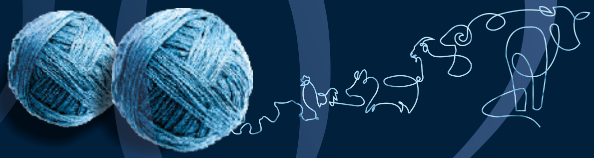
14th
INTERNATIONAL
SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

2023



PROCEEDINGS

4 - 6 October 2023, Belgrade, Serbia

Institute for Animal Husbandry
Belgrade - Zemun, SERBIA

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GUEST SPEAKER PRESENTATION

THE SCIENTIFIC CONFERENCES OF THE ZEMUN INSTITUTE - A REVIEW AND OUTLOOK

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Abstract: The congresses and symposiums of the Zemun Institute for Animal Husbandry look back on a 30-year tradition. That deserves respect and congratulations. In all years, a demanding scientific program with close practical relevance was offered. The conferences were in the past a reflection of the changes in agriculture. This is also the case today and will continue to be so in the future. As a podium for scientific and practical discussions, the meetings proved to be guideposts for future developments. The conferences had an international focus from the start. Guests from 35 countries gave lectures and presented new scientific results. In cooperation with the relevant ministries, the tasks of the institute have expanded. Here is the centre of breeding and husbandry for the important livestock breeds. New topics and methods are increasingly determining the farm animal industry. Major challenges arise from changes in societal attitudes towards agriculture and animal-based foods. In the complex field of tension between economic efficiency, new social perspectives on agriculture, alternative production processes and sustainability, but also internationality and competitiveness with market presence, viable solutions must be developed.

Historical Review

The scientific conferences of the Zemun Animal Breeding Institute have a long tradition. The first event took place in 1993. The 30th anniversary is to be celebrated this year.

It is said that starting a new project is difficult. This also applies to the start of a conference series. You have to stand out from previous and proven processes and programs and start something new. It requires a topic that remains topical in the long term. If the "child", i.e. the conference is "born", the "raising" follows.

This "rearing" requires a lot of patience, energy, strength, ideas, and perseverance over the years. There are often worries, difficult phases, and the question of costs, you don't really know where you're going. Ultimately, persevering becomes a challenge that usually requires much more energy than the start. Here at the Institute for Livestock Husbandry in Zemun, they had the strength and the ideas, the perseverance. In all the eventful years, it was not always easy to be able to hold the conference. It has managed to organize interesting scientific events with challenging programs for 30 years. Congratulations and thanks for that!

But we also remember interesting excursions to agricultural and cultural destinations. Exuberant social evenings with music, dance and culinary delights offered the opportunity for further specialist discussions and private conversations. This was the beginning of friendships for many colleagues. This is an enrichment that in addition to the specialist program everyone will remember fondly and is part of the program of such a scientific conference.

It remains to be hoped that this series of conferences will continue to be successful. We wish you the necessary strength and commitment for the exchange of ideas on a scientific and practical level. There will be no shortage of topics in the future, because agriculture, especially livestock farming, is undergoing a far-reaching transformation process. This situation calls for meetings of this kind.

The meetings here in Zemun-Belgrade have been organized as a symposium or as a congress. They are the podium for the interdisciplinary exchange of ideas on a scientific and practical level. It was always the goal and profile of the event to bring scientists and practitioners together. Congratulations on that, because you have realized that. The exchange of scientific results, experiences and the discussion about practicable ways mostly led to improved or new solutions and to new developments. It was always about the effective and sustainable production of food and raw materials of animal origin in sufficient quantities and high quality. That is the primary task of agriculture in order to earn money.

The German agricultural scientist JULIUS KÜHN (1825 – 1910) was already pursuing exactly this concern, namely the scientific-practical content. He understood agriculture like this:

“Natural scientific research alone is not agriculture. The true goal is the maximum production of food and clothing. Thus our science has a duty to try to meet the needs of mankind for food and clothing. We have to recognize the laws of nature, we have to apply the laws of nature with the best possible return (profit) for material conversion. The highest scientific goal is the practical goal... Our task is the benefit”.

This goal is still relevant today. The philosopher IMMANUEL KANT (1724-1804) said 100 years earlier: "*There can be nothing more practical than a good theory*". That is exactly what the meetings here in Belgrade and Zemun did.

Internationality right from the start. The series of conferences is, so to speak, a reflection of the changes over time in agricultural livestock husbandry. The symposia and congresses were guideposts and pointers for the future. From the outset, the focus was on beyond Serbia in order to see national issues in an international context. Speakers were specifically invited for this purpose. Results and experiences of scientists of different generations and countries of origin were exchanged for use in Serbia and the surrounding countries.

The large number of participants and speakers from science and practice from over 35 countries is proof of the importance of the conference series. From this, international cooperation projects developed. One of the main tasks of the institute today is to further promote this cooperation.

The focal points of the conferences were and are animal breeding, animal nutrition, animal husbandry, and operational management in close connection with veterinary medicine. The further and more extensive interdisciplinary cooperation with other national and international institutes is therefore an important task for the future. Ultimately, the results of this cooperation must be put into practice in the spirit of JULIUS KÜHN and IMMANUEL KANT. It was and has always been a concern of the institute's work to work together with animal owners and breeders and with veterinary medicine in the country.

Current situation

The wealth and diversity of the institute's tasks have increased over the years. The Institute reports directly to the Ministry of Science, Technological Development and Innovation. It works for him for the purpose of decision-making. The same applies to cooperation with the Ministry of Agriculture, Forestry and Water Management. Here in the institute, the threads come together of breeding for the important agricultural animal species and breeds, but also for autochthonous breeds in the country. Four complexes are pursued:

- Maintaining and improving the performance of the farm animals, taking animal health into account,
- Improvement of profitability, especially competitiveness, of animal production,

- Ensuring the quality requirements of the products obtained from the animals and
- Conservation of genetic diversity.

The newly developed “Open-Science-Concept” for national and international access to scientific results and publications is an important innovation. This meets the modern requirements and expectations of a scientific institute with an international focus.

The open-access scientific journal “Biotechnology in Animal Husbandry” also serves this purpose. Both enable the use of scientific results and the incorporation of the results into the international pool of animal science knowledge.

Future

In order to ensure the continued existence of farms, work must be done economically. The preservation of the farms and companies is the original meaning of "sustainability". This term is often used in an inflationary way today. It is clear to differentiate:

- Sustainability in the economic field, i.e. economic work for the purpose of investment and continued existence of the company,
- Sustainability in the ecological field, such as towards the environment and in relation to animal welfare and the use of resources,
- Sustainability in the social field to preserve human relationships, family and business traditions, and business continuity.

In recent decades, social attitudes have clearly developed in the direction of more environmental awareness, greater rejection of violence and greater appreciation of animals. This does not only apply to agriculture. Food security has been assured in Europe since the mid-1980s. The percentage of people working in agriculture is very low. In any country, there is almost no public interest in increasing production because food price reductions are no longer necessary. Increases in productivity no longer bring any significant benefits to society, namely freeing up labour for industry.

The changing framework conditions force progressive adjustments in order to maintain and open up markets. The national and international market situation changed. The general political conditions tended in other directions, and last but not least, people's eating habits changed. This is undoubtedly influenced by the media. The impact on primary agricultural production is noticeable.

The topics and methods in agricultural livestock husbandry have changed. They will continue to change. In farm animal breeding breeding, genomic breeding value estimation is established for the important animal species. We speak of "Precision Farming" and use the Microbiome for information about the animal. New forms of performance testing and the digitization of processes in animal husbandry have found their way. Breeding, husbandry, animal nutrition and management were evaluated years ago from an economic point of view. Today, in addition to the individual company goals, animal welfare, animal protection with the addition of "sustainability" with regard to breeding goals and performance characteristics play an important role.

Here the ambivalent attitude of society towards animal breeding becomes clear. Even today, the animal owner, the farmer has to earn money with their animals. This is possible with high performance, sophisticated management and the stable health of the livestock.

We must also bear in mind that on a global scale, different criteria apply to the breeding orientations of the performance characteristics of farm animals than on a national scale. In this respect, programs for farm animal breeding and husbandry in local countries do not always correspond to the ideas and possibilities of other countries far from Europe. This is particularly true for animal exports. Finding viable solutions here is a very special challenge for agriculture with farm animals in order not to lose international market share.

Quality of products. The national and European requirements for agricultural production in terms of food safety and product quality are very high. This is a great and important good of our time. In addition to product quality, process quality is playing an increasingly important role today and will continue to do so in the future. Consumers ask how animals are bred, kept, fed and slaughtered without changing the quality of the product. Alternative methods of agricultural livestock farming, such as so-called "eco-, bio-agriculture, or organic farming", occupy a larger market share than in previous years. Last but not least, the higher proportion of vegetarians and vegans with the result of new, alternative foods on the market is affecting commercial animal production.

Consequences

Animal husbandry is increasingly under public pressure. Significant changes will be necessary to make livestock farming sustainable and good for the future. The meaningful place of farm animals in human nutrition is undisputed, also from an environmental point of view. This will pose new challenges for agricultural research in terms of breeding, husbandry, animal nutrition, management, animal health and human-animal relationships.

When new barns are built, more emphasis is placed on forms of husbandry with more space. In order to keep emissions under control, consequences in the stocking density are necessary, which will also have a positive effect on animal behaviour. The human-animal relationship is meaningful with hopefully positive effects on animal health and drug use.

The adaptability of livestock to different husbandry conditions will be a focus for breeding. This applies in particular to the robustness feature. Forms of performance testing will change. In animal nutrition, reducing food competition with humans and the associated consequences will play an increasing role. For a sustainable acceptance of agricultural livestock farming, the establishment of convincing nutrient cycles is necessary. Selected keywords are animal husbandry, humus formation, and CO_2 binding.

Conclusion

Modern agricultural animal husbandry in most European countries is facing major changes or is undergoing profound structural change. This has to happen if it doesn't want to be crushed between the two megatrends "substitutes" and "sustainability". The steps include:

- Adaptation of the animal stocks to the extent necessary with a view to the forms of husbandry,
- Do not use increased efficiency to expand sectoral production (increase in inventories),
- Forms of husbandry with high ethical standards and effective control over,
- Responsibility of the legislator with regard to the social consequences of structural changes, - Find solutions for domestic production in the face of ongoing European competition that do not overstrain consumers' willingness to pay.

The social and temporal pressure to tackle this variety of topics and the problems they contain force scientifically sound discussions. The direct practical reference is at the top so that the task, form and dimensions for agricultural animal husbandry are redefined and acceptable in accordance with their importance for human nutrition and for material cycles. This is where the Institute for Animal Husbandry Zemun has its task. The conferences today and in the following years are the national and international podiums for constructive scientific-practical discussion. I am sure that the conference series is in very good hands with colleagues in Zemun today and in future.

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SUSTAINABILITY OF A PLANT-BASED DIET

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Invited paper

Abstract: Considering that recently there are more and more different options and dietary trends, vegetarianism is presented as a possible solution for quality nutrition with less impact on the environment, it is necessary to analyze how true such a premise really stands. A plant-based diet implies the consumption of foods that come from plants with some or no components of animal origin. This diet could have some positive effects on the human health of adults (lower risk of chronic diseases such as heart disease, type 2 diabetes, certain cancers, and obesity) but only well balanced and supplemented while in infants, children, and adolescents could cause severe deficiencies in vitamin 9 and vitamin 12 affecting the growth, cognition, social development, and expression of depression. From the point of environmental sustainability, further research is necessary to distinguish the accurate footprint of a plant-based diet as well as of animal production. Finally, it is up to each person to decide, hopefully, based on knowledge and responsibility, what kind of diet will follow. From the point of those engaged in animal production, it is up to us to ensure efficient and sustainable production of high-quality animal products, because the market is only growing.

