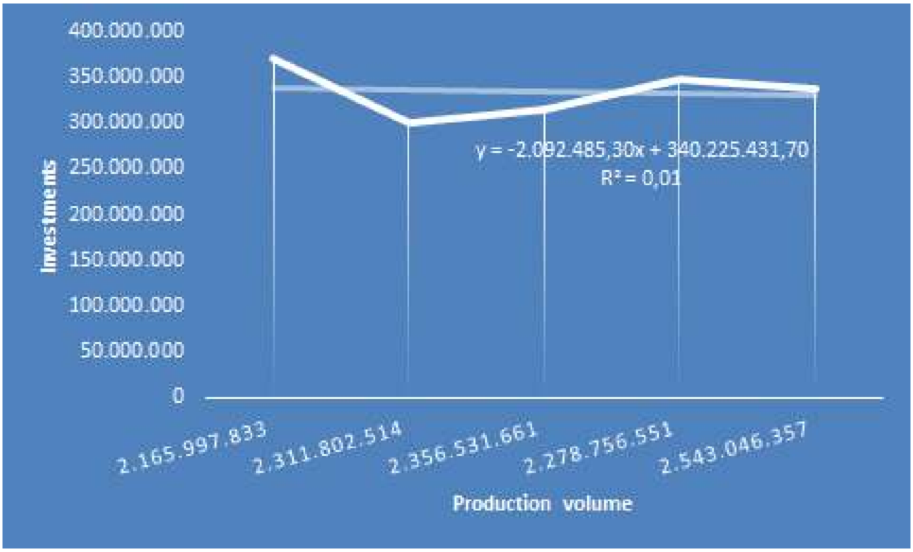


Figure 1. Linear trend of investment dependence on production volume in the Republic of Croatia (developed by the author based on the data from Table 1)

Based on the Figure 1 it could be observed that if the volume of production increased by 1, we can expect a decrease in investment by - 2,092,485.30. Also, 10% of the relationship between production volume and investment is explained by projected linear model.

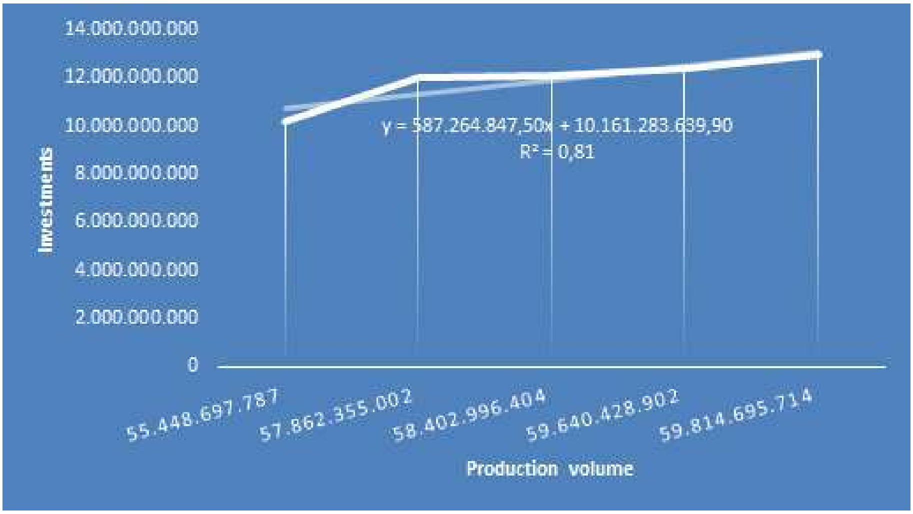


Figure 2. Linear trend of investment dependence on the volume of production in the EU (developed by the author based on the data from Table 1)

Based on the trend presented at the Figure 2 it could be observed that if the production volume increased by 1, we can expect an increase in investment by 587,264,847.50. Also, 81% of the relationship between production volume and investment is explained by used linear model.

Graphic results show that agricultural producers in the EU, unlike agricultural producers in the Republic of Croatia, maintain a positive proportion according to the current concept of the ratio of the volume of field production and investment in tangible assets. Any additional increase in production volume will result in an increase in investment. The trend of the ratio of the volume of field production and investment in tangible assets in the Republic of Croatia shows a discrepancy, because although the volume of production increases, the volume of investment decreases. The reasons can be multiple, but in general such a situation can be attributed to a large fiscal burden in relation to disposable income.

Conclusion

Investments are a generator of economic growth. However, if there is no favourable market climate, ie if monetary and fiscal policies are not implemented in the function of creating stimulating investment conditions, investments do not have sufficient strength to cause positive market shifts. In the conducted research, this is shown by the results in the Republic of Croatia. Capital-intensive production requires the engagement of machines, equipment and sophisticated technological production systems in the production process, on a larger scale than simple work. Capital-intensive production requires a higher level of investment, a higher amount of funds and financial resources. The capital-intensive production process is mostly automated and as such is able to generate a continuous increase in production volume. Since capital-intensive production relies mainly on machinery and equipment, such a concept requires long-term investments that pay off over the years. This statement is also supported by the results of the research, which is visible for the EU.

Efekti ulaganja u proizvodnju kapitalnih useva - uporedna analiza Republike Hrvatske i Evropske Unije

Dragan Dokić, Maja Gregić, Mirna Gavran, Vesna Gantner

Rezime

Stvaranje preduslova za ekonomski razvoj danas je nezamislivo bez ulaganja. Sa ekonomskog aspekta, investicije utiču na promet kapitala, što stvara materijalno-tehničke preduslove za lokalni razvoj. Uočljivo je da se u praksi ulaganjima daje značaj samo u teorijskom smislu, drugim rečima, o njima se mnogo priča i spekuliše u medijima. Zbog toga je potrebno unaprediti poslovanje u smislu modernizacije materijalne imovine, a sve radi stvaranja novih vrednosti, kao neophodnog preduslova za dalji opstanak. Rad je tematski fokusiran na značaj ulaganja i njihov smer u poboljšanju proizvodnog procesa, posebno na uvođenje inovacija i značaj modernizacije proizvodnje. Povećanje iznosa ulaganja, s jedne strane, određeno je povećanjem kapitalne opreme, ali s druge strane mora biti popraćeno investicionom potrošnjom. Zato rad ima za cilj da prikaže ekonomski značaj ulaganja u tehnologije koje modernizuju proizvodni proces, što dugoročno doprinosi jačanju konkurentske pozicije.

Ključne reči: investicije, ratarska proizvodnja, kapitalno intenzivna proizvodnja, materijalna sredstva, obim proizvodnje

References

BELAK V. (1995): Menadžersko računovodstvo. RRiF Zagreb, pp. 207.
BELL J., SENGE P. (1980): System dynamics and scientific method. In: Randers J. Waltham editor. Elements of the system dynamics method Williston, VT: Pegasus Communications, 141-145.
DRŽAVNI ZAVOD ZA STATISTIKU, DZS (2020): https://www.dzs.hr
EUROSTAT (2020): https://ec.europa.eu
HIRT G., BLOCK S. (2005): Managing Investments, McGraw-Hill, New York, pp. 91.
JONES C.P. (2007): Investments. John Wiley & Sons, Hoboken, pp. 88.
JOVANOVIĆ P. (2003): Upravljanje investicijama. Grafoslog Beograd, Beograd, pp. 15.
LITTERMAN B. (2003): Modern Investment Management. John Wiley & Sons, Hoboken, New York, pp. 61.
MIKAC T., BLAŽEVIĆ D. (2007): Planiranje i upravljanje proizvodnjom. Tehnički fakultet Rijeka, pp. 13.
PIKE R. (2009): Corporate finance and investment. Pearson Education, Harlow, pp. 148.
SELAKOVIĆ M. (1994): Organizacija poslovnih sistema. Tehnički fakultet Rijeka, Rijeka, 135- 149.
SISEK B. (2005): Strane izravne investicije u Hrvatskoj – uzroci neuspjeh. Zbornik Ekonomskog fakulteta u Zagrebu, Zagreb, 93-97.
VUKADINOVIĆ P., JOVIĆ P. (2012): Investicije. Univerzitet Singidunum, Beograd, 5-27.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

MYCOPOPULATION OF ALFALFA AND RED CLOVER HAY IN SERBIA

Sanja Živković, Tanja Vasić

Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Republic of Serbia
Corresponding author: Sanja Živković, gajicsanja43@gmail.com

Abstract: Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) belong to the family *Fabaceae* (*Papilionaceae*). Alfalfa and red clover have a special place in the crop rotation. After plowing the areas under alfalfa and red clover, large amounts of nitrogen and organic matter remain in the soil, whose decomposition and mineralization improve the physical, chemical and microbiological properties of the soil. So far, there have been no more detailed researches of the mycoflora of alfalfa hay and red clover in Serbia. In this paper, we present the results of preliminary research on the mycopopulation of 200 samples of alfalfa and red clover hay. A total of 4 genera of fungi were isolated from alfalfa and red clover hay, namely: *Fusarium, Phoma, Rhizoctonia and Verticilium.*

Key words: alfalfa, red clover, mycopopulation

Introduction

Alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) belong to the order *Fabales* (*Leguminosae*), the family *Fabaceae* (*Papilionaceae*) and the genus *Medicago* and *Trifolium*, respectively (*Kojić, 1984; Yli-Mattila et al., 2010*).

Considering field crops used as livestock feed, alfalfa has the most important role so it is refered as the "queen of fodder plants". As one of the oldest and the most important perennial fodder plants, alfalfa gives a high yield of fodder of excellent quality.

Alfalfa and red clover have a special place in the crop rotation. After plowing of areas under alfalfa and red clover, large amounts of nitrogen and organic matter remain in the soil. The decomposition and mineralization of this organic matter improve the physical, chemical and microbiological properties of the soil (*Tapia et al., 2005; Yli-Mattila et al., 2010*).

Alfalfa and red clover are usually grown independently, although they can also be used as components of grass-legume mixtures. Alfalfa and red clover hays are rich in proteins, with excellent amino acid composition and high digestibility (*Tapia et al., 2005; Yli-Mattila et al., 2010*). Alfalfa hay proteins are the cheapest source of protein in animal feed (*Ocokoljić et al., 1983*). In Serbia, alfalfa is the most important fodder perennial legume and is grown on about 190,000 ha. In the years of full exploitation, alfalfa can achieve high yields of green mass (70-90 tha^{-1}) and about 20 t ha^{-1} of dry matter in four to five cuts per year (*Lugić and Dinić, 2010*). Important regions for its cultivation in our country are: Vojvodina, especially Banat (Vršac, Kikinda, Zrenjanin), Bačka (Bečaj, Subotica, Sombor, Senta, Kula, Odžaci) and Srem (Sremska Mitrovica, Šid, Ruma, Stara Pazova). In central Serbia, the most significant alfalfa production areas are in Posavina: Mačva, Pomoravlje, Stig, Šumadija and Timočka Krajina (*Lukić, 2000*).

Red clover (*Trifolium pratense* L.) is a very important fodder crop. It is especially grown where the soil is not suitable for growing alfalfa. Red clover is considered a short-term fodder crop due to it lasting 2 to 4 years. It tolerates moist and acidic soils better than alfalfa. It is also important to note that red clover has less resistance to various diseases compared to other forage crops (*Yli-Mattila et al., 2010*).

Alfalfa and red clover are very important and productive fodder crops in agriculture, but like all crops, they are susceptible to infectious diseases that can limit their production. Disease control of these crops is very important in order to achieve higher economical production of protein-rich livestock feed.

Alfalfa and red clover diseases can reduce yields and quality. Also, diseases can reduce lifespan of composition of these crops. Sometimes, due to the disease, there can be a sudden deterioration of alfalfa and clover, which causes irreparable damage in productions to farmers.

Relatively low yields of alfalfa and red clover in Serbia are certainly a consequence of the impact of inadequate agricultural techniques and, sometimes, utilization of inadequate soil, but also of the occurrence of pests and plant pathogens (*Mijušković, 1993; Yli-Mattila et al., 2010*). Many diseases are characterized by plant death, which reduces the yield and quality of these crops. Disease pathogens attack individual parts or the whole plant in various developmental phenophases (*Vučković, 1999; Yli-Mattila et al., 2010*). For many years, the reduced longevity of alfalfa and red clover crops has been one of the main problems in the production of these fodder crops around the world. Frost damage, root mycosis, and bacterial wilt are all common factors that cause this problem (*O'Rourke and Millear, 1966; Yli-Mattila et al., 2010*).

The aim of this research was to determine phytopathogenic fungi on alfalfa and red clover hay, with the task to clarify causes of reduced quality and yield of these forage crops, as well as premature decay of their composition.

Material and Methods

Alfalfa and red clover hay was sampled on the territory of the Republic of Serbia in Rasina district (Globoder, Gaglovo, Selo Varvarin, Kobilje, Veliki Šiljegovac) and Pomoravlje district (Bobovo, Gložane, Tropanje) in the period of 2017 and 2018. Samples were taken by cutting off parts of alfalfa hay and red clover. The samples obtained in that way were carefully washed under running water. After rinsing, stems were carefully cut into 0.5 to 1 cm long pieces. The prepared stem sections were disinfected with 96% ethanol solution for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 min and then washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. Five pieces of the plant parts (roots and stems) were placed in individual Petri dishes in four replications. They were kept in a thermostat at 25 °C in 12 h light / 12 h night regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial growth, the peak part of the mycelium was reseeded on PDA again.

Microscopic examination was performed using microscope Olympus CX31. Morphological identification of fungi to the genus was carried out using a standard key. The frequency of isolation in % was calculated according to the formula by *Vrandečić et. al. (2011)*:

$$\text{(\%) Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results

In this study, mycopolation on alfalfa and red clover hay was monitored in the period from 2017 to 2018. On the territory of the Republic of Serbia, a total of 200 samples of alfalfa and red clover hay were collected in two districts: Rasina district (Globoder, Gaglovo, Selo Varvarin, Kobilje, Veliki Šiljegovac) and Pomoravlje district (Bobovo, Gložane, Tropanje). Out of a total of 200 samples, 160 samples of alfalfa and red clover hay developed a fungal colony, while 40 samples did not develop any fungal colonies (Table 1). A total of 120 alfalfa samples were tested, of which 92 samples developed fungal colonies and 28 alfalfa hay samples did not. Out of 80 tested samples of red clover hay, 68 samples developed fungal colonies and 12 samples did not.

Fungi from the genera *Fusarium*, *Phoma*, *Rhizoctonia* and *Verticilium* were isolated from alfalfa and red clover hay (Table 1).

Table 1. Frequency of fungal isolation on alfalfa and red clover on the hay

District	Year of sampling	Number of samples Plant part - stem	Fungal species - stem	(%) Isolation frequency
Red clover – Globoder	2017	20	*Fusarium* sp. *Phoma* sp.	60 35
Red clover – Gaglovo	2017	20	*Fusarium* sp. *Rhizoctonia* sp. *Phoma* sp	15 40 20
Red clover – Selo Varvarin	2018	20	*Fusarium* sp. *Verticillium* sp. *Phoma* sp.	20 60 15
Red clover – Kobilje	2018	20	*Fusarium* sp. *Verticillium* sp. *Phoma* sp.	15 40 20
Alfalfa - Gložane	2017	20	*Fusarium* sp. *Rhizoctonia* sp.	65 20
Alfalfa - Kobilje	2017	20	*Fusarium* sp. *Phoma* sp.	55 20
Alfalfa - Globoder	2017	20	*Fusarium* sp. *Phoma* sp.	45 40
Alfalfa - Bobovo	2018	20	*Verticillium* sp. *Rhizoctonia* sp.	45 40
Alfalfa - Veliki Šiljegovac	2018	20	*Fusarium* sp.	60
Alfalfa - Tropanje	2018	20	*Fusarium* sp.	70

A difference in the frequency of isolations of individual genera of fungi was noticed in these researches, depending on the site from which alfalfa and red clover hay originates.

Table 1 clearly shows that fungi from the genus *Fusarium* were present in all examined site, except in the site Bobovo - Pomoravlje district. Namely, representatives of the genus *Fusarium* were not isolated on alfalfa hay from this site, but only isolates belonging to the genera *Verticilium*. and *Rhizoctonia* were found. It can be concluded that the representatives of the genus *Fusarium* are most often isolated from alfalfa and red clover hay in almost all examined sites on the territory of the two districts of Rasina and Pomoravlje (Table 1). Fungi of the genus *Rhizoctonia* were isolated from the hay of red clover from the site Gaglovo - Rasina district, as well as from the hay of alfalfa at the location Gložane and Bobovo - both belong to the Pomoravlje district. Out of 10 examined sites, fungi of the genus *Phoma* were isolated from alfalfa and red clover hay samples at 6 localities (Table 1).

Discussion

Genera *Fusarium*, *Rhizoctonia*, *Phoma* are dominant in annual and perennial legumes worldwide (*Tivoli et al., 2006; Villegas-Fernández and Rubiales, 2011; Salam et al., 2011; Sillero and Rubiales, 2014, Vasić et al., 2015, Vasić et al., 2017*). *Al-Jaradi et al. (2018)* in Oman detected *Fusarium equiseti* on *Phaseolus vulgaris*.

Lukezić (1973), in his research, did not isolate *Colletotrichum trifolii* from alfalfa stem 90 days after harvest, most likely due to high humidity which favors the development of other pathogens. He isolated a large number of *Fusaurium* sp. from the same alfalfa hay samples, which is in accordance with the results of these studies.