Key words: plant-based diet, environmental impact, sustainability

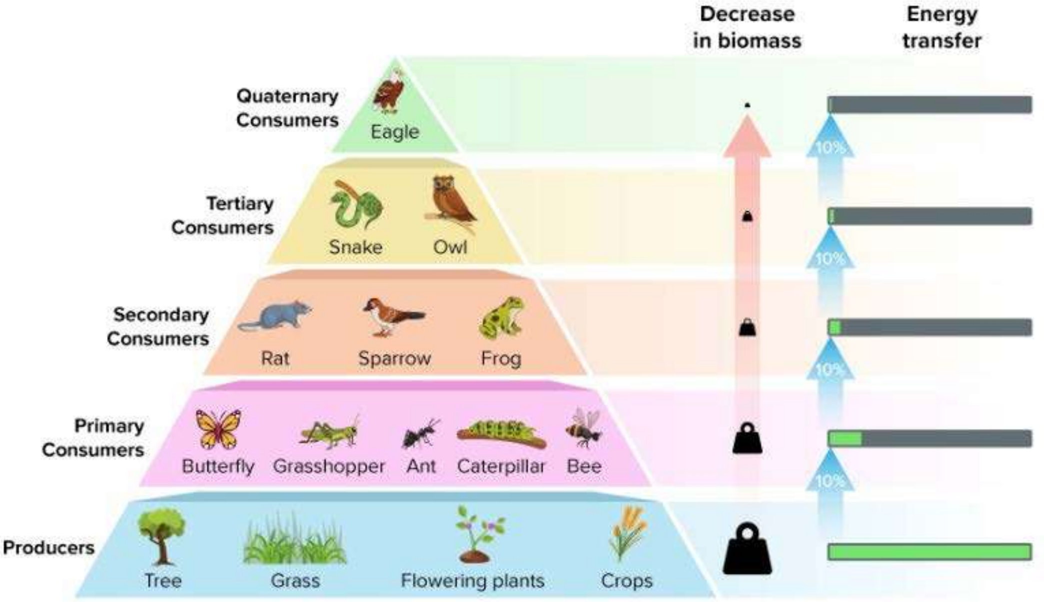
The basics of living beings

All living beings have several basic characteristics: cellular organization (all living beings are composed of one or more cells), genetic material (all living beings have genetic material, DNA or RNA), homeostasis (all living beings maintain a stable inner conditions despite changes in the environment), growth and development (all living beings increase in size and complexness), reproduction (all living beings reproduce, sexually or asexually depending on species and environmental conditions), adaptation (all living beings can adapt and evolve accordingly to the environmental conditions), response to stimulants (all living beings can detect and respond to various environmental factors: light, sound, temperature, etc.), and metabolism (all living beings metabolize, that is they utilize necessary energy from their environment). All living organisms need energy implying that all living beings need to eat.

Furthermore, accordingly, to the source of energy they use, all living beings can be classified into distinct categories: autotrophs (organisms that can produce their food and energy using inorganic substances); photoautotrophs (primary source of energy is sunlight, photosynthesis), chemoautotrophs (obtain energy by oxidizing inorganic sulfur or iron compounds), heterotrophs (organisms that obtain energy by consuming other organisms or organic matter); herbivores (consume plant material as their source of energy), carnivores (consume other animals), omnivores (have a mixed diet and consume both plant and animal materials), decomposers (obtain their energy through decomposition of dead organic materials, crucial for the nutrients recycling).

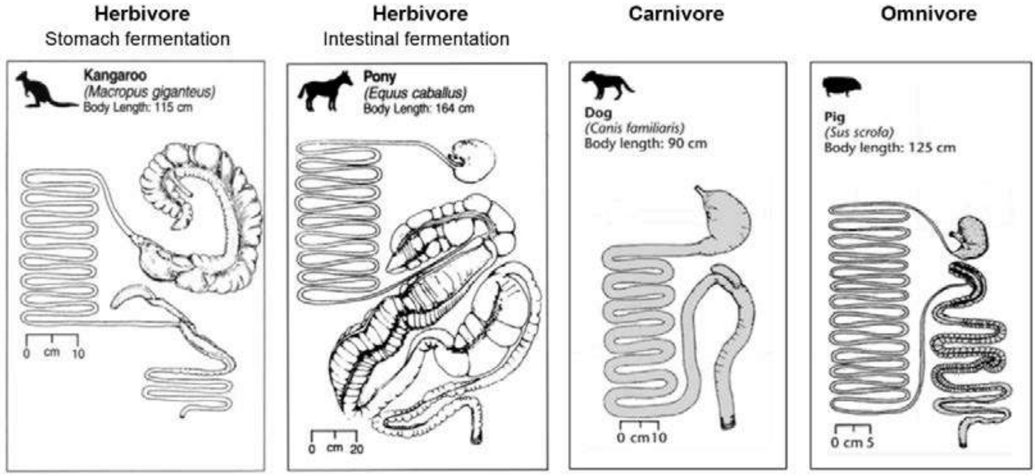
Furthermore, accordingly, to the source of energy they use, living beings in an ecosystem are classified to their trophic level that defines their place in the food chain. Each trophic level represents a different level of energy transfer within an ecosystem. Accordingly to trophic levels living beings are: producers (the first trophic level, primary producers, autotrophs: mainly plants and some algae), primary consumers/herbivores (the second trophic level, organisms that consume the primary producers (plants) as their source of energy, they extract energy from plants, secondary consumers/carnivores (the third trophic level, organisms that primarily consume other animals as their source of energy), tertiary consumers (the fourth trophic level, carnivores that feed on other carnivores). In all ecosystems, energy is transferred and transformed through the trophic levels. Each level extracts energy from the level below it, while some energy loses as heat. Therefore, the total biomass and number of individuals decrease as the trophic level increase (Picture 1).

Omnivores represent organisms that have a mixed diet and consume food of both plant and animal origin. The term "omnivore" comes from the Latin words *omnis* (all) and *vorare* (to consume), implying their ability to eat diverse types of food. The flexibility in food selections allows omnivores to adapt to diverse environments and use available food resources. In various ecosystems, omnivores play significant ecological roles. For instance, omnivores can contribute to the dispersal of plant seeds through fruit consumption, while controlling the overpopulation of smaller animals. Omnivores can be mammals, birds, reptiles, insects, and of course humans. Commonly, the human diet includes fruits, vegetables, grains, nuts, seeds, and animal-based products such as dairy, meat, eggs, and fish.



Picture 1. Variation in biomass and energy transfer regarding the trophic level *(Ansari, 2023)*

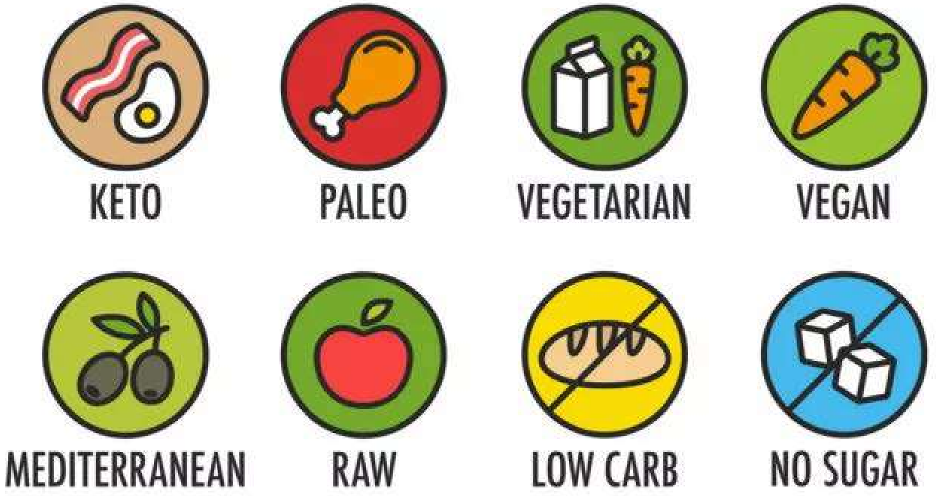
The structure of the digestive systems of heterotrophs varies significantly depending on their specific dietary habits and evolutionary adaptations (Picture 2). Herbivores have longer digestive tracts to facilitate the decomposition of plant material (longer tracts allow for more time and space for microbial activities and the breakdown of cellulose) and could have specialized compartments, some herbivores, like ruminants, have specialized pre-stomachs (microbial fermentation occurs before further digestion), and efficient nutrients extraction (due to large cecum or pre-stomachs). Carnivores have shorter digestive tracts compared to herbivores because animal tissues are normally easier to digest and need less processing, have high acidity of stomachs (for the decomposition of proteins and neutralization of potentially harmful bacteria), and reduced or absent cecum (in cecum primarily occur the fermentation of plant material). Omnivores have a combination of digestive adaptations from herbivores and carnivores. They have a moderate-length digestive tract, a lower acidity of the stomach compared to carnivores, and a shorter cecum compared to herbivores.



Picture 2. Differences in digestive system between herbivores, carnivores and omnivores ***(Kiezebrink, 2022)***

Human diet

The human diet can vary depending on cultural, regional, and individual choices. A balanced and healthful human diet is supposed to include the following components: carbohydrates (as an energy source; grains, starchy vegetables, legumes, and fruits), proteins (essential for growth and maintenance of body tissues; meats, eggs, dairy products, legumes, and plant-based proteins (tofu and tempeh), fruits and vegetables (as a source of vitamins, minerals, and dietary fiber), healthy fats (source of energy, help absorb fat-soluble vitamins, and sustain overall health), whole grains (source of fiber, vitamins, and minerals; wheat, brown rice, quinoa, and oats), dairy (source of calcium, protein, and different essential nutrients; milk, cheese, yogurt, etc.), hydration (enough water). It is crucial to have a balanced diet that provides all the required nutrients, vitamins, and minerals for ensuring optimal condition and health.

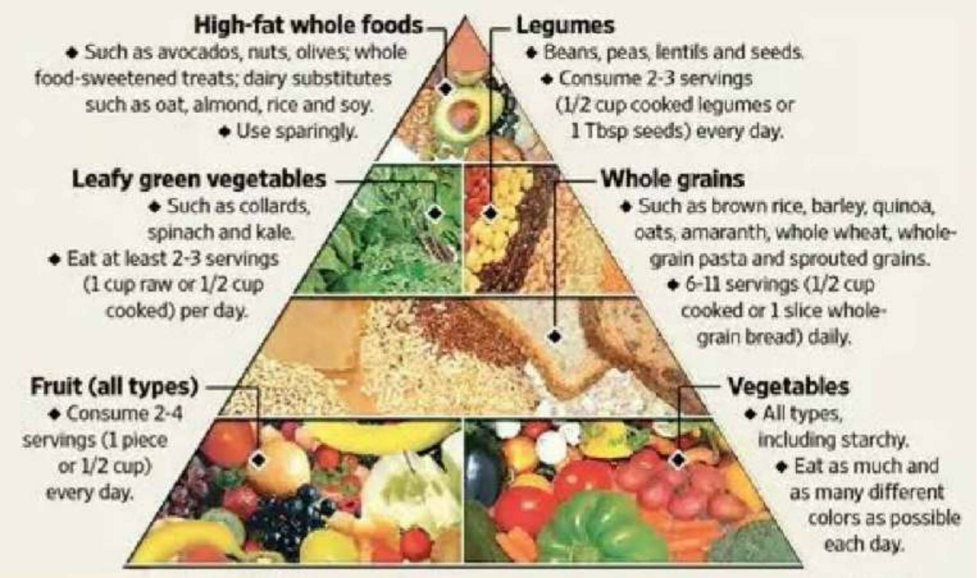


Picture 3. Different types of human diet *(Karlsen, 2015)*

There are various types of human diets that somebody could select for different causes: health, religion, ethical relations, or weight maintenance. Some of the frequently described and most fashionable types of human diets are Mediterranean, Vegetarian, Vegan, Paleo, Gluten-Free, Keto, etc... Mediterranean diet emphasizes fruits, vegetables, whole grains, legumes, nuts, seeds, olive oil, and moderate consumption of fish, poultry, and dairy products. A vegetarian diet *(Klemm, 2021)* excludes meat, poultry, and seafood but may include eggs and dairy products; lacto-ovo-vegetarian (includes plant-based foods, dairy products, and eggs); lacto-vegetarian (includes plant-based foods and dairy products, excludes eggs), ovo-vegetarian (includes plant-based foods and eggs, excludes dairy products). A vegan diet excludes all animal products and focuses on plant-based foods (fruits, vegetables, legumes, whole grains, nuts, and seeds). A pescatarian diet include fish and/or shellfish, while semi-vegetarians (or flexitarians) include some meat, seafood, poultry, eggs and dairy. A paleo diet or the caveman diet is based on consuming lean meats, fish, fruits, vegetables, nuts, and seeds while excluding grains, legumes, dairy products, processed foods, and refined sugars). A gluten-free diet eliminates foods containing gluten, a protein found in wheat, barley, rye, and their derivatives. A ketogenic diet represents a high-fat, and low-carbohydrate diet based on various meats, fish, eggs, avocados, nuts, seeds, and healthy oils, and excluding or restricting grains, sugar, fruits, starchy vegetables, and legumes.