Krnjaja et al. (2004) in their research divided the mycopopulation on seeds of 3 varieties of red clover K9, K17 and NS Kolubara, and two seed treatments - rinsing and surface disinfection (T1) and surface disinfection of seeds (T2). They found that the frequency of species of the genus *Fusarium* sp. varied from 0 to 13%, considering the presence of *Fusarium* sp. on the seeds of any of the tested varieties. Using T2 treatment, the incidence of *Fusarium* species varied from 1 to 13%, depending on the variety.

Vasić et al. (2011) studied mycopopulation on alfalfa seed. In the pathogenicity test, treated *P. vulgaris* showed yellowing symptoms with presence of lesions and root rot on the taproot. *F. equiseti* is one of the causal agents of foot and root rot disease which infects *Phaseolus vulgaris, Pisum sativum* and other crops.

Miličević et al. (2013), in Croatia, determined two *Fusarium* species, *F. verticillioides* and *F. proliferatum* on bean seeds. So, for these reasons, it is recommended to utilize crop rotation of four years, when it comes to the sowing of faba bean and pea (*Salam et al., 2011*). *Salam et al. (2011*) also cited *Phoma medicaginis* var. *pinodella* as significant pathogen in pea. *Rhizoctonia solani* Kühn is soil parasite that can cause serious problems in many legumes, especially on faba bean (*Assunção et al., 2011*). In Canada, 304 faba bean genotypes were tested on the resistance to *R. solani* and only five of them were identified with high resistance (*Rashid and Bernier*, *1993*). *Al-Jaradi et al. (2018*) also isolated *Rhizoctonia solani* from *Vigna unguiculata* in Oman.

Ligoxigakis et al. (2002) determined *V. dahliae* in Greece as a parasite on beans and other legumes.

Rhizoctonia solani Kühn is a soil parasite that can cause serious problems on many legumes especially on beans (*Assunção, 2011*). In Canada, 304 bean genotypes were tested for resistance to *R. solani* and only five were identified with high resistance (*Rashid and Bernier*, *1993*). *Ligoxigakis et al. (2002*) determined *V. dahliae* in Greece as a parasite on beans and other legumes.

According to *Tegegn, (2017)* taproot, chocolate spot reduces yield by up to 61%, with the presence of the probability of complete crop failure due to the disease. *Tegegn, (2017)* reported that rust can incur a maximum yield loss of up to 21%. So far, the control of these diseases had been attempted through the use of improved varieties, cultural practices, chemical fungicides and integration of two or more of the above options in Integrated Disease Management (IDM) scheme.

In these researches, a difference in the frequency of isolation of individual genera of fungi was noticed, depending on the locality from which alfalfa and red clover hay originates.

Conclusion

This paper presents the preliminary results considering mycopopulations on alfalfa and red clover hay, originating from two districts in Serbia - Rasina and Pomoravlje. Alfalfa and red clover are very important fodder crops for the production of hay and silage for livestock feed in our country. These researches are just the beginning of the research of phytopathogenic fungi on alfalfa and red clover hay.

Alfalfa and red clover are important forage crops and their importance as a livestock feed is growing within our country. This research is the beginning of a more comprehensive study of phytopathogenic fungi on alfalfa and red clover hay. So far, there were no significant researches in this direction in Serbia, so the future researches related to the hay of alfalfa and red clover will go in the direction of selection of genotypes with increased tolerance to fungal diseases.

Mikopopulacija na senu lucerke i crvene deteline u Srbiji

Sanja Živković, Tanja Vasić

Rezime

Lucerka (*Medicago sativa* L.) i crvena detelina (*Trifolium pratense* L.) pripadaju familiji *Fabaceae* (*Papilionaceae*). Lucerka i crvena detelina imaju posebno mesto u plodoredu. Nakon razoravanja površina pod lucerkom i crvenom detelinom u zemljištu ostaju velike količine azota i organskih materija, čijim se razlaganjem i mineralizacijom popravljaju fizičke, hemijske i mikrobiološke osobine zemljišta. Detaljnijih istraživanja mikoflore sena lucerke i crvene deteline u Srbiji do sada nije bilo. U ovome radu iznosimo rezultate preliminarnih istraživanja mikopopulacije 200 uzoraka sena lucerke i crvene deteline. Iz sena lucerke i crvene deteline ukupno je izolovano 4 roda gljiva, i to: *Fusarium, Phoma, Rhizoctonia* i *Verticilium*.

Ključne reči: lucerka, crvena detelina, mikopopulacija

References

ASSUNÇÃO I. P., NASCIMENTO L.D., FERREIRA M.F.; OOLIVIRA F.J., MICHEREFF S.J., LIMA G.S.A. (2011): Reaction of faba bean genotypes to *Rhizoctonia solani* and resistance stability. Horticultura Brasileira, 29, 492-497.

AL-JARADI A., AL-MAHMOOLI I., JANKE R., MAHARACHCHIKUMBURA S., AI-SAADY N., AL-SADI A.M. (2018): Isolation and identification of pathogenic fungi and oomycetes associated with beans and cowpea root diseases in Oman. PeerJ 6:e6064. http://doi.org/10.7717/peerj.6064

KOJIĆ M. (1984): Botanika. Naučna knjiga, Beograd. pp 519.

KRNJAJA V., LEVIĆ J., IVANOVIĆ M., TOMIĆ Z., MRFAT-VUKELIĆ S. (2004): Incidence of *Fusarium* species on red clover seed. Biotechnology in Animal Husbandry, 20, 1-2, 101-108.

LIGOXIGAKIS E. K., VAKALOUNAKIS D. J., THANASSOULOPOULOS C. C. (2002): Host range of *Verticillium dahliae* in cultivated species in Crete. Phytoparasitica, 30, 2, 141-146.

LUGIĆ Z., DINIĆ B. (2010): Proizvodnja, konzervisanje i iskoriščavanje kabaste stočne hrane kao osnovni uslov održivog razvoja stočarstva. Zbornik radova simpozijuma "Tradicija i budućnost stočarstva u brdsko planinskom području sa posebnim osvrtom na sjeničko peštersku visoravan", 22-24. jun, Sjenica, 20-49.

LUKEZIĆ F.L. (1973): Dissemination and survival of *Colletotrichum trifolii* under field conditions. Phytopathology, 64, 57-59.

LUKIĆ D. (2000): Lucerka. Naučni institut za ratarstvo i povrtarstvo, Novi Sad.

MILIČEVIĆ T., KALITERNA J., IVIĆ D., STRIČAK A. (2013): Identification and occurrence of *Fusarium* species on seeds of common vetch, whithe Lupine and some wild legumes. Agriculture, 19, 25-32.

MIJUŠKOVIĆ M. (1993): Najčešće mikoze lucerke u Crnoj Gori. Poljoprivreda i šumarstvo, XXXIX, 3-4, 55-64.

OCOKOLJIĆ S., MIJATOVIĆ M., ČOLIĆ D., BOŠNJAK D., MILOŠEVIĆ P. (1983): Višegodišnje leptirnjače. Prirodni i sejani travnjaci. Nolit, 118-141.

O'ROURKE C.J., MILLEAR R.L. (1966): Root rot and root microflora of alfalfa as affected bz potassium nutrition. Frequencz of cutting, and leaf infection. Phytopathology, 56, 1040-1046.

RASHID K. Y., BERNIER C. C. (1993): Genetic diversity among isolates of *Rhizoctonia solani* and sources of resistance in *Vicia faba*. Canadian Journal of Plant Pathology, 15, 23-28. https://doi.org/10.1080/07060669309500845

SALAM M.U., DAVIDSON J.A., THOMAS G.J., FORD R., JONES R.A.C., LINDBECK K. D., MACLEOD W. J., KIMBER R.B.E., GALLOWAY J., MANTRI N., van LEUR J.A. G., COUTTS B.A., FREEMAN A.J., RICHARDSON H., AFTAB M., MOORE K.J., KNIGHTS E.J., SILLERO J.C., ROJAS-MOLINA M.M., EMERAN A.A., RUBIALES D. (2011): Rust resistance in faba bean. Grain Legumes, 56, 27-28.

SILLERO J.C., RUBIALES D. (2014): Response of *Vicia* species to *Ascochyta fabae* and *Uromyces viciae-fabae*. Czech J. Genet. Plant Breed., 50, 109-115.

TAPIA S., PARDO F., PERICH F., QUIROZ A. (2005): Clover root borer *Hylastinus obscurus* (Marsham) (Coleoptera: Scolytidae): has no preference for volatiles from root extracts of disease infected red clover. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 55, 158-160.

TEGEGN ADDISU (2017): Effect of aerated and non-aerated compost steepages on the severity and incidence of major fungal diseases of faba bean; *Botrytis fabae*, *Uromyces vicia fabae* and *Ascochyta fabae*. Plant, 5, 6, 85-92. doi: 10.11648/j.plant.20170506.11

TIVOLI B., BARANGER A., AVILA C.M., BANNIZA S., BARBETTI M., CHEN W.D., DAVIDSON J., LINDECK K., KHARRAT M., RUBIALES D., SADIKI M., SILLERO J.C., SWEETINGHAM M., MUEHLEAUER F.J. (2006):

Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica, 147, 223-253.