Plant-based diet

A plant-based diet focuses on foods that come from plants (including vegetables, whole grains, legumes, nuts, seeds, and fruits) with some or no components of animal origin *(BDA, 2023)*. As already defined, there are several types of vegetarians, but all of them primarily consume plant-based foods. There are a variety of reasons why to switch to meat-free *(Klemm, 2021)*: personal preference, health considerations, ethical reasons (they do not want animals killed or harmed), environmental concerns (based on the premise that animal production has a high environmental impact), religious beliefs (Hindus, Buddhists, etc. ...).



Picture 4. A food pyramid in a plant-based diet *(Plant-Based, 2023)*

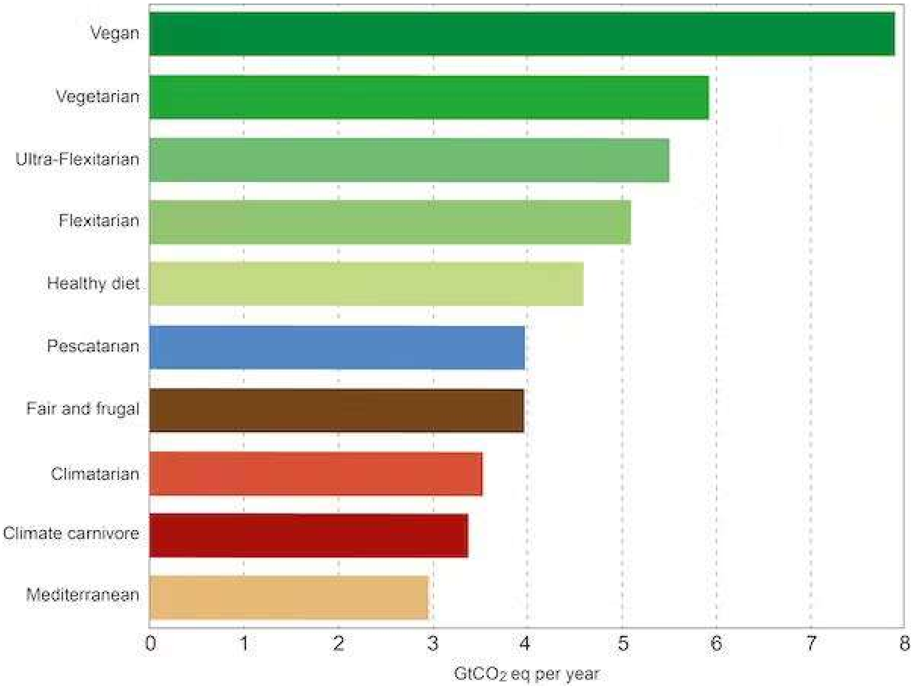
Many people switch to some kind of plant-based diet due to the expectation of potential health improvements. Accordingly to *Klemm (2021)*, vegetarianism is related to health improvements through lower frequency obesity, reduced risk of heart disease, and lower blood pressure. Since plant-based diets commonly have lower calories level and a lower share of saturated fats in meals, with a higher share of dietary fiber and higher amounts of some vitamins and minerals comparable to a standard American diet, these diets could contribute to better health status. Accordingly to *(BDA, 2023)* plant-based diet is a way to improve health your health. This kind of diet could reduce the risk of diabetes type 2, hypertension, cardiovascular disease, and some cancers, and could result in weight loss. Furthermore, in the case of excluding animal-based food from your diet, you need to pay attention to the possible deficit of some nutrients. You can easily satisfy *protein* needs by consuming protein-rich foods (lentils, beans, chickpeas, seeds, nuts, nut-butters, tofu, and tempeh). Meat substitutes (soya burgers and soya sausages, mycoprotein, and seitan) are high-quality protein sources but often could have a high content of salt and fat. Furthermore, long-chain *omega-3 polyunsaturated fatty acids* are substantial for human health and the richest source is oily fish (pilchards, salmon, and mackerel). In the case of excluding fish from the diet, the body can transform the essential fats from walnuts, flaxseeds, chia seeds, and hemp seeds into long-chain omega-3 fatty acids. *Vitamin B12* (present in eggs and dairy foods) represents one of the important segments of a balanced diet, so if you have a lack of B12, you could suffer fatigue, anemia, and nerve damage, as well as the risk of cardiovascular disease could increase. If you are vegan, the only reliable sources of vitamin B12 are fortified foods and supplements. Also, *Vitamin D* and *Calcium* are crucial for sustaining bone health. Dairy foods are rich in calcium, but if you are not consuming dairy you have to include fortified plant-based alternatives (figs, nuts such as almonds, leafy green vegetables such as kale, red kidney beans, sesame seeds, tempeh, and calcium-set tofu). *Iron* represents one of the most important minerals for human health, and the lack of Fe induces anemia and consequently poor general health. Animal-based products are rich in iron while in a plant-based diet, iron could be found in dried fruits, whole grains, nuts, green leafy vegetables, seeds, peas, beans, and lentils. Since the form of iron in plant foods have lower absorption efficiency it is supposed to be consumed with food rich in vitamin C (citrus fruits, strawberries, berries, green leafy vegetables, and peppers). Another important mineral that is necessary for the growth and development and the immune system is *Zinc* (which could be found in high amounts in eggs and milk). Adequate substitutes for animal-based products are wheat germ, beans, nuts, seeds, mushrooms, and some fortified breakfast cereals but the absorption efficiency is lower in comparison to animal-based products. *Iodine* is crucial for the functioning of the thyroid gland. The major sources of iodine in the human diet are dairy products and seafood, so in the case of a plant-based diet, you may experience the risk of deficiency. So, depending on the type of vegetarian diet, there is a possibility of a lack of the following micronutrients: vitamin B1, vitamin B6, vitamin B12, vitamin D, calcium, iodine, iron, omega-3, omega-6, and zinc. Therefore, as with any other diet, and in a plant-based diet, to ensure an adequate state of health and condition of the organism, it is necessary to know the characteristics of the food and the needs of the organism to ensure proper balance.

Although many sources suggest that plant-based diet does not represent a harmful choice for the human organism, certain studies indicate serious disadvantages and consequences of insufficient consumption of animal-based food. For instance, studies conducted in Guatemala on mothers and newborns *(Casterline et al., 1997)*, as well as on school-age children *(Rogers et al., 2003)* documented a high deficiency of vitamin B12 concentrations as a consequence of inadequate dietary intake that is low or no consumption of animal-source foods. Severe deficiencies in folate (vitamin B9) and cobalamin (vitamin B12) have a significant negative effect on brain development during infancy, as well as on depression prevalence in adulthood *(Black, 2008)*. Furthermore, *Black (2008)* emphasized that adequate folate intake during pregnancy reduces the risk of a neural tube defect (NTD) in infants while severe cobalamin deficiency has negative effect on behavioral and psychoeducational functioning of adolescences. The deficiencies in folate and cobalamin are mainly correlated to malabsorption or a vegetarian diet. *Black (2008)* concluded that vitamin B12 deficiency affects infant growth, cognition, social development, and symptoms of depression, therefore, it is extremely important to develop strategies to prevent deficiency of B12, especially in developing countries where nutrition is not a matter of choice but of lack of sufficient amount of food.

Environmental impact

A plant-based diet is often considered a more sustainable dietary choice due to its potential positive environmental impacts: reduced greenhouse gas emissions (plant-based diets generally have a lower carbon footprint since plant production needs fewer resources (land, water, and energy) than animal production), resources conservation (since fewer resources are required than in animal production, natural resources, ecosystems, and biodiversity could be preserved in greater extent), deforestation decrease (plant-based production requires less agricultural land contributing to the forest protection), water conservation (animal production requires substantial amounts of water for animal drinking, feed crops, and cleaning, while plant production is less water demanding), mitigation of pollution (animal production contributes to water and air pollution while plant-based diets can help mitigate pollution by lowering the environmental impact from animal production).

Maslin (2022) stated that the food we consume has a huge effect on our environment, therefore changing what we eat can help decrease carbon emissions and enable sustainable agriculture. Furthermore, the same author implies the necessity for a new diet, the climatarian diet (a diet that is healthy, climate-friendly, and nature friendly). As it is shown in Picture 5., a standard meat-based diet delivers about 7.2 kilograms of CO_2 equivalent per day, while a vegetarian diet delivers 3.8 kg and a vegan diet 2.9 kg. So, in case the whole world shifted to vegan, it would result in 8 billion tonnes of CO_2e (60% of all food emissions from the current 13.7 billion tonnes of CO_2e a year.



Picture 5. How much CO_2e (in billions of tonnes, or Gt) would be saved if the whole world switched to each of these diets *(Maslin, 2022; IPPC, 2023)*

Furthermore, *Maslin (2022)* stated that if we want to preserve our planet we also have to think about water and land usage. Beef production requires about 15,000 liters of water per kg, while avocados and almonds also have a huge water footprint. Overall a plant-based diet requires about half the water consumption compared to a standard meat-based diet (Picture 6).

Picture 6. How thirsty is our food? Liters of water required to produce one kg of the food product *(IO, 2019)*

Is a plant-based diet really better for the environment?

Plant-based diets mainly rely on the soybean products (like tofu and tempeh) that are mainly growth in Brazil and India where it significantly contributes to overall deforestation and loss of habitats *(Trauger, 2022)*. Accordingly to a new investigation *(Jordan et al., 2022)*, more than 400 sq miles (1,000 sq km) of Amazon rainforest have been cleared out to expand soya-growing farms in the Brazilian state of Mato Grosso in 10 years.