VASIĆ T., ANĐELKOVIĆ S., ŽIVKOVIĆ S., ANĐELKOVIĆ B., TERZIĆ D., MILENKOVIČ J. (2011): Appearence and frequence of fungi on alfalfa seed in Serbia. Biotechnology in Animal Husbandry, 27, 4, 1579-1584.

VASIĆ T., MILENKOVIĆ J., LUGIĆ Z., TERZIĆ D., STANISAVLJEVIĆ R., BLAGOJEVIĆ M., DJOKIĆ D. (2015): Mycopopulation of different vetch genotypes in Serbia. Plant Protection, Belgrade, 66, 1, 291, 45-50.

VASIĆ T., MILENKOVIĆ J., ANDJELKOVIĆ S., TERZIĆ D., MARKOVIĆ J., DJOKIĆ D., SOKOLOVIĆ D. (2017): Fungal pathogens of vetch genotypes in Serbia. Journal of Mountain Agriculture on the Balkans, 20 2, 192-200.

VILLEGAS-FERNÁNDEZ A. M., RUBIALES D. (2011): Chocolate spot resistance in faba bean. Grain Legumes, 56, 29-30.

VRANDEČIĆ K., ĆOSIĆ J., JURKOVIĆ D., POČTIĆ J. (2011): Mycopopulation of medicinal plants in Croatia . Agriculture, 17,2, 18-21.

VUČKOVIĆ S. (1999): Krmno bilje. Institut za istraživanja u poljoprivredi “Srbija”, Beograd, Bonart, Nova Pazova. pp 1-553.

YLI-MATTILA T., G. KALKO G., HANNUKKALA A., PAAVANEN-HUHTALA S., HAKALA K. (2010): Prevalence, species composition, genetic variation and pathogenicity of clover rot (*Sclerotinia trifoliorum*) and *Fusarium* spp. in red clover in Finland. Eur J. Plant Pathol., 126, 13-27. doi 10.1007/s10658-009-9516-1

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

AN INSIGHT INTO THE MYCOTOXICOLOGICAL SITUATION – RECENT EXPERIENCE AND CLOSE PREDICTION

Ksenija Nešić, Nikola Pavlović, Marija Pavlović, Jelena Vlajković, Aleksandra Tasić, Vladimir Radosavljević, Božidar Savić

Institute of Veterinary Medicine of Serbia, Autoput 3, 11070 Belgrade, Serbia
Corresponding author: Ksenija Nešić, ksenija.nesic@gmail.com

Abstract:Mycotoxins are secondary metabolites of various fungi, primarily of *Aspergillus*, *Penicillium* and *Fusarium* genera. Fungal species commonly enter the food chain through contaminated food and feed, mainly cereals, which get infested prior to and at the harvest, or during (improper) storage. Although there are over 300 mycotoxins that have been isolated and chemically characterized, worldwide research has focused on those which significantly impact humans and animals. This paper presents test results of 340 samples of feedingstuffs and complete feed mixtures for different species and categories of farm animals randomly analyzed during 2019 and 2020 (120 and 220 respectively) for the presence of aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin and T-2/HT-2 toxin. Deoxynivalenol was the most frequently detected, while 8.82% of results in 2019 and 7.46% in 2020 were above the permitted levels. Pigs, as the most sensitive species of animals, are particularly affected by this. One sample of mixture for piglets, which contained deoxynivalenol 1.76 mg/kg, was the most contaminated, while the overall maximal value was determined in barley (4.73 mg/kg). The aim of this paper was to give a brief insight into the recent mycotoxicological situation with animal feed on the Serbian market. The results indicate the need for regular and comprehensive monitoring of fungal contaminants and their detrimental impacts on animal and human health, as well as the implementation of predicting models in the prevention strategies.

Key words*:* aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin, T-2/HT-2 toxin, feed

Introduction

Mycotoxins are produced by various fungal species, belonging primarily to genera *Aspergillus, Penicillium* and *Fusarium,* as their secondary metabolites. There are also other fungal genera, including *Alternaria, Cladosporium, Chaetomium, Claviceps, Diplodia, Myrothecium, Phoma, Phomopsis, Pithomyces* and *Stachybotrys,* that contain mycotoxigenic fungi. Under favorable environmental conditions, when temperature and moisture are suitable, fungi proliferate and may produce these toxic substances. The functions of mycotoxins have not been clearly established, but they are believed to play a role in eliminating other microorganisms competing in the same environment. They are also believed to help parasitic fungi invade host tissues. Toxigenic fungi (molds) are known to produce one or more secondary metabolites, but not all fungal species are toxigenic and not all secondary metabolites are toxic (*Brase et al., 2009*). Fungi commonly enter the food chain through contaminated food and feed, mainly cereals, which get infested prior to and during harvest, or during (improper) storage (*Nesic et al., 2021*). Although there are over 300 mycotoxins that have been isolated and chemically characterized, worldwide research has focused on those types causing significant harm to humans and animals (*Nesic et al., 2014*).

Even though there are geographic and climatic differences in the production and occurrence of mycotoxins, exposure to these substances is worldwide (*Eskola et al., 2020*). The accumulation of mycotoxins is known to reflect weather conditions. The two most important factors that affect the life cycle of all microorganisms, including mycotoxigenic fungi, are water availability and temperature. Given such a strong influence of meteorological situation, it is clear that climate change is very much reflected on mycotoxins (*Battilani et al., 2016; Nesic, 2018; Perrone et al., 2020).* Rising temperatures, changes in precipitation, rising sea levels, floods and droughts are widely present. As a result of the increase in temperature, more insects will appear on the crops, which are an important factor in the contamination with toxigenic fungi. The number of birds consuming these insects can also increase, which also contribute to crop damage and greater synthesis of mycotoxins. Changes in crop phenology are also predicted. Therefore, climate change is expected to strongly affect the geographical distribution of crops, as well as their mycoflora. So, predictive modeling is a very promising approach, with necessity to consider as many combinations of mycotoxins and crops at one locality as possible (*Van der Fels-Klerx, 2016).*

The aim of this paper was to evaluate the mycotoxicological situation of feed samples on the Serbian market collected during 2019 and 2020, as well as to point out the need to implement preventive measures and predictive models against the presence of toxigenic fungi and their metabolites in the food chain.

Materials and Methods

Analysis of 340 samples of feedingstuffs and complete feed for different species and categories of farm animals were carried out in the Institute of Veterinary Medicine of Serbia during 2019 and 2020. Quantification of aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin and T-2/HT-2 toxin was done using commercial ELISA (enzyme-linked immunosorbent assay) kit according to the manufacturer's instructions (Neogen Veratox), with the detection range 1-8 μg/kg , 0,25-2 mg/kg, 25-500 μg/kg, 2-25 μg/kg and 25-250 μg/kg, respectively. The absorbance was determined at a wavelength of 650 nm on an ELISA plate reader (Tecan Sunrise, Switzerland). Results were interpreted in relation to the levels permitted by the Serbian *Regulation on the quality of animal feed.*

Results and Discussion

Results of mycotoxicological examination of 340 feed samples for different species and categories of farm animals during 2019 and 2020 showed the presence of mycotoxins, as presented in Table 1. The most frequent presence and the highest concentrations were determined for deoxynivalenol. In 2019 8.82% of results and in 2020 7.46% were above the permitted levels. This is especially important for raising pigs, which are the most susceptible species. Therefore, the highest level of contamination, which was determined in the mixture for piglets, and was 1.76 mg/kg, poses a serious threat. The overall maximal value of deoxynivalenol was determined in barley (4.73 mg/kg).

Table 1. Mycotoxin content (mg/kg) in feed analyzed in 2019 and 2020

Mycotoxin	2019		2020	
	Maximum	Mean	Maximum	mean
Aflatoxin B1	0.089	0.012	0.013	0.002
Zearalenone	0.239	0.063	0.081	0.073
Ochratoxin	0.008	0.007	0.050	0.037
T-2/HT-2 toxin	0.078	0.042	0.093	0.057
Deoxynivalenol	3.6 (wheat)	1.255	4.73 (barley)	0.748

Deoxynivalenol (vomitoxin) is produced by *F. graminearum, F. culmorum, F. crookwellense, F. sporotrichioides, F. poae, F. tricinctum, and F. acuminatum (Nesic et al., 2014).* Intoxication with this metabolite is manifested by a decrease in food intake or its refusal, vomiting, and digestive disorders with subsequent losses of weight gain. From a practical viewpoint deoxynivalenol is of outstanding importance among the B type trichothecenes because of its frequent occurrence at levels high enough to cause adverse effects, especially in pigs *(EFSA, 2013).* Other animals are regarded to be less sensitive, but they still suffer from negative consequences, primarily in terms of performance *(Santos et al., 2021).*

Considering results for aflatoxin presence the situation was different from that in 2013 when most of the samples, about 75%, were highly contaminated and as many as 35% of them exceeded the maximum permitted levels, so did not correspond to the Serbian regulation at that moment *(Nesic and Pavlovic, 2013).* It also differed from the results of *Krnjaja et al. (2019)* obtained for aflatoxin B_1 in poultry feed in 2016 when 14.29% were above the regulation limits. Tests performed in 2019, and especially in 2020, for aflatoxin B_1 detection revealed low and mostly undetectable concentrations. For other mycotoxins, as shown in Table 1, average content was slightly above limit of detection (LOD) for the applied methods (LOD: 0.002 mg/kg for ochratoxin, 0.025 mg/kg for T-2/HT-2 and zearalenone), while most of the values were below the LOD of the used ELISA protocols.