Picture 7. Deforestation in Nova Xavantina, Mato Grosso state, Brazil (Photograph: Amanda Perobelli/Reuters)

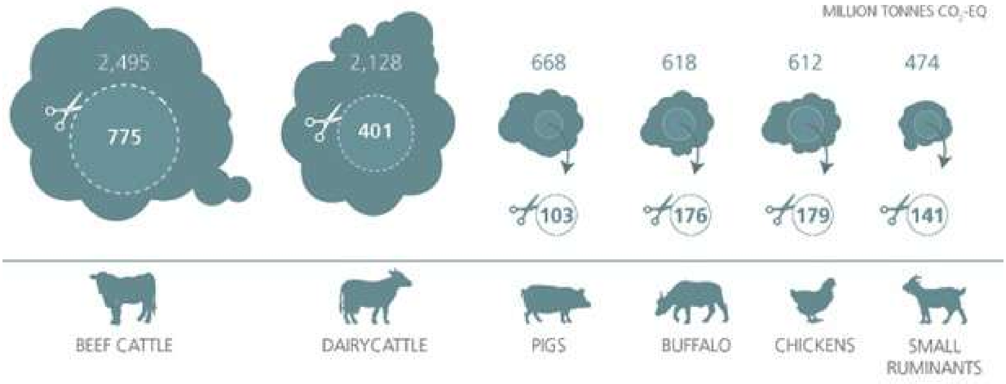
Furthermore, accordingly to *Trauger (2022)* palm oil as one of the main components of plant-based diet is mostly produced and imported from Indonesia, Malaysia, Thailand, and Nigeria where local ecosystems there have been destroyed by deforestation and biodiversity loss since millions of hectares of forests are razed for palm oil production. Besides the environmental impact, the palm oil industry has been the subject of numerous allegations of human rights violations (child labor, rampant sexual abuse and rape, exposure to dangerous pesticides, etc.). Furthermore, *Sabajo et al. (2017)* stated that the expansion of oil palm and other cash crops causes an increase in the land surface temperature in the Jambi province in Indonesia. Similarly, *Wright et al. (2019)* reported that the world's demand for palm oil is driving deforestation in Indonesia.

Picture 8. Deforestation in Borneo (*Future Environment Defenders, 2023*)

Mitigation potential of the animal production sector

The animal production sector, within agriculture, has to some degree impact on the environment. The global animal production sector contributes to anthropogenic GHG emissions, but on the other hand, the animal production sector has high mitigation potential. The animal production sector with 7.1 gigatons of CO2-eq/year (the year 2005) emits 14.5% of all anthropogenic emissions (49 gigatons CO2-eq for the year 2004; *IPCC, 2007*). Analyzing the species, cattle contribute to 65% of sector emissions, while small ruminants have much lower emission levels in the interval from 7-10% of sector emissions (0.47 gigatons CO2-eq). The main origin of GHG emissions in the ruminants' production systems represents enteric fermentation and feed production. Furthermore, 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. Also, in ruminant production systems, there is a strong negative relationship between productivity and emission intensity that is emission intensity decreases with the increase in animal production.

The decline of the impact of the animal production sector on the environment that is the decrease in sectors emission could be achieved by reduction of production and consumption, lowering the emission intensity of production, and by the combination of mentioned above. Technical options for the mitigation of GHG emissions in animal production could be divided into the several categories: options related to feed supplements and feed/feeding management (for CH4 only), options for manure management (dietary management, storage, handling and application phases of manure management), and animal husbandry options (animal and reproductive management practices and technologies). Also, practices and technologies that decrease the GHG emissions usually results in productivity incerase, consequently contributing to food security and economic development *(FAO, 2013a).* By the application of different mitigation techniques could result in large environmental benefits with a mitigation potential of 14 to 41% depending on the selected species, production system, and world's region (Picture 6).

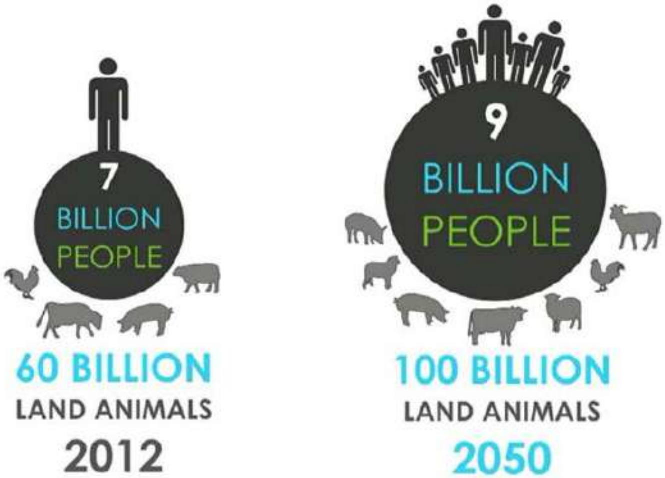


Picture 9. The potential for mitigation regarding the animal species *(FAO, 2016)*

Furthermore, it is very important to emphasize the role of domestic animals in soil preservation and improvement through nutrient cycling (animals consume plant material and return nutrients to the soil through their manure that enrich the soil and enhance its fertility), soil aeration, and compaction prevention (grazing animals with their hooves create small channels in the soil, improving water infiltration and allowing air to reach plant roots), tillage and soil disturbance (chickens or pigs are scratching or rooting in the soil that improve soil structure, and promote better water infiltration and root penetration), weed and pest control, green manure and cover crops (these plants fix atmospheric nitrogen, enrich the soil with organic matter when incorporated, and provide erosion control and weed suppression).

The future of food production

It is unrealistic to expect that the human population will switch to the food of plant origin, especially in that part of the world population that, due to the expected increase in income, will finally be able to provide their families with food of animal origin. Accordingly to the forecasts, the world human population will increase to 9.6 billion by the year 2050 *(Census Bureau, 2016)* while in combination with expected rising incomes and urbanization, the demand for meat and milk will increase by 73 and 58% in the year 2050, comparing the levels in the year 2010 *(FAO, 2011)*. The expected increase in the human population generates the need for significant increase in the number and productivity of domestic animals (Picture 10).



Picture 10. Expected increase of human and domestic animals' population *(FAO, 2011)*

Finally, some pros and cons of a plant-based diet could be emphasized. As pros following could be pointed out: health benefits (plant-based diets could be rich in fiber, vitamins, minerals, and antioxidants with low levels of saturated fats and cholesterol resulting in lower risk of chronic diseases such as heart disease, type 2 diabetes, certain cancers, and obesity, environmental sustainability (plant-based diets have a lower environmental impact compared to diets rich in animal products; which showed to be questionable), and ethical concerns. Furthermore, the cons are as follows: nutritional concerns (lack of vitamin B12, iron, calcium, zinc, and omega-3 fatty acids and necessity for supplements), social and practical challenges, and probable higher costs of food.

Instead of a Conclusion

A plant-based diet implies the consumption of foods that come from plants (including vegetables, whole grains, legumes, nuts, seeds, and fruits) with some or no components of animal origin. This diet could have some positive effects on the human health of adults (lower risk of chronic diseases such as heart disease, type 2 diabetes, certain cancers, and obesity) but only well balanced and supplemented while in infants, children, and adolescents could cause severe deficiencies in vitamin 9 and vitamin 12 affecting the growth, cognition, social development, and expression of depression. From the point of environmental sustainability, further research is necessary to distinguish the accurate footprint of a plant-based diet as well as of animal production.

Finally, it is up to each person to decide, hopefully, responsibly and based on knowledge regarding the anatomical and physiological predispositions of the human population, the consequences of not consuming meat, and what constitutes a balanced meal for a certain age, what kind of diet will follow. From the point of view of those engaged in animal production, it is up to us to ensure efficient and sustainable production of high-quality animal products, because the market is only growing.

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THE INFLUENCE OF NUTRITION ON CANINE BEHAVIOR AND THE ROLE OF THE GUT-BRAIN AXIS: A COMPREHENSIVE REVIEW

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Invited paper

Abstract: The emotional well-being of our animals plays a crucial role in their overall quality of life, which in turn affects the lives of their owners. The evidence of existence of a gut-brain axis has prompted studies to understand the complex interactions between intestinal microbiota, central nervous system, autonomic nervous system, and other communication pathways, in order to contribute to the improvement of psychological and behavioral balance through diet and targeted supplements. Indeed, studies are investigating the potential of nutritional intervention on behavior by employing nutrients such as amino acids, fatty acids, or additional dietary supplements, which may provide benefits through the interaction with the gut-brain axis.

Key words: gut-brain axis, canine nutrition, intestinal microbiota, central nervous system, behavior, supplements

Introduction

Behavioral disorders in dogs are often challenging to manage (Niyyat *et al*., 2018). They are mainly represented by increased aggression towards people or other dogs, anxiety, stress, fear, disobedience, and depression *(Bosch et al., 2007).* Sometimes, these disorders are more prevalent during the aging phase, and in some cases, it can be referred to as "Cognitive Dysfunction Syndrome" (CDS), associated with specific neurochemical, metabolic, and neuroanatomical alterations *(Pan, 2011).* This condition leads to a progressive decline in brain functions, similar to what characterizes Alzheimer's disease in humans. Behaviorally, CDS mainly affects memory, learning, awareness, reasoning, and perception *(Landsberg et al., 2005),* resulting in various abnormal behaviors in elderly dogs. In recent years, there has been a growing focus on studying the mental health of dogs, as it constitutes an issue of animal welfare *(Manteca, 2011)* that negatively impacts not only the dogs' quality of life but also that of their owners *(Pan, 2011).* Studies are now investigating the influence of diet on behavior, exploring certain bacterial metabolites like serotonin, specific nutrients such as the amino acids tryptophan and tyrosine, and polyunsaturated fatty acids like EPA and DHA. Moreover, the discovery of the gut-brain axis, a connection involving neuronal, immunological, hormonal, and metabolic pathways *(Mayer et al., 2022)*, has strengthened the idea that associating nutritional support, integrating dietary supplements or certain bacterial strains, could be beneficial for individuals with these issues. Furthermore, in elderly dogs with CDS it can be beneficial supporting brain function with alternative energy sources like medium-chain triglyceride fatty acids. Additionally, it can be helpful for overall well-being integrating the diet with mitochondrial enzymatic cofactors, that counteract oxidative damage caused by free radicals in brain tissue, antioxidants such as vitamins C and E, carotenoids, polyphenols, flavonoids, and minerals like selenium, zinc, copper, and manganese.

The gut-brain axis

The research regarding gut-brain axis is at the beginning with a primary focus on rat and human species, but potentially these studies hold significant potential and serve as valuable inspiration for future therapeutic interventions in dog as well.

The gut-brain axis activity is mediated by several pathways *(Dinan and Cryan, 2012)*, such as:

1. Central Nervous System – CNS, and afferent vagal and spinal fibers;
2. Neuroendocrine System – NES;
3. Autonomic Nervous System – ANS and, in particular, Enteric Nervous System – ENS composed by myenteric (Auerbach's) and submucosal (Meissner's) plexuses, and sympathetic and parasympathetic fibers;
4. Intestinal Microbiota – IM, and specifical metabolites derived from catabolism of nutrients (such as short-chain fatty acids – SCFAs), neurotransmitters and neuroactive metabolites produced in the gastrointestinal (GI) tract (Strandwitz, 2018) and microbial cell wall components too *(Needham et al., 2020).*

Independently from the CNS, ANS nerve cells regulate motility and secretion of digestive system. They establish synaptic connections with NES and smooth muscle cells *(Sjaastad et al., 2013)* and, at the same time, communicate with the CNS and influence brain function through afferent pathways *(Dinan and Cryan., 2012).*

Moreover, IM can impact brain activity through local effects on enteric neurons or via activation of vagal and sympathetic afferent receptors. Vagal fibres are involved also in the communication between IM and hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response *(Sudo et al., 2004; Foster and Mcvey Neufeld, 2013)* but it also modulates alimentary function during digestion *(Tsigos and Chrousos, 2002).*

Additionally, the IM interacts with the immune system through the release of cytokines and chemokines, which play a role in regulating the inflammatory response.

The influence of bacterial metabolites on CNS

As mentioned earlier, IM can communicate with the CNS through catabolites produced by IM bacterial populations *(Kubinyi et al., 2020).*

The SCFA, for example, can influence serotonin production (that also depends on the amount of tryptophan in diet) by increasing the mRNA expression of tryptophan-hydroxylase enzyme in enterochromaffin cells.