Zearalenone was the second most frequently detected mycotoxin, after deoxynivalenol. In practice, the co-occurrence of deoxynivalenol and zearalenone, or even additional mycotoxins in contaminated cereals exacerbates the management of affected animals *(Döll and Dänicke 2011).* A group of Italian researchers *(Palumbo et al., 2020)* collected and published data on the content of mycotoxins in cereals in Europe in the period from 2010 to 2018. They concluded that mycotoxins fumonisin, deoxynivalenol, aflatoxins and zearalenone were mainly found in wheat and maize. An important fact is that in 54.9% of the samples, the simultaneous appearance of two or more mycotoxins was proven. This, due to the synergistic effect, drastically complicates the situation, as well as the methods of struggle.

One of the newer approaches in prevention of mycotoxicological problems includes the possibility of predicting their occurrence and the use of so-called "predictive models". These are the scenarios of the effects of climate change on mycotoxins, i.e. studies of the effects of changes in precipitation, temperature, etc. on mycotoxin contamination. They combine data on meteorological conditions, pathogens (fungi and mycotoxins) and hosts (plant species). Thus, the predictions are highly specific to one locality, plant species and even strain, as well as type of fungi and their metabolites *(Nesic, 2018).*

Climate change forecasts for Europe by the end of this century have been published in a document called the Green Paper *(European Commision, 2007).* The temperature will rise drastically. In the very south, locally up to 5.5^{0}C, while in Serbia approximately 3.5–4.5^{0}C. Precipitation will be reduced in the south, in some localities even up to 40%, while towards the north of the continent it will increase to almost drastic proportions. In general, the so-called desertification will occur in

Southern and Southeastern Europe which will contribute to the synthesis of aflatoxins, while in Central Europe, due to the higher moisture, and even more frequent floods, contamination with *Fusarium* species and their metabolites will increase.

Conclusion

Based on the results of the mycotoxicology tests in 2019 and 2020, as well as previous years of analytical experience, the continuous presence of mycotoxins (one or more), with occasional specific outbursts, is evident. Mycotoxins remain an unavoidable problem that needs to be fought in the best possible way, while a constant good management system for these natural hazards is still required. Due to climate change, the risk to human and animal health is transforming and growing, and strategies to mitigate adverse effects are becoming more complex. This also has economic consequences. Therefore, the multidisciplinary scientific community has recently been given the task of enabling the prediction of the occurrence of mycotoxins and the preparation of targeted prevention.

Uvid u mikotoksikološku situaciju – nedavna iskustva i bliska predviđanja

Ksenija Nešić, Nikola Pavlović, Marija Pavlović, Jelena Vlajković, Aleksandra Tasić, Vladimir Radosavljević, Božidar Savić

Rezime

U ovom radu su predstavljeni rezultati ispitivanja 340 uzoraka komponenti i kompletnih smeša za ishranu životinja koji su analizirani tokom 2019. (120 uzoraka) i 2020. godine (220 uzoraka) na prisustvo aflatoksina B_1, deoksinivalenola, zearalenona, ohratoksina i T-2/HT-2 toksina, sa ciljem da se stekne uvid u mikotoksikološku situaciju u hrani za životinje koja se koristi u Srbiji. Mikotoksini su sekundarni metaboliti različitih plesni, pre svega vrsta iz rodova *Aspergillus, Penicillium* i *Fusarium*. U lanac hrane oni obično dospevaju preko kontaminiranih žitarica, koje se infestiraju pre i tokom žetve ili u uslovima nepravilnog skladištenja. Iako postoji više stotina različitih mikotoksina koji su izolovani i hemijski identifikovani, praktični značaj imaju oni sa najvećim uticajem na zdravlje ljudi i životinja. U hrani za životinje u navedenom periodu najčešće je bio prisutan deoksinivalenol, i to u nedozvoljenim koncentracijama u 8,82%

uzoraka iz 2019. godine i 7,46% iz 2020. godine. Ovakva situacija je naročito nepovoljna za svinjarstvo, s obzirom da su na deksinivalenol svinje najosetljivija vrsta životinja. Maksimalna koncentracija od 1,76 mg/kg je bila utvrđena u uzorku smeše za prasad, dok je ukupna maksimalna vrednost ustanovljena u uzorku ječma (4,73 mg/kg). Svi ostali mikotoksini su bili u okviru propisima dozvoljenih granica, čak uglavnom ispod limita detekcije primenjenih metoda, dok je drugi po učestalosti bio zearalenon. Dobijeni rezultati ukazuju na potrebu za redovnim i sveobuhvatnim nadzorom usmerenim na kontrolu sadržaja ovih prirodnih kontaminenata koji imaju veliki globalni uticaj na bezbednost hrane za životinje i ljude, odnosno njihovo zdravlje. Takođe je poželjno da se primeni i mogućnost predviđanja i prognoze prisustva mikotoksina, pri čemu bi prediktivni modeli postali obavezan deo preventivnih strategija.

Key words*:* aflatoksin B1, deoksinivalenol, zearalenon, ohratoxin, T-2/HT-2 toksin, hrana za životinje

Acknowledgment

This paper is published under the research contract with the Ministry of Education, Science and Technological Development, Republic of Serbia No. 451-03-9/2021-14/200030.

References

BATTILANI P., TOSCANO P., VAN DER FELS-KLERX H.J., MORETTI A., CAMARDO LEGGIERI M., BRERA C., RORTAIS A., GOUMPERIS T., ROBINSON T. (2016): Aflatoxin B1 contamination in maize in Europe increases due to climate change. Scientific Reports, 6, 24328.

BRASE S., ENCINAS A., KECK J., NISING, C.F. (2009): Chemistry and biology of mycotoxins and related fungal metabolites. Chemical Reviews, 109, 9, 3903–3990.

DÖLL S., DÄNICKE S. (2011): The Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON) in animal feeding. Preventive Veterinary Medicine, 102, 132–145.

EFSA (2013): Deoxynivalenol in food and feed: occurrence and exposure. EFSA Journal, 11, 10, 3379,

ESKOLA M., KOS G., ELLIOTT C.T., HAJŠLOVÁ J., MAYAR S., KRSKA, R. (2020): Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited ‘FAO estimate’ of 25%. Critical Reviews in Food Science and Nutrition, 60, 16, 2773-2789.

EUROPEAN COMMISION (2007): Green Paper from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions - Adapting to climate change in Europe – options for EU action. 1-27.
KRNJAJA V., PETROVIĆ T., STANKOVIĆ S., LUKIĆ M., ŠKRBIĆ Z., MANDIĆ V., BIJELIĆ Z. (2019): Mycobiota and aflatoxin B1 in poultry feeds. Biotechnology in Animal Husbandry, 35, 1, 61-69.
NEŠIĆ K., HABSCHIED K., MASTANJEVIĆ K. (2021): Possibilities for the Biological Control of Mycotoxins in Food and Feed. Toxins, 13, 3, 198.
NEŠIĆ K. (2018): Mycotoxins – climate impact and steps to prevention based on prediction. Acta Veterinaria – Beograd, 68, 1, 1-15.
NEŠIĆ K., IVANOVIĆ S., NEŠIĆ V. (2014): Fusarial Toxins - Secondary Metabolites of *Fusarium* Fungi. Reviews of Environmental Contamination and Toxicology, 228, 101-120.
NESIC K., PAVLOVIC N. (2013): Current mycotoxicological profile of Serbian feed. Proceedings of the International 57th Meat Industry Conference “Meat and meat products – perspectives of sustainable production”; June 10-12, Belgrade, 320-323.
– PALUMBO R., CRISCI A., VENÂNCIO A., CORTIÑAS ABRAHANTES J., DORNE J-L., BATTILANI P., TOSCANO P. (2020): Occurrence and Co-Occurrence of Mycotoxins in Cereal-Based Feed and Food. Microorganisms, 8, 1, 74.
PERRONE G., FERRARA M., MEDINA A., PASCALE M., MAGAN N. (2020): Toxigenic Fungi and Mycotoxins in a Climate Change Scenario: Ecology, Genomics, Distribution, Prediction and Prevention of the Risk. Microorganisms, 8, 10, 1496.
REGULATION ON THE QUALITY OF ANIMAL FEED. Official Gazette of RS, 4/2010, 113/2012, 27/2014, 25/2015, 39/2016 and 54/2017, Belgrade.
SANTOS R.R., OOSTERVEER-VAN DER DOELEN M.A.M., TERSTEEG-ZIJDERVELD M.H.G., MOLIST F., MÉZES M., GEHRING R. (2021): Susceptibility of Broiler Chickens to Deoxynivalenol Exposure via Artificial or Natural Dietary Contamination. Animals, 11, 4, 989.
VAN DER FELS-KLERX H.J., LIU C., BATTILANI P. (2016): Modelling climate change impacts on mycotoxin contamination. World Mycotoxin Journal, 9, 5, 717-726.

Proceedings of the 13th International Symposium
Modern Trends in Livestock Production
October 6 – 8, 2021, Belgrade, Serbia

FUSARIUM SPP. AND DEOXYNIVALENOL CONTAMINATION OF RYEGRASS SEEDS

Vesna Krnjaja[1], Violeta Mandić[1], Zorica Bijelić[1], Slavica Stanković[2], Milica Nikolić[2], Tanja Vasić[3], Nikola Delić[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
[3]University of Niš, Faculty of Agriculture, Kosančićeva 4, 37000, Kruševac, Serbia
Corresponding author: Vesna Krnjaja, vesnakrnjaja.izs@gmail.com

Abstract: The aim of this study was to evaluate fungal infection, with a special focus on *Fusarium* spp. and deoxynivalenol (DON) presence, as a participant in Fusarium head blight (FHB) pathogenesis in two cultivars of Italian ryegrass K-13 and K-29. A total of 24 seed samples were collected during harvest in 2019.

By mycological analyses, *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. and non-sporulating species (Mycelia sterilia) were isolated on the seeds of both cultivars of Italian ryegrass. Among *Fusarium* spp., four species, *F. graminearum*, *F. poae*, *F. proliferatum* and *F. subglutinans* were identified in cultivar K-13 and three species, *F. graminearum*, *F. poae* and *F. subglutinans*, in cultivar K-29. *F. graminearum* and *F. poae* were identified as FHB pathogens, of which *F. graminearum* was dominant in both cultivars with 20.5% (cultivar K-13) and 32% (cultivar K-29) compared to *F. poae* which was present in both cultivars with a frequency of 0.5%. The frequency of DON positive samples was 100%. A statistically significant difference in DON level was found between the two tested cultivars, with a higher DON level in cultivar K-29 (5334.33 $\mu g\ kg^{-1}$) compared to cultivar K-13 (4738.58 $\mu g\ kg^{-1}$).

The obtained results indicate that two *Fusarium* species, *F. graminearum* and *F. poae*, were FHB pathogens, with *F. graminearum* as the predominant species in both cultivars of Italian ryegrass. High DON levels (>3000 $\mu g\ kg^{-1}$) in the tested seed of Italian ryegrass indicate on potentially significant participation of DON in FHB pathogenesis, as well as a potential risk for the quality seed production, feed safety and the food chain in general. In Serbia, this is the first report about *Fusarium* infection and DON presence in ryegrass seed.

Key words: *Fusarium* spp., deoxynivalenol, Italian ryegrass

Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is annual and also biennial or short-lived perennial bunchgrass. It is a high-yielding and high-quality fodder crop native to southern Europe. It is an integral part of pasture for hay and silage production. Its high nutrition, digestibility, and palatability, conducives to the diet of ruminants (dairy, sheep) (*Simić et al., 2005; 2012*). This crop grows best between 20 and 25°C and requires a cool climate with high rainfall and well-drained soils. It has the fastest growth and regeneration and produces a lot of forage in a short time (*Delić et al., 2012; Kostrzewska et al., 2020*). It is sensitive to strong frosts and quite sensitive to drought, and longer droughts can bring it very low yields (*Simić et al., 2005*).

In recently year, due to global warming, the occurrence of pathogenic and potentially toxigenic fungi from *Fusarium*, *Aspergillus* and *Penicillium* genera has become more frequent on cereal grains and other grasses (*Silva et al., 2014; Torres et al., 2019*). In addition, agro-ecological and environmental conditions in Serbia are favorable to growth of these fungal species on cereal grains, with *Fusarium* species as predominant (*Lević, 2008).* Members of *Fusarium graminearum* species complex (FGSC) cause Fusarium head blight (FHB) disease of small grains and several grasses, including annual ryegrass (*Machado et al., 2015*). *F. graminearum* is the most common pathogen which cause FHB of small grains. The first symptoms of FHB appear after flowering at anthesis. Spikelets are bleached, and with development of mycelium, the disease spreads throughout the head (spike), causing shrivelled seeds (*Francesconi et al., 2019*). *F. graminearum*-infected seeds have poor germination with slow emergence and can cause seedling blight disease (*Wiewióra, 2012*). *F. graminearum* is a hemibiotroph, with the biotrophic (short last from 24 to 32 h after infection) and necrotrophic phase (the pathogen grows and extends within the head through the vascular bundles and parenchyma) (*Bai et al., 2002; Goswami and Kistler, 2004; Trail, 2009*).

In addition to causing FHB disease, *Fusarium* species produce a wide range of toxic secondary metabolites (mycotoxins), which are serious constraints for feed/food production (*Richardson et al., 1996*). The occurrence of these fungal metabolites in food/feed poses healt risks to the consumers (*Haidukowski et al., 2005*). Deoxynivalenol (DON), primarily produced by *F. graminearum,* is the most associated with FHB. It belongs sesquiterpene epoxides, namely trichothecenes. The most commonly DON found in grain/seed from FHB-diseased cereal and grass heads (*Gunupuru et al., 2017*). Negative effects of DON ingestion by experimental animals are manifested as acute (emesis) and chronic poisoning (anorexia, growth retard, neurotoxicity, immunotoxicity as well as reproduction disorders. Likewise, DON can be associated with human gastroenteritis (*Pestka, 2010*). Reffering to participation of DON in FHB pathogenesis, it also has the ability to be virulent and facilitates the spread of fusariosis within the head. Like FHB, DON may cause premature bleaching of head and leaves. DON-producing *F. graminearum* strains can supress programmed cell death (apoptosis) in the leaves and support biotrophic and necrotrophic phases of *Fusarium* infection (*Diamond et al., 2013*). According to *Bushnell et al. (2010),* DON-producing strains inhibited the formation of the wall thickenings, allowing the pathogen to spread in the head.

According to European Regulations EC No. 1126/2007 amended the Regulation (EC) No. 1881/2006 and EC No. 576/2006, the maximum limits of DON level are 1250 μg kg^{-1} in unprocessed cereal other than durum wheat, oats and maize and 8000 μg kg^{-1} in unprocessed cereal and cereal products except in maize by-products intended for animal feeding, respectively. Quality and healthy seeds are a prerequisite for high yields of ryegrass as well as for safe feed production. The high yield of seed and fodders is correlated with the genetic potential of the selected varieties (*Velijević et al., 2016*). Grass (cereal) cultivars have different types of resistance to FHB (from I to V resistance types), there is no unsusceptible genotype. Types I and II are the most described. Type I resistance is resistance to initial infection, type II is resistance to spread of pathogen within the head. Other types include resistance to seed infection (type III), tolerance to FHB and DON (type IV), and resistance to DON accumulation (Type V) (*Mesterházy, 1995; Boutigny et al., 2008*). In addition to growing resistant cultivars, crop rotation, fertilization, tillage, and the application of fungicides and biological agents can also significantly reduce *Fusarium* seed infestation (*Shah et al., 2018*).

Since the quality and healthy seed is important for ryegrass, livestock and feed production, the main aim of this work was to identify and quantify *Fusarium* spp. associated with ryegrass seeds, as well as to determine DON levels in seed samples of two ryegrass cultivars and to assess its potential risk in FHB pathogenesis.

Materials and Methods

A total of 24 seed samples of two commercial Italian ryegrass cultivars (12 samples of cultivar K-13 and 12 samples of cultivar K-29) were analyzed by mycological and toxicity tests. About 1 kg of ryegrass intended for seed production were randomly taken during the 2019 harvest season. The area of tested ryegrass cultivars was on about one hectare and located on the experimental fields of Institute for Animal Husbandry, Belgrade-Zemun in Serbia.

The each of 24 samples was divided into two sub-samples, so, it was 12 sub-samples per cultivar. In mycological analyses, per each cultivar, the first 12 seed sub-samples were pooled in one representative sample. To evaluate *Fusarium* spp., 600 seeds per cultivar from representative samples were analyzed. First, the seeds were surface-disinfected for 3 minutes with 1% sodium hypochlorite, washed twice with sterilized distilled water and dried on filter paper. Then, the seeds were plated, 10 seeds per 90 mm Petri-dishes with Potato Dextrose Agar (PDA), and incubated at room temperature during 7-10 days. Based on microscopic observations, *Fusarium* species were identified using fungal keys of *Burgess et al. (1994)* and *Leslie and Summerell (2006)*. Incidence of fungal species was presented as percentage values in pooled seed samples.