Serotonin plays a crucial role in the gut-brain axis as the primary neurotransmitter *(Margolis et al., 2021).* It is responsible for regulating local immune responses, peristalsis, and secretions in the GI tract. Approximately 90% of serotonin production occurs within the digestive system, after which it is transported throughout the body via platelets *(Spohn and Mawe, 2017).* The action of *Streptococcus* spp. is known to contribute to the production of serotonin through tryptophan-synthase activity *(Portune et al., 2016).* Serotonin is not only an important chemical messenger in CNS and ENS because it extends the dopamine action in cerebral cortex and hippocampus *(Palestrini et al., 2019),* but it also plays a crucial role in regulating behavior, mood, pain sensitivity, cardiovascular function, hormone release and sleep. These effects are mediated through afferent-vagal fibers, as discussed by *Reigstad et al. (2015)* and *Bonaz et al. (2018).* Serotonin plays a role in regulating behavioral responses and is negatively associated with aggression *(Manteca, 2011).* It promotes a sense of well-being and calmness, and its depletion can lead to impulsivity, hyperexcitability, hyperreactivity, and disinhibition *(Riva et al., 2011).*

The SCFA can also influence various other pathways including, for example, those associated with satiety. In fact, their amount impacts on tyrosine-tyrosine peptide (PYY) and glucagon-1-like peptide (GLP-1), secreted by “L” intestinal cells. Acetate and propionate, primarily detected by the GPR43 receptor, play a significant role in this process. Specifically, acetate and propionate induce PYY release, facilitate the blood-brain barrier crossing, and inhibit neurons in the arcuate nucleus of the hypothalamus, which regulate hunger and eating behavior *(Bosch et al., 2009).*

Another relevant neurotransmitter is the dopamine that can also be produced, such as norepinephrine, by *Bacillus* genus bacteria in GI tract: it has been demonstrated that the IM is capable of locally producing biologically active catecholamines, such as dopamine and norepinephrine; *Asano et al. (2012)* conducted a study comparing germ-free mice, lacking of commensal microbiota, with normal mice, and found reduced concentrations of both hormones in germ-free mice, present in biologically inactive forms.

Lastly, another fundamental neurotransmitter is the inhibitory γ-aminobutyric acid (GABA), produced by *Lactobacillus* and *Bifidobacterium* spp in GI tract, via decarboxylation of glutamate, by the glutamate-decarboxylase enzyme *(Portune et al., 2016; Strandwitz, 2018).* Apparently, specific GI bacterial populations can influence the expression of some GABA receptors in CNS particularly during the developmental age *(Bravo et al., 2011).* As previously mentioned, another form of communication between IM and CNS is through the HPA axis: *Foster and Mcvey Neufeld (2013)* demonstrated that certain bacterial microorganisms are able to activate stress circuits, contributing to the development of mood disorders, such as depression and anxiety. In a study, the influence of the IM on the HPA axis was investigated by comparing germ-free mice with free from specific pathogens mice. Both groups were subjected to mild stress induced by containment inside a glass tube, and the plasma levels of ACTH and corticosterone were measured afterward. The results highlighted an overstated stress response in germ-free mice which, however, could be attenuated by colonization with *Bifidobacterium infantis* or with a fecal microbiota transplantation *(Sudo et al., 2004).*

The relationship between IM and behavior

The bidirectional communication between the intestine and the brain is a complex process. On one hand, the brain can influence the peristalsis, sensory perception, and secretory activity of the intestine, as highlighted by *Dinan and Cryan (2012).* On the other hand, signals originating from the enteric nervous system and IM can have a profound impact on brain function, particularly on cognitive and emotional-behavioral processes. This interaction can sometimes lead to emotional, mental, and behavioral disorder. According to *Kubinyi et al. (2020),* behavior can be influenced by metabolites synthesized by intestinal bacterial microorganisms that transfer information to the brain, leading to neurophysiological changes in human *(Foster et al., 2016).* These changes can affect behavior and anxiety levels (O'Mahony *et al*., 2015), and have also been implicated in the development of neurodegenerative diseases such as Parkinson and Alzheimer diseases, as well as other neuropsychological disorders including depression and autism *(Ambrosini et al., 2019).* There is emerging evidence supporting these concepts even in dogs. For example, *Kirchoff et al. (2019)* evaluated for the first time the relationship between IM and aggression in dogs, comparing two groups of Pitbull dogs that had been saved from organized fighting. Dogs were divided in two groups based on whether they were aggressive or not and were fed the same commercial diet. Results showed that predominant phyla in both groups were *Firmicutes, Fusobacteria*, *Bacteroidetes* and *Protebacteria* but it was also observed that the genera *Fusobacterium, Dorea, Bacteroides* and *Proteobacterium* were more abundant in the feces of non-aggressive dogs, while bacteria belonging to the *Lactobacillus* genus and *Firmicutes* were more abundant in aggressive dogs.

The relationship between IM and behavior was evaluated also by *Mondo et al. (2020)* by comparing three groups of forty-two dogs of different breeds, aged between 1 and 13, from three different shelters but fed with the same diet. Based on diagnostic evaluations conducted by a veterinary behaviorist, eleven of these dogs were identified as aggressive, thirteen as fearful, and eighteen as normal. The statistically significant findings from the comparison of the IM among the three groups revealed that aggressive dogs exhibited a distinct biodiversity pattern, with a relative abundance of *Megamonas* and *Catenibacterium*, and a reduction in *Bacteroides* and *Coprobacillus* compared to phobic or normal dogs. Moreover, *Lactobacillus* was found to be more abundant in the fearful group. These findings do not align with previous studies, such as those conducted by *Dinan and Cryan (2012),* which also suggested a potential calming effect of *Lactobacillus,* associated with probiotic and psychobiotic properties and with the production of GABA.

Results from these studies suggest that analyzing IM may help predicting aggressive behaviors and that these behaviors may be reduced by supporting IM health and balance with specific probiotics.

Nutrients' role in behavioral modulation

In recent years, there has been increasing focus on the modulation of animal behavior through diet or dietary supplements. As already mentioned, certain neurotransmitters play an important role in the development of some behavioral pathologies, for this reason a controlled supply of amino acids precursor of these neurotransmitters can aid in behavior and mood modulation *(Bosch et al., 2007; Riva et al., 2007).*

For example, tryptophan (Trp) is an essential amino acid in dog, so it must be obtained through the diet. Once introduced into the CNS with the help of a carrier protein, it is converted into 5-hydroxytryptophan (5-HTP) by tryptophan-hydroxylase. Subsequently, 5-HTP is decarboxylated by 5-HTP-decarboxylase enzyme resulting in the production of serotonin, also known as 5-HT *(Felten et al., 2016).*

Starting from findings in rats, studies are gradually multiplying in dogs to evaluate how the Trp integration is able to bring benefits to behavior, when provided above the minimum recommended levels. *De Napoli et al. (2000)* compared the effect of four diets based on different protein and Trp levels in 33 dogs diagnosed with aggression or hyperactivity. They found that diets with moderate protein levels or Trp supplementation were helpful in managing aggression. *Puurunen et al. (2016)* examined plasma metabolites levels in a group of 22 dogs with clinical signs similar to attention deficit hyperactivity disorder (ADHD) in human, finding that individuals with more accentuated ADHD-like behaviors had high plasma levels of kynurenic acid, metabolite of Trp catabolism, and consequently a reduced availability of Trp for the CNS and serotonin as well. In fact, it has been shown that oral administration of Trp can alleviate ADHD symptoms *(Banerjee and Nandagopal, 2015).* Trp was also tested *in vivo* in a blend with *Valeriana officinalis* and *Melissa officinalis (Cannas et al., 2013)*: dogs that received this blend, compared with a group that received a placebo, showed a decreasing frequency and intensity of anxiety-related behaviors such as house soiling, compulsive following the owner, vocalizing, destroying objects and having aggressive tendencies.

Generally, increasing Trp levels in the CNS and thus enhancing serotonin synthesis not only depends on elevated Trp levels in the plasma (which are influenced by Trp bound to albumin) but also on reducing the plasma concentrations of other neutral amino acids that compete for the same carriers. In addition to the dietary protein content, carbohydrates content also seems to play an important role. An increase in insulin levels promotes the absorption of neutral amino acids in skeletal muscles without affecting Trp, which remains protected by its binding to albumin. Consequently, the variation of insulin levels promotes the passage of Trp into CNS, due to lack of competitors *(Bosch et al., 2007; Tynes and Landsberg, 2021).* Factors such as sex, breed, age, activity, and social status of dogs, as well as inflammatory processes and certain medications like corticosteroids that increase Trp metabolism, also influence the amount of Trp in circulation and in the CNS *(Bosch et al., 2007; Dipace, 2015).*

Another amino acid that may affect behavior is tyrosine (Tyr), which has shown efficacy in managing stressful events in humans and rodents. Tyr is involved in the synthesis of adrenaline, which predominantly functions as a hormone, and norepinephrine and dopamine, which are important neurotransmitters in the CNS *(Sjaastad et al., 2013). Lehnert et al. (1984)* administered a Tyr-enriched diet to a group of rats and evaluated their reaction after tail shock stress. Thanks to the Tyr supplementation, there was no stress-induced depletion of norepinephrine levels, suggesting the hypothesis that, under conditions of stress and high catecholaminergic demand, a Tyr-rich diet may prevent the depletion of catecholamines precursors *(Lehnert et al., 1984).* Moreover, supplementation of Tyr in human has been associated with limiting cognitive and behavioral declines during stressful conditions and improving cognitive functions, memory, attention, and reasoning skills *(Mahoney et al., 2007; Attipoe et al., 2015; Jongkees et al., 2015).* Surely, future studies are necessary to fully understand the potential role of Tyr as a dietary supplement.

Another category of nutrients that may influence behavior is represented by specific polyunsaturated fatty acids like eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), considered crucial for neurological development, CNS functioning, maintenance of brain membrane integrity and fluidity and transmission of signals between neurons *(Parletta et al., 2013; Tynes and Landsberg, 2021)*. EPA and DHA act also as neuroprotectors and allow to enhance cognitive functions and memory *(Kaur et al., 2020).* Dietary supplementation with DHA may be beneficial in puppies because it improves perceptual, cognitive, motor and neurosensory systems development *(Kidd, 2007; Zicker et al., 2012).* Moreover, in senior dogs, DHA, in combination with other protective nutrients, aids to prevent cognitive aging-associated decline, characterized by progressive cognitive deterioration *(Dodd et al., 2003; Hadley et al., 2017; Palestrini et al., 2019).*

In dogs, as in human beings and rats, the relationship between lipid metabolism and aggression has been investigated. For example, comparing triglyceride and total cholesterol levels (*Sentürk and Yalçin, 2003)* or DHA levels in plasma *(Re et al., 2008)* of aggressive and non-aggressive dogs, it has been found that dogs with dominance aggression exhibit hypocholesterolemia, advancing the hypothesis that low cholesterol concentration may negatively affect serotonin uptake by reducing cerebral membrane fluidity and serotonin receptor exposure. Results also showed significantly lower DHA concentration, higher ω-6 to ω-3 ratio and lower bilirubin levels in aggressive animals. More recently, *Niyyat et al. (2018)* evaluated the efficacy of a DHA, EPA, magnesium citrate and zinc sulphate supplement in dogs suffering from behavioral problems. The dietary supplement was administered daily for six weeks to dogs with aggressive, hyperactive, fearful behaviors and with inappropriate elimination and destructive attitudes. At the end of the treatment, authors observed an improvement in fear, destructiveness and incontinence, based on behavioral assessment questionnaires.

A specific protein hydrolysate called α-casozepine (α-CZP) has also been studied with the aim of controlling anxious and aggressive behaviors of dogs. In fact, α-CZP, a casein derivative, is an allosteric modulator of GABA-A receptors with an activity comparable to diazepam, an anxiolytic benzodiazepine. The α-CZP binds to GABA-A receptors, enhancing the inhibitory signal of GABA, involved in the development of anxiety *(Miclo et al., 2001; Beata et al., 2007)*. Studies in dogs have confirmed a decrease of anxiety signs, cortisol plasmatic levels and heart rate *(Palestrini et al., 2010)* and a general reduction of aggressiveness and fear signs when α-CZP was associated to Trp in the diet *(Kato et al., 2012).*

Other dietary supplements with a similar activity on behavior include α-lactalbumin, containing a significant amount of Trp, tested in rat and human species *(Orosco et al., 2004; Markus et al., 2000)* and used in dogs in blends with other nutraceuticals, and L-theanine, a precursor for GABA biosynthesis, that increases GABA concentrations and enhances its inhibitory effect *(Cannas et al., 2021).* L-theanine also increases the concentration of serotonin and dopamine in some specific brain regions in rats *(Yokogoshi et al., 1998; Nathan et al., 2006)* and studies in dogs have demonstrated its relaxing effects and ability to alleviate stress and fear responses induced by noise or associated with certain places, objects, or people *(Berteselli et al., 2007; Michelazzi et al., 2015).*

Probiotics’ role in behavioral modulation

Probiotics are living beneficial microorganisms (bacteria and yeasts) that are able to resist the digestive processes along the gastrointestinal tract and reach the intestine in a viable state. Once in the intestine, probiotics attach to the mucosa, produce metabolites, modulate the immune system, and improve the IM stability, ensuring a more balanced composition and efficient activity. The Food and Agriculture Organization (FAO) defined probiotics as “live microorganisms that, when administered in adequate quantities, bring a benefit to the health of the host” *(FAO and WHO, 2006).* At present, the European Community has included in the list of permitted dog feed additives *E. faecium* NCIMB 10415, *L. acidophilus* CECT 4529, *B. velezensis* DSM 15544, *L. reuteri* DSM 32203 and the yeast strain *S. cerevisiae* MUCL 39885, *(Register of Feed Additives Pursuant to Regulation (EC) No 1831/2003*). Interestingly, some bacterial strains, for example certain *Lactobacillus* and *Bifidobacterium* species, are capable of producing significant amounts of neuroactive substances that may positively influence behavior and mental health via gut-brain axis *(Foster et al., 2016)* making them referred to as "psychobiotics".

Based on findings on rats, *Lactiplantibacillus plantarum* PS128 has been tested *(Yeh et al., 2022)* to modulate behavior disorders in a group of 45 dogs, selected based on data reported by owners and assessed using the EDED emotional disturbance rating scale and the CBC - Canine Behavioral Checklist questionnaire, which include targeted questions for subgroups such as "aggression", "separation anxiety", "compulsive disorders" and “unclassified categories” *(Landsberg et al., 2013).* At the end of supplementation period, dogs showed a significant improvement in separation anxiety and aggression disorders. Moreover, the analysis of the 5-HT and its metabolite 5-HIAA plasma levels revealed a decreased 5-HIAA to 5-HT ratio in dogs affected by separation anxiety, suggesting a reduced catabolism of 5-HT and its increased availability.

The same bacterial strain, when tested in germ-free mice demonstrated similar effects on behavior, neurotransmitter levels (5-HT and dopamine), and neuromodulation *(Liu et al., 2016).*

Starting from findings in rats, various *Bifidobacterium* strains have been tested in dogs as well: for example, *B. longum* was found to be effective in reducing anxiety-related behaviors in dog according to *Bercik et al. (2011).*

Other bacterial strains with potential “psychobiotic” features tested in rats include *L. rhamnosus* JB-1 which modulates GABA receptors and has a decreasing effect on stress-induced corticosterone concentrations and anxiety-related behavior *(Bravo et al., 2011)*; *L. paracasei* CCFM1229 and *L. rhamnosus* CCFM1228 *(Xu et al., 2022)* that significantly improved synaptic plasticity, astrocyte function and CNS myelin structure and role in depressed mice; a blend composed by *B. longum* and *L. helveticus,* that displayed anxiolytic-like activity in a preclinical study on rats and beneficial psychological effects in a clinical study on healthy human volunteers *(Messaoudi et al., 2011); B. infantis* that showed a Citalopram-like effect, a selective serotonin reuptake inhibitor, when tested in rats with altered IM partially associated to a post-traumatic stress from separation *(García-Ródenas et al., 2006; Desbonnet et al., 2010);* a blend of *L. reuteri, L. rhamnosus* and *B. infantis* which showed modulatory effects on biomarkers of oxidative stress and inflammation (such as IL-1β e TNF-α) and Aβ plaques in an Alzheimer's disease rat model *(Mehrabadi and Sadr, 2020).*

Conclusion

The influence of diet on behavior is becoming increasingly evident in various species, including rats, humans, and dogs. This involves nutrients or dietary supplements capable of regulating behavioral disorders such as anxiety, stress, fear, and aggressiveness. The nature of these compounds is various, including essential amino acids, polyunsaturated fatty acids, as well as other functional ingredients derived from amino acids or polypeptide chains, and even specific bacterial strains. Some of these interact with neural, immunological, and endocrinological mediators, which influence the gut-brain axis activity. Certainly, further studies are necessary to fully understand these interactions and their implications.

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EFFECT OF THE FATTY ACID COMPOSITION OF THE MATERNAL DIETS OF SWISS LARGE WHITE SOWS ON THE FATTY ACID COMPOSITION OF THE BACKFAT OF THE PROGENY

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Abstract: The study aims to determine the effects of two dietary fats (coconut fat [CF] and soy oil [SO]), which differ in their saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid (FA) content, supplemented to the gestation and lactation diet of 16 multiparous Swiss Large White sows on the FA composition of the adipose tissue (AT) of their progeny at 105 kg body weight (BW). At weaning, four females, two with the lowest and two with the highest birth weight (BtW) within the litter from each CF and SO sow were selected and fed a standard starter and grower diet from 9 to 63 kg BW. In the finishing period (63 to 105 kg BW), one low and one high BtW pig was fed a finisher diet with the same FA composition (expressed as % total FA; SFA: 25.8%; MUFA: 26.6%; PUFA: 47.6%) as the grower diet (high degree of unsaturation; dUS-H), whereas one low and one high BtW pig was fed a more saturated (low degree of unsaturation; dUS-L) finisher diet (SFA: 28.8%; MUFA: 25.1%; PUFA: 46.1%). Sow reproduction traits, mature milk FA profile, growth performance, carcass characteristics, and FA composition of the progenies' AT were assessed. Sow reproduction traits and litter performance during lactation were not affected by the dietary FA composition of the gestation diet. Compared to the SO group, the milk of CF sows contained more SFA and MUFA ($P < 0.01$) and less PUFA ($P < 0.01$) than the sow milk of the SO group. Regardless of the diet fed in the finisher period, CF and low BtW pigs were less efficient ($P \leq 0.04$), and low BtW pigs grew slower ($P = 0.02$). The dUS-L pigs had lower ($P = 0.02$) carcass yield, greater ($P = 0.01$) defatted shoulder portions, lighter ($P \leq 0.05$) hearts and kidneys, and heavier livers ($P = 0.02$). The maternal diet and the BtW had minimal effects on the FA composition of the AT of slaughtered pigs. The AT of the dUS-L pigs contained greater amounts of SFA and MUFA ($P < 0.01$) and lower amounts of PUFA ($P < 0.01$) than the AT of dUS-H pigs. These differences were primarily due to greater

($P < 0.01$) levels of stearic, palmitoleic, and oleic acid, and lower ($P < 0.01$) levels of linoleic acid. However, compared to the dUS-H diet feeding, the dUS-L diet decreased the linoleic acid and PUFA content to a greater extent in the pigs AT born from CF than SO sows and increased the stearic and SFA level only in progenies AT of the CF sow (maternal feeding × finisher diet interaction; $P = 0.04$). These findings confirm that the FA composition of the AT depends strongly on the supplied dietary FA in the grower and finisher diet. Furthermore, we show that the FA composition of the gestation and lactation diet has lasting effects and influences how the dietary FA of the grower and finisher diet impacts the AT's FA profile of slaughtered pigs.

Key words: birth weight, dietary fat, maternal nutrition, grower diet, pigs

Introduction

There is evidence that the source, as well as the level of dietary fats offered during gestation and lactation, influence the sow's reproductive performance, piglet growth *(Tanghe and De Smet, 2013)* and the development of the offspring's adipose tissue (AT) *(Gerfault et al., 1999; Boone et al., 2001; Mourot, 2001)*. Increasing the fat content of the gestation and lactation diet from 2.5% to 5% increased the fat content of the carcass and liver of newborn and seven-day-old piglets. The offspring from the 5% fat group had fewer but larger fat cells in the subcutaneous fat *(Mourot, 2001)*. At 100 kg body weight (BW), the number of fat cells was probably increased due to the greater number of preadipocytes on day 7 of life. However, the diameters of adult fat cells were smaller, and the fat content of the carcass was not changed at 100 kg BW. Apart from the fat content, the fatty acid composition of the fat added to the gestation and lactation diet also influences the prenatal development of the foetal AT as well as the characteristics of the AT of the slaughtered pigs *(Gerfault et al., 1999; Boone et al., 2001)*. In these studies, coconut fat (high in saturated fatty acids [SFA]), sunflower oil (high in polyunsaturated fatty acids [PUFA]), and pork fat (similar relative proportion of SFA and monounsaturated fatty acids [MUFA]) were compared. The carcasses of newborn piglets of sows fed the PUFA-rich gestation diet were fatter than those of the pork fat group. In addition, the fatty acid composition of the newborn piglets' AT was similar to that of the supplemented fat of the gestation diet. This suggests that PUFA, which can only be of dietary origin, penetrates the placental barrier. At 100 kg BW, offspring of sows fed the coconut-fat-supplemented diet had more but smaller fat cells than offspring of sows fed the sunflower-oil- or pork-fat-supplemented diet. The amount of deposited fat at the slaughter of finishing pigs did not differ. These results indicate that the amount and fatty acid composition of the dietary fat offered to the gestating sows affects *in utero* hyperplasia and hypertrophy of the fat cells of the offspring.

In the postnatal period, three major factors impact the amount of deposited AT: the birth weight (BtW), the feeding intensity (restricted vs *ad libitum* feed access) and the fatty acid composition of the supplemented dietary fat. In agreement with previous studies *(Gondret et al., 2006; Rehfeldt and Kuhn, 2006)*, we have demonstrated that the fat deposition rate from birth to slaughter depends on the BtW *(Bee, 2004)*. Compared to their siblings with a heavier BtW, pigs with a low BtW have significantly more AT and consequently a lower lean meat content when offered *ad libitum* access to the same starter and grower-to-finisher diets. Compared to feed restriction, *ad libitum* feed access commonly leads to a greater fat deposition *(Bee et al., 1999; Bee et al., 2002)*. In addition to the feeding regime, the fatty acid (FA) composition, more than the amount of dietary fat offered from weaning to slaughter, has a marked impact on the fatty acid composition of the AT *(Bee et al., 1999; Bee et al., 2002)*. Furthermore, *Averette Gatlin et al. (2002)* have reported that by altering the dietary FA composition, the FA profile of the AT can be modulated fairly easily within weeks. These dietary interventions can be relevant for pig production labels where specific FA profiles are requested for their products.

With these influencing factors in mind, we conducted a study comparing two dietary fats used in the gestation and lactation diets, differing in the degree of unsaturation (dUS; low dUS = coconut oil; high dUS = soy oil), and their impact of growth performance, carcass composition and the AT's FA composition of low and high BtW offspring. These pigs were offered the same starter and grower diets, whereas the FA composition of the finisher diet differed in the dUS. We hypothesized that, compared to the heavier siblings, low BtW pigs would deposit less AT and that the dUS of the gestation and lactation diets partly and that of the finisher diet more significantly increase the dUS of the AT.

Materials and Methods

The Swiss Federal Committee for Animal Care and Use approved all procedures involving animals.