For mycotoxicological analyses, a total of 24 samples, 12 sub-samples per cultivar, were ground in an analytical mill (IKA A11, Staufen, Germany). Using the moisture analyzer (OHAUS MB35, USA), the moisture content of tested samples was determined. ELISA assay for determining DON levels was done according to the manufacturer's instructions Celer Tecna® ELISA kits. The limit of detection for DON was 40 μg kg^{-1}.

Data were statistically analyzed using the independent-samples T-test (IBM SPSS Statistic 20). Pearson's correlation coefficients between DON levels and moisture contents were determined.

Results and Discussions

The results of mycobiota isolated from the seed of two Italian ryegrass cultivars, K-13 and K-29, are shown in Table 1. The fungal species isolated were *Acremonium* spp., *Alternaria* spp., *F. graminearum*, *F. poae*, *F. proliferatum*, *F. subglutinans* and non-sporulating fungi (Mycelia sterilia) on the seed of both cultivars, except on seed of cultivar K-29, in which *F. proliferatum* was not identified. Among isolated species, *Alternaria* spp. was the most present with 70% and 61.67% on the seed of K-13 and K-29 cultivar, respectively. Considering incidence of *Fusarium* species, *F. graminearum* was the most common at both cultivars, with higher incidence on the seed of cultivar K-29 (32%) than cultivar K-13 (20.50%). *F. graminearum* is followed by *F. subglutinans* which was isolated in a higher percent on the seed of cultivar K-13 (7.50%) compared to cultivar K-29 (2%). A low incidence of *F. poae* (0.50%) was found on the seed of both cultivars, as well as the incidence of *F. proliferatum* on the seed of cultivar K-13 (0.17%). In a similar study, *Wiewióra (2012)* has established *Alternaria alternata* as the most common species on the seed of perennial ryegrass and identified 10 *Fusarium* species of which *F. avenaceum* and *F. solani* were the most frequent. Further, the same autor stated that the most species such as *Alternaria* spp., *Epicoccum* spp., *Septonema* spp. and *Penicillium* spp. were saprophytes or weak parasites, while *Drechslera* spp., *Fusarium* spp., *Phoma* spp., *Curvularia* spp. and *Bipolaris* spp. were presented as pathogenic species. Similar results were reported by *Varga and*

Fischl (2005) on the ryegrass seeds and *Pathak and Zaidi (2013)* on wheat seeds. According to reported data of *Torres et al. (2019)*, the major FHB pathogens include FGSC members and related species such as *F. avenaceum*, *F. culmorum* and *F. poae*. Similarly, in this study, *F. graminearum* and *F. poae* identified as FHB pathogens from which *F. graminearum* was more presence than *F. poae* on the seed of both tested cultivars. *Machado et al. (2015)* have identified three FGSC species, *F. graminearum*, *F. asiaticum* and *F. cortaderie* from diseased ryegrass spikes and confirmed their pathogenicity to ryegrass. It was the first report of FGSC members as head blight pathogens of ryegrass (*L. multiflorum* L.) in Brazil. The results of this study were also the first report of *Fusarium* spp. incidence on the seed of two Italian ryegrass cultivars (K-13 and K-29) in Serbia.

Table 1. Incidence of fungal species on the seed of two tested ryegrass cultivars

Fungal species	Incidence (%)	
	Cultivar of ryegrass	
	K-13	K-29
Acremonium spp.	0.50	0.83
Alternaria spp.	70	61.67
Fusarium graminearum	20.5	32
Fusarium poae	0.50	0.50
Fusarium proliferatum	0.17	0
Fusarium subglutinans	7.50	2
Mycelia sterilia	0.83	3

By mycotoxicological assays, DON was found in all tested seed samples of two ryegrass cultivars, respectively the incidence of DON positive samples was 100%. It has been established the statistically significant higher mean level of DON on the seed of cultivar K-29 (5334.33 μg kg^{-1}) compared to cultivar K-13 (4738.58 μg kg^{-1}). There were no statistically significant differences between means of moisture contents of seeds in tested cultivars (Table 2). By investigating the participation of DON in FHB pathogenesis, *Bai et al. (2002)* have stated that DON production had a significant role in the spread of FHB within a spike. According to the reports of *Diamond et al. (2013)*, relatively low DON levels might inhibite plant programmed cell death (PCD), while high DON levels might induce cell death. So, DON-producing *F. graminearum* strains with low and 10,000 μg kg^{-1} DON levels might contribute to biotrophic and necrotrophic phases development in *Fusarium* infection leading to FHB disease symptoms (*Gunupuru et al., 2017*). In Brazil, the first report of DON-producing *F. graminearum* isolated from ryegrass spikes, as well as their pathogenicity on annual ryegrass reported by *Machado et al. (2015)*. In this study, high DON levels in all seed samples of both cultivars indicating that there was potentially risk of isolated *F. graminearum* strains in FHB pathogenesis.

Table 2. Means of DON level and moisture content in tested seed samples of two ryegrass cultivars

	DON level (μg kg^{-1})		Moisture content (%)
Item	Mean ± S.D.	Range	Mean ± S.D.
Cultivar K-13	4738.58 ± 244.17^{a}	3157 – 5586	11.74 ± 0.32
Cultivar K-29	5334.33 ± 147.26^{b}	4382 – 5886	11.30 ± 0.53
Level of significance	*	-	ns

Means followed by the same letter within a column are not significantly different at P ≤ 0.05 level; *, ** - significant at the 0.05 and 0.01 probability levels; ns - not significant.

Temperature, moisture and relative humidity are the most important environmental factors influencing the development of FHB and therefore DON accumulation in cereal grains *(Wegulo, 2012)*. Weak positive correlations were found for levels of DON with moisture contents on seeds of tested cultivars K-13 (r = 0.348) and K-29 (r = 0.380) (data not presented). Similarly, positive correlations between moisture-related variables with biological variables such as DON accumulation in wheat cultivars were established by *Cowger et al. (2009)*, *Kriss et al. (2010)* and *Hernandez Nopsa et al. (2012)*.

Conclusion

This study presents a natural occurrence of fungal infection and DON mycotoxin in the seed of two ryegrass cultivars, K-13 and K-29, as a participant in FHB pathogenesis. *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. and non-sporulating fungal species (Mycelia sterilia) were isolated at both cultivars. Among *Fusarium* spp., *F. graminearum*, *F. poae*, *F. proliferatum*, and *F. subglutinans* and *F. graminearum*, *F. poae*, and *F. subglutinans* were identified on the seed of cultivars K-13 and K-29, respectively. *F. graminearum* and *F. poae* were identified as FHB pathogens, with *F. graminearum* as predominant in both cultivars. All tested seed samples were DON positive. DON level in cultivar K-29 was statistically significant higher (5334.33 μg kg^{-1}) than in K-13 (4738.58 μg kg^{-1}). High DON levels (>3000 μg kg^{-1}) in tested seed samples were indicated on potential significant participation of DON in FHB pathogenesis. Both ryegrass cultivars were susceptible to FHB pathogens. These results indicate on importance of health of seeds, especially for ryegrass seeds production as well as forage production. Ryegrass as the weed may also be potentially a source of inoculum for FHB epidemics in cereal crops.

Fusarium spp. i deoksinivalenol kontaminacija semena italijanskog ljulja

Vesna Krnjaja, Violeta Mandić, Zorica Bijelić, Slavica Stanković, Milica Nikolić, Tanja Vasić, Nikola Delić

Rezime

Cilj rada bio je da se oceni gljivična infekcija sa specijalnim fokusom na *Fusarium* spp. i prisustvo deoksinivalenola (DON) kao učesnika u patogenezi fuzarioze klasa (FHB) kod dve sorte italijanskog ljulja, K-13 i K-29. Ukupno 24 uzoraka semena sakupljeno je tokom žetve u 2019. godini. Mikološkim analizama izolovane su *Acremonium* spp., *Alternaria* spp., *Fusarium* spp. i nesporulišuće vrste (Mycelia sterilia) na semenu obe ispitivane sorte italijanskog ljulja. Među *Fusarium* spp., identifikovane su četiri vrste, *F. graminearum*, *F. poae*, *F. proliferatum* i *F. subglutinans*, kod sorte K-13 i tri vrste, *F. graminearum*, *F. poae* i *F. subglutinans*, kod sorte K-29. *F. graminearum* i *F. poae* su identifikovane kao FHB patogeni, od kojih *F. graminearum* je dominantnija kod obe ispitivane sorte sa 20,5% (sorta K-13) i 32% (sorta K-29) u odnosu na *F. poae* koja je bila prisutna kod obe sorte sa učestalošću od 0,5%. Učestalost DON pozitivnih uzoraka bila je 100%. Utvrđena je statistički značajna razlika u koncentraciji DON između dve ispitivane sorte, s tim da je utvrđena veća koncentracija DON kod sorte K-29 (5334,33 μg kg^{-1}) u odnosu na sortu K-13 (4738,58 μg kg^{-1}). Dobijeni rezultati ukazuju da su dve *Fusarium* vrste, *F. graminearum* i *F. poae*, FHB patogeni, s tim da je *F. graminearum* preovladjujuća vrsta kod obe ispitivane sorte italijanskog ljulja. Visoke koncentracije DON mikotoksina (>3000 μg kg^{-1}) u ispitivanim uzorcima semena italijanskog ljulja ukazuju na potencijalno značajno učešće DON u FHB patogenezi, kao i na potencijalni rizik za proizvodnju kvalitetnog semena, bezbednost hrane za životinje i lanca ishrane uopšte. U Srbiji, ovo je prva objava o *Fusarium* infekciji i prisustvu DON u semenu italijanskog ljulja.