Experimental lactation and gestation diets and sows' rearing condition

Two basal gestation and lactation diets were formulated to cover the nutrient requirements of sows specifically in the production period *(Agroscope, 2017)*. The gestation diets were supplemented with 3.8% coconut fat (CF) or 3.8% soy oil (SO). The lactation diets were supplemented with 5% CF or 5% SO. All basal diets contained no additional fat supplements.

Eighteen multiparous Swiss Large White sows were allocated to one of the two experimental groups based on their BW at weaning. They were then mated by artificial insemination to one boar and kept for the 110 d of the gestation period in group pens equipped with an automatic feeder system and individual pig recognition system (Schauer Maschinenfabrik Ges.m.b.H. & CoKG – A-4731 Prambachkirchen, Austria). The feed allowance was 2.9 kg/d, and the actual feed intake was recorded daily. On d 111 of gestation, the sows were transferred to the nursery and kept in individual pens until weaning on d 32 of lactation. Feed allowance was 2.5 kg on d 1 after birth and was gradually increased to 5.2 kg within 6 d after birth. The daily feed allowance was increased by 200 g/d per piglet for sows with more than eight suckling piglets. Sows' BW was determined on d 0 (start of the experiment) and d 110 of gestation, and the end of lactation. The day of farrowing and at weaning, all piglets of the litters were individually weighed. Milk samples were collected on d 28 of lactation, after the piglets had been separated from the sows for at least one hour. The milk samples were stored at -20°C until analysis.

Experimental grower and finisher diets and selected progenies

At weaning, two females born with the lowest (L, but > 800 g) and two females born with the highest (H) birth weight (BtW) were selected from each litter. From weaning (8.9 ± 2.11 kg BW [mean ± SD]) to the start of the grower period (22.2 ± 1.43 kg BW) and during the grower period (22.2 ± 1.43 kg to 62.6 ± 1.81 kg BW), the 72 pigs received the same starter and finisher diets (Table 1). In the finisher period (62.6 ± 1.81 kg to 106.3 ± 2.93 kg BW), two finisher diets were prepared. One had a similar dUS (dUS-H = 2.88) as the grower diet, and the other had a lower dUS (dUS-L = 2.47). Half of the L and H pigs were assigned to either the dUS-H or the dUS-L diet. Pigs were group penned from weaning to slaughter. As for the gestating sows, pens for pigs in the starter, grower, and finisher periods were equipped with an automatic feeder system and individual pig recognition system (Schauer Maschinenfabrik Ges.m.b.H. & CoKG – A-4731 Prambachkirchen, Austria). Daily individual feed intakes were recorded, and BW was determined weekly. In the grower and finisher periods, feed access was restricted. Aiming to reach an average growth rate of 700 g/d, the daily digestible energy allowance was adjusted weekly based on the BW using the Agroscope feeding curve *(Agroscope, 2017)*. One week after the pigs reached a BW of 100 kg, they were slaughtered at the research abattoir. Prior to slaughter, the pigs were fasted for 12 h. Slaughter and carcass dissection were carried out as described previously *(Bee et al., 2004).*

Tissue sampling and preparation

During the gestation, lactation, and grower-to-finisher periods, feed samples were regularly collected and pooled into three feed samples for each period and experimental treatment. Collection of mature sow milk took place at 11:00 for sows who farrowed in the morning or at 15:00 for those who farrowed in the afternoon. Milk (10 mL) was obtained by milking several udders after milk let-down was induced by intramuscular injection of 3 mL oxytocin (Oxytocin-20, Graeub, Switzerland). Samples were frozen immediately for lipid extraction and fatty acid analysis.

The day after slaughter, 100 g samples from the AT at the 10^{th}-rib level were taken from the left side of the carcasses, lyophilized, homogenized, vacuumed, and stored at -20°C until the determination of total fat content and the fatty acid profile.

Sample analysis of feed, mature milk and adipose tissue

Dry matter, ash, CP, crude fat, and crude fiber analyses of pooled feed samples were carried out according to methods described by the Association of Official Analytical Chemists *(AOAC; 1995)*. Milk and AT lipids were extracted, as described by *Winter (1963)*, with minor modifications. Briefly, 5 g of milk and 300 mg of AT were homogenized in 1 mL of triundecanine (internal standard) and 60 mL of dichloromethane/methanol (1:2, v/v) for 30 s. After 15 min, the filtered sample was added to 1 mL $MgCl_2$ (20 g/L) and 20 mL water. The organic phase containing the lipid extract was removed and reduced in volume under vacuum (550 × 102 Pa at 40°C). The fatty acid methyl esters were prepared by transesterification by methanolic sodium hydroxide and boron trifluoride according to the method of *Metcalfe and Schmitz (1961).* The fatty acid methyl esters were determined using a gas chromatograph (HP 5860 A GC, Hewlett-Packard, Urdora, Switzerland), equipped with a flame ionization detector, and separated on a 30 m × 0.32 mm internal diameter (i.d.) Supelcowax TM 10 fused-silica capillary column (Supelco, Bellefonte, PA). The oven temperature was as follows: initial temperature 170°C for 1 min; raised to 250°C at 2.5°C/min; 250°C held for 7 min. The detection temperature was at 250°C and split at 250°C.

Statistical analysis

Data on sow reproduction, litter performance and FA profile of the mature milk were analyzed as a randomized complete block design using the PROC MIXED of SAS (Version 9.1, SAS Inst. Inc., Cary, NC) with an individual sow as the experimental unit. The data on offspring's growth performance, carcass characteristics and the FA profile of the AT were analyzed using the PROC MIXED of SAS (Version 9.1, SAS Inst. Inc., Cary, NC) as a split-split-split plot design, where sow treatment was the whole plot, BtW the sub-plot and dietary treatment in the finisher period the sub-sub plot. Litter of origin was defined as the random effect. Least square means were calculated, and the PDIFF option of SAS was used to determine differences between treatment groups. Differences were considered as significant at $P < 0.05$ and as a tendency at $P < 0.10$.

Results and Discussion

Gestation, lactation, grower and finisher diets

Despite the different amounts of fat supplemented to the gestation (3.80%) and lactation (5.06%) diets, the dUS were similar in the CF gestation and lactation diets and in the SO gestation and lactation diets (Table 1)

The dUS of the CF gestation and lactation diets were 0.66 and 0.61, respectively, and those of the SO gestation and lactation diets were 5.04 and 4.90, respectively. The addition of coconut fat elevated the SFA level by up to 44.1% in the gestation and lactation diets and lowered that of MUFA and PUFA by up to 12.3% and 31.4% in the CF compared with the SO diets. These differences were mainly caused by higher levels of dodecanoic (12:0) and myristic acid (14:0) and lower levels of oleic [18:1(n-9)], linoleic [18:2(n-6)] and linolenic acid [18:3(n-3)].

As per the design, the dUS of the finisher diet differed (Table 1). The greater dUS of the dUS-H compared to the dUS-L diet resulted from higher inclusion levels of corn and soybean meal, both rich sources of MUFA and PUFA. As a result, 18:1(n-9), 18:2(n-6), total MUFA and total PUFA levels were higher, and palmitic acid (16:0) and total SFA were lower in the dUS-H compared with the dUS-L diet.

Table 1. Diet composition and nutrient and digestible energy content of the gestation and lactation diets of the sows and grower-to-finisher diets of the progenies

	Maternal diets[1]		**Maternal diets[1]**		**Diets of progeny[2]**		
	Gestation		**Lactation**		**Grower**	**Finisher**	
	Coconut fat (CF)	**Soy oil (SO)**	**Coconut fat (CF)**	**Soy oil (SO)**	**G**	**dUS-H**	**dUS-L**
Feed ingredients[3]							
Barley			1.00	1.00	19.90	21.90	24.80
Wheat	18.60	18.60	28.20	28.20	36.00	34.90	37.90
Corn	17.20	17.20	22.60	22.60	13.40	17.90	
Oat	20.00	20.00					
Sugar Beet molasses	0.80	0.80					
Corn gluten	4.00	4.00					
Wheat starch							5.00
Potato protein							3.10
Soybean meal	8.60	8.60	25.70	25.70	15.50	10.70	9.40
Flax cake	2.60	2.60					
Rapeseed meal			5.00	5.00			
Barley bran					10.20	10.20	
Wheat bran	1.80	1.80					
Dry sugar beet pulp	20.00	20.00	10.00	10.00			11.80
Apple pomace							4.10
Coconut fat	3.80		5.06				
Soy oil		3.80		5.06			
Animal fat blend					1.22	1.16	0.90
Lysine-HCl					0.36	0.29	0.11
L-Methionine					0.04		
L-Threonine					0.10	0.06	
NaCl	0.30	0.30	0.40	0.40	0.45	0.37	0.35
Dicalcium phosphate	1.10	1.10	0.48	0.48	0.26	0.03	0.79
Calcium carbonate	0.40	0.40	0.70	0.70	1.86	1.80	1.05
Pellan	0.40	0.40	0.40	0.40	0.30	0.30	0.30
Vitamin-mineral-premix	0.40	0.40	0.40	0.40	0.40	0.40	0.40

Nutrient (g/kg DM) and energy content (MJ/kg DM)[4]

Crude protein	165	162	221	219	189	171	171
Crude fat	64	64	88	91	40	40	26
Crude fiber	94	104	48	49	40	38	55
DE, MJ/kg DM	14.5	14.5	16.6	16.6	15.3	15.3	15.3
Fatty acid profile, % of total fatty acids							
8:0	4.49	-	4.85	-	-	-	-
10:0	3.46	-	3.50	-	-	-	-
12:0	26.83	-	27.00	-	-	-	-
14:0	10.97	0.10	10.98	0.07	0.89	0.89	1.04
16:0	11.67	12.26	10.57	1.11	18.10	18.25	20.58
18:0	2.66	3.14	2.69	3.00	6.37	6.37	7.22
20:0	0.10	0.36	0.19	0.37	-	-	-

Table 1. (continue)

Total SFA	60.25	16.56	59.37	15.29	25.66	25.80	28.84
16:1(n-7)	-	-	-	0.15	0.84	0.89	0.99
18:1(n-9)	15.60	27.35	15.37	27.00	25.00	25.21	23.61
20:1(n-9)	0.14	0.40	0.20	0.34	0.48	0.49	0.52
Total MUFA	15.82	28.10	15.57	27.49	26.33	26.60	25.12
18:2(n-6)	20.05	48.40	22.53	52.20	44.35	44.29	41.49
18:3(n-3)	3.74	6.81	1.97	5.01	3.66	3.31	4.54
Total PUFA	23.93	55.35	20.49	47.48	4.02	47.60	46.03
16:1(n-7)/16:0	-	-	-	0.14	0.05	0.05	0.05
18:1(n-9)/18:0	5.86	8.71	5.71	9.00	3.92	3.96	3.27
US ratio	0.66	5.04	0.61	4.90	1.18	2.88	2.47

[1] CO = gestation and lactation diets supplemented with 2% coconut oil; SO = gestation and lactation diets supplemented with 2% soy oil

[2] G = standard grower diet formulated for a body weight of 40 kg *(Agroscope, 2017)*; dUS-H: finisher diet formulated for a body weight of 80 kg *(Agroscope, 2017)* with an unsaturated-to-saturated fatty acids ratio of 2.88; dUS-L: finisher diet formulated for a body weight of 80 kg *(Agroscope, 2017)* with an unsaturated-to-saturated fatty acids ratio of 2.47

[3] Pellan = binder that aids in pellet formation; animal fat blend = composed of 50% lard and 50% tallow; vitamin-mineral-premix = supplied the following nutrients per kg of diet: 20000 IU vitamin A, 200 IU vitamin D_3, 39 IU vitamin E, 2.9 mg riboflavin, 2.4 mg vitamin B_6, 0.010 mg vitamin B_{12}, 0.2 mg vitamin K_3, 10 mg pantothenic acid, 1.4 mg niacin, 0.48 mg folic acid, 199 g choline, 0.052 mg biotin, 52 mg Fe as FeS, 0.16 mg I as $Ca(IO)_3$, 0.15 mg Se as Na_2Se, 5.5 mg Cu as $CuSO_4$, 81 mg Zn as ZnO_2, 15 mg Mn as MnO_2

[4] DE = the digestible energy coefficients from each feed ingredient were obtained from the Swiss Feed database (http://www.feedbase.ch). Taking into account the relative amount of each feed ingredient in the diet, digestible contents were calculated. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; degree of unsaturation (dUS) = the sum of total PUFA and MUFA expressed as a ratio of the sum of SFA

Sow reproductive performance and mature milk composition

Supplementing the sow diets with identical amounts of coconut fat or soy oil did not affect the average daily gain (ADG) during gestation (

. This result was unsurprising, as the average feed allowance in the gestation period was based on a restricted feeding regime, resulting in a similar average daily feed intake (ADFI). The litter size at birth did not differ between CF and SO sows (

Our findings agree with those of other researchers who also failed to show any significant effects of dietary FA composition on sow reproduction traits *(Tanghe and De Smet, 2013; Quesnel et al., 2015)*. By contrast, *Corson et al. (2008)* reported larger and also heavier litters at birth when sows ingested, in the first half of pregnancy, a diet supplemented with 10% palm oil, a source rich in medium-chain FA, compared with a diet supplemented with soy oil, containing a high level of n-6 PUFA. The authors explained these differences by the fact that diets containing sunflower oil, rich in PUFA, promote pro-inflammatory responses, which can deleteriously affect piglet development. By contrast, the medium-chain FA present in palm oil are more easily combusted and could have provided a readily available energy source. In the present study, the more saturated diet had a rather harmful impact on litter BtW, as litters of CF sows tended to be 170 g lighter (P = 0.07) than litters of SO sows. An explanation could be that coconut fat is more saturated, less digestible, and does not deliver the same combusting energy as palm oil. In addition, the amounts used differed between the study of *Corson et al. (2008)* and the current study. We offered both supplements during the whole gestation period, which might further explain the different outcomes.

Table 2. The feed intake, body weight development and reproduction performance of multiparous sows fed gestation and lactation diets supplemented with coconut fat (CF) or soy oil (SO)

	Sow treatment[1]			**Effect**
Item[2]	**CF**	**SO**	**SEM[3]**	**P-values**
Gestation period				
BW at the start of gestation, kg	**206.6**	**210.7**	**10.55**	**0.79**
BW at the end of gestation, kg	**258.2**	**261.7**	**7.95**	**0.76**
ADG, kg/d	**0.492**	**0.492**	**0.039**	**0.99**
ADFI, kg/d	**2.96**	**2.97**	**0.053**	**0.89**
Lactation period				
BW at the end of lactation, kg	**226.9**	**218.7**	**8.42**	**0.50**
ADFI, kg/d	**5.07**	**5.41**	**0.175**	**0.18**
Lactation length, d	**32.8**	**32.3**	**0.51**	**0.55**
Reproduction performance				
Litter size, n	**14.6**	**13.9**	**1.05**	**0.66**
BW at birth, kg	**1.37**	**1.54**	**0.062**	**0.07**
BW at weaning, kg	**9.09**	**8.76**	**0.33**	**0.49**
ADG, g/d	**0.143**	**0.171**	**0.015**	**0.20**

[1] The CF gestation diets were supplemented with 3.80 and 5.06% coconut fat, respectively; the SO gestation diets were supplemented with 3.80 and 5.06% soy oil, respectively.

[2] BW = body weight, ADG = average daily weight gain; ADFI = average daily feed intake

[3] SEM = pooled standard error of the mean

Mature sow milk from CF sows contained similar amounts of fat as that of SO sows, whereas the milk fat was more saturated, as evidenced by the greater levels (P ≤ 0.03) of 12:0, 14:0, pentadecanoic acid (15:0) and total SFA and the lower levels (P ≤ 0.03) of myristoleic acid (14:1n-5), palmitoleic acid (16:1n-7), 18:2n-6, 18:3n-3, total MUFA and total PUFA concentrations

Table 3. The total fatty acid content and the fatty acid profile of the mature milk of multiparous sows fed gestation and lactation diets supplemented with coconut fat (CF) or soy oil (SO).

	Sow treatment[1]			Effect
Item[2]	**CF**	**SO**	**SEM[3]**	**P-values**
Total fatty acids, g/100 g	**9.02**	**9.05**	**0.168**	**0.81**
Fatty acids, g/100 g total fatty acids				
10:0	**0.22**	**0.18**	**0.036**	**0.08**
12:0	**3.79**	**0.24**	**0.378**	**< 0.01**
14:0	**6.99**	**3.02**	**0.484**	**< 0.01**
15:0	**0.16**	**0.13**	**0.016**	**0.03**
16:0	**28.49**	**28.50**	**2.310**	**0.99**
17:0	**0.28**	**0.28**	**0.034**	**0.83**
18:0	**3.86**	**4.24**	**0.478**	**0.26**
20:0	**0.09**	**0.11**	**0.004**	**< 0.01**
Total SFA	**42.45**	**35.42**	**3.327**	**< 0.01**
14:1(n-5)	**0.47**	**0.16**	**0.034**	**< 0.01**
16:1(n-7)	**8.90**	**6.49**	**0.562**	**< 0.01**
18:1(n-9)	**26.67**	**25.86**	**1.288**	**0.66**
18:1n-11	**2.17**	**1.92**	**0.107**	**0.12**
20:1(n-9)	**0.28**	**0.26**	**0.024**	**0.68**
Total MUFA	**36.32**	**32.76**	**1.074**	**0.03**
18:2(n-6)	**17.48**	**27.08**	**1.947**	**< 0.01**
20:2(n-6)	**0.33**	**0.41**	**0.033**	**0.10**
20:4(n-6)	**0.47**	**0.50**	**0.028**	**0.45**
18:3(n-3)	**1.68**	**2.63**	**0.164**	**< 0.01**
20:3(n-3)	**0.10**	**0.12**	**0.014**	**0.37**
22:5(n-3)	**0.22**	**0.21**	**0.028**	**0.64**
Total PUFA	**20.56**	**31.21**	**2.182**	**< 0.01**
Σ n-6 fatty acids	**18.35**	**28.06**	**1.992**	**< 0.01**
Σ n-3 fatty acids	**2.14**	**3.08**	**0.191**	**< 0.01**
n-6/n-3 fatty acid ratio	**8.89**	**9.07**	**0.610**	**0.61**
16:1(n-7)/16:0	**0.35**	**0.26**	**0.039**	**< 0.01**
18:1(n-9)/18:0	**6.95**	**6.36**	**0.645**	**0.17**

[1] The CF gestation diets were supplemented with 3.80 and 5.06% coconut fat, respectively; the SO gestation diets were supplemented with 3.80 and 5.06% soy oil, respectively.

[2] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

[3] SEM = pooled standard error of the mean

It is well established that milk FA composition reflects the FA composition of the dietary fat sources *(Kim, 2013)*. It is interesting to note that the 18:1(n-9) level in the milk was similar for the two sow treatments, although the dietary concentrations were half as low in the CF than in the SO diet. This can only be due to the fact that more SFA were desaturated *(Zhe et al., 2023)*. The difference in the FA composition of the milk had no effect on the growth performance of the offspring from birth to weaning, as neither ADG nor weaning BW differed between the two groups.

In this study, sow milk was the sole energy and nutrient source for the piglets during the lactation period. The similar growth rates and weaning BW suggest that milk intake and/or milk yield were unaffected by the two dietary fat sources. This hypothesis is in line with findings indicating that the milk yield and the daily energy output were not altered *(Lauridsen and Danielsen, 2004)* and had no effect on weaning BW *(Wilkinson et al., 2014)* when sows were offered lactation diets supplemented with either saturated or highly unsaturated fat.

Growth performance of selected offspring

To our knowledge there are only a few studies investigating the long-term effect of the dietary FA composition of the gestation and lactation diets on the growth rate from birth to market weight of the progeny. The results of an earlier study suggest that, regardless of the dUS of the dietary fat source used in the lactation diet, the growth rate of pigs from birth to slaughter was unaffected *(Stahly et al., 1980)*. These findings are in disagreement with the results of the current study. The ADFI in the grower and finisher period was similar, whereas the ADG in the overall grower and finisher period was lower (P = 0.02) in pigs from CF sows than from SO sows

Table 4. Growth performance of high (H) and low (L) birth weight progenies born from sows fed gestation and lactation diets supplemented with coconut fat (CF) or soy oil (SO) and offered, in the finisher period, a diet with a high (dUS-H) or low (dUS-L) degree of fatty acid unsaturation

	Maternal nutrition[1]		Birth weight[2]		Finisher diet[3]		*P*-value[4]			
	CF	SO	H	L	dUS-H	dUS-L	SEM[5]	M	BtW	F
Birth weight, kg	1.49	1.61	1.80	1.30	1.56	1.55	0.042	0.12	<0.01	0.71
BW, kg										
At weaning	9.05	8.84	10.25	7.64	8.96	8.93	0.334	0.70	<0.01	0.93
Start grower period	22.2	22.3	22.1	22.4	21.9	22.6	0.23	0.72	0.45	0.10
Start finisher period	62.5	62.5	62.7	62.3	62.7	62.3	0.33	0.87	0.54	0.49
At slaughter	105.9	106.2	106.6	105.5	105.5	106.5	0.58	0.61	0.17	0.32
ADG, kg/d										
Weaner period	0.38	0.38	0.39	0.37	0.38	0.38	0.010	0.81	0.14	0.57
Grower period	0.59	0.61	0.61	0.59	0.60	0.59	0.011	0.15	0.17	0.46
Finisher period	0.72	0.77	0.78	0.71	0.74	0.75	0.018	0.08	0.02	0.95
Grower-finisher period	0.65	0.68	0.68	0.65	0.67	0.66	0.012	0.08	0.02	0.76
Overall	0.53	0.55	0.56	0.52	0.54	0.54	0.009	0.17	<0.01	0.85
ADFI, kg/d										
Grower period	1.38	1.42	1.40	1.40	1.41	1.39	0.192	0.26	0.70	0.33
Finisher period	2.10	2.17	2.18	2.09	2.15	2.11	0.031	0.17	0.05	0.32
Grower-finisher period	1.71	1.76	1.75	1.72	1.75	1.73	0.022	0.19	0.15	0.28
G/F ratio, kg/kg										
Grower period	0.42	0.43	0.44	0.42	0.43	0.43	0.005	0.28	0.02	0.80
Finisher period[6]	0.34	0.36	0.36	0.34	0.34	0.35	0.005	0.04	0.03	0.23
Grower-finisher period	0.38	0.39	0.39	0.38	0.38	0.038	0.004	0.06	<0.01	0.50
Total feed intake, kg										
Grower period	95.7	93.2	93.6	95.3	95.3	93.6	1.33	0.28	0.33	0.46
Finisher period	127.0	123.9	124.4	126.6	125.5	125.4	1.84	0.25	0.45	0.98
Grower-finisher period	222.6	216.9	217.8	221.8	220.6	219.0	2.25	0.09	0.22	0.62
Day, d										
Grower period	69.8	65.8	67.3	68.3	67.9	67.7	1.40	0.10	0.53	0.85
Finisher period	61.3	57.3	57.6	61.0	59.2	59.5	1.52	0.09	0.19	0.89
Grower-finisher period	130.1	123.0	124.7	129.2	126.9	127.1	2.40	0.05	0.20	0.96
Age, d	199.7	191.0	188.5	202.3	194.8	196.0	3.07	0.09	<0.01	0.76

[1] The CF gestation diets were supplemented with 3.80 and 5.06% coconut fat, respectively; the SO gestation diets