Ključne reči: *Fusarium* spp., deoksinivalenol, italijanski ljulj

Acknowledgment

Research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, Agreement on the realization and financing of scientific research work of SRO no. 451-03-9/2021-14/200022.

References

BAI G.H., DESJARDINS A.E., PLATTNER R.D. (2002): Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia, 153, 91-98.

BOUTIGNY A.L., RICHARD FORGET F., BARREAU C. (2008): Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. European Journal of Plant Pathology, 121, 411-423.

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory manual for *Fusarium* research. Third edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, pp. 133.

BUSHNELL W.R., PERKINS-VEAZIE P., RUSSO, V.M., COLLINS J., SEELAND T.M. (2010): Effects of deoxynivalenol on content of chloroplast pigments in barley leaf tissues. Phytopathology, 100, 33-41.

COWGER C., PATTON-ÖZKURT J., BROWN-GUEDIRA G., PERUGINI L. (2009): Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99, 320-327.

DELIĆ D., STAJKOVIĆ-SRBINOVIĆ O., ŽIVKOVIĆ S., PROTIĆ N., RASULIĆ N., KUZMANOVIĆ DJ., SIMIĆ A. (2012): Growth promotion of Italian ryegrass (*Lolium multiflorum* Lam.) by application of plant growth promoting rhizobacteria. Plant Protection, 63, 280, 93-99.

DIAMOND M., REAPE T.J., ROCHA O., DOYLE S.M., KACPRZYK J., DOOHAN F.M., MCCABE P.F. (2013): The *Fusarium* mycotoxin deoxynivalenol can inhibit plant apoptosis-like programmed cell death. PloS One, 8, 7, e69542.

FRANCESCONI S., MAZZAGLIA A., BALESTRA G.M. (2019): Different inoculation methods affect components of Fusarium head blight resistance in wheat. Phytopathologia Mediterranea, 58, 3, 679-691.

GOSWAMI R.S., KISTLER H.C. (2004): Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology, 5, 515-525.

GUNUPURU L.R., PEROCHON A., DOOHAN F.M. (2017): Deoxynivalenol resistance as a component of FHB resistance. Tropical Plant Pathology, 42, 175-183.

HAIDUKOWSKI M., PASCALE M., PERRONE G., PANCALDI D., CAMPAGNA C., VISCONTI A. (2005): Effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. Journal of the Science of Food and Agriculture, 85, 191-198.

HERNANDEZ NOPSA J., BAENZIGER P.S., ESKRIDGE K.M., PEIRIS K.H.S., DOWELL F.E., HARRIS S.D., WEGULO S.N. (2012): Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Canadian Journal of Plant Pathology, 34, 380-389.

KOSTRZEWSKA M.K., JASTRZĘBSKA M., TREDER K., WANIC M. (2020): Phosphorous in spring barley and Italian rye-grass biomass as an effect of inter-species interactions under water deficit. Agriculture, 10, 329.

KRISS A.B., PAUL P.A., MADDEN L.V. (2010): Relationship between yearly fluctuations in Fusarium head blight intensity and environmental variables: A window-pane analysis. Phytopathology 100, 784-797.

LESLIE J.F., SUMMERELL B.A., (2006): The *Fusari*um Laboratory Manual. Blackwell Publishing Ltd, Oxford, pp. 388.

LEVIĆ J. (2008): Vrste roda *Fusarium* u oblasti poljoprivrede, veterinarske i humane medicine. Cicero, Beograd. pp. 1226.

MACHADO F.J., MÖLLER P.A., NICOLLI C.P., Del PONTE E.M. (2015): First report of *Fusarium graminearum*, *F. asiaticum*, and *F. cortaderiae* as head blight pathogens of annual ryegrass in Brazil. Plant Disease, 99, 12, 1859-1859.

MESTERHÁZY A. (1995): Types and components of resistance to Fusarium head blight of wheat. Plant Breeding, 114, 377-386.

PATHAK N., ZAIDI R. (2013): Fungi associated with wheat seed discolouration and abnormalities in *in-vitro* study. Agricultural Sciences, 4, 9, 516-520.

RICHARDSON M.J. (1996): Seed mycology. Mycological Research 100, 4, 385-392.

SHAH L., ALI A., YAHYA M., ZHU Y., WANG S., SI H., RAHMAN H., MA C. (2018): Integrated control of fusarium head blight and deoxynivalenol mycotoxin in wheat. Plant Pathology, 67, 532-548.

SILVA A.E.L., REIS E.M., TONIN R.F.B., DANELLI A.L.D., AVOZANI A. (2014): Identification and quantification of fungi associated with seeds of ryegrass (*Lolium multiflorum* Lam.). Summa Phytopathologica, 40, 2, 156-162.

SIMIĆ A., VUČKOVIĆ S., SABOVLJEVIĆ R. (2005): Seed yield and quality of Italian ryegrass (*Lolium italicum*) containing different ploidy produced in the first harvest year. Plant Breeding and Seed Production, XI, 1-4, 19-24.

SIMIĆ A., VUČKOVIĆ S., SOKOLOVIĆ D., STANISAVLJEVIĆ R., DURONIĆ G. (2012): Response of Italian ryegrass seed crop to spring nitrogen application in the first harvest year. African Journal of Biotechnology, 11, 26, 6826-6831.

TORRES A.M., PALACIOS S.A., YERKOVICH N., PALAZZINI J.M., BATTILANI P., LESLIE J.F., LOGRIECO A.F., CHULZE S.N. (2019): *Fusarium* head blight and mycotoxins in wheat: prevention and control strategies across the food chain. World Mycotoxin Journal, 12, 4, 333-355.

TRAIL F. (2009): For blighted waves of grains: *Fusarium graminearum* in the postgenomics era. Plant Physiology, 149, 103.110.

VARGA Z., FISCHL G. (2005): Infection rates of perennial ryegrass seeds (*Lolium perenne* L.) with different fungi. Communications in Agricultural and Applied Biological Sciences, 70, 195-198.
VELIJEVIĆ N., SIMIĆ A., VUČKOVIĆ S., ĐUKANOVIĆ L., POŠTIĆ D., ŠTRBANOVIĆ R., STANISAVLJEVIĆ R. (2016): Varijabilnost dormantnosti, klijavosti semena i vigora klijanaca sorti crvene deteline i italijanskog ljulja. XXI Savetovanje o biotehnologiji, 11. – 12. mart 2016. godine, Čačak, Srbija. Zbornik radova, 21, 23, 73-79.
WEGULO S.N. (2012): Influencing deoxynivalenol accumulation in small grain cereals. Toxins, 4, 1157-1180.
WIEWIÓRA B. (2012): The effect of seed health of perennial ryegrass (*Lolium perenne* L.) on germination capacity. Plant Breeding and Seed Science, 65, 51-62.

==============================

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

636/638(082)(0.034.2)
631/635(082)(0.034.2)

INTERNATIONAL Symposium Modern Trends in Livestock Production (13 ; 2021 ; Beograd)
Proceedings [Elektronski izvor] / 13th International Symposium Modern Trends in Livestock Production, 6 -8 October 2021, Belgrade, Serbia ; [organizer] Institute for Animal Husbandry, Belgrade - Zemun ; [editor Zdenka Škrbić]. - Belgrade : Institute for Animal Husbandry, 2021 (Belgrade : Institute for Animal Husbandry). - 1 USB fleš memorija ; 1 x 3 x 6 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 100. - Bibliografija uz svaki rad.

ISBN 978-86-82431-77-0

а) Сточарство -- Зборници б) Пољопривреда -- Зборници

COBISS.SR-ID 46411785

==============================

13th INTERNATIONAL SYMPOSIUM
MODERN TRENDS IN LIVESTOCK PRODUCTION
6 - 8 October 2021 - Belgrade, Serbia

PROCEEDINGS

INSTITUTE FOR ANIMAL HUSBANDRY
Autoput 16, P. Box 23, 11080, Belgrade - Zemun, Serbia
www.istocar.bg.ac.rs

ISBN 978-86-82431-77-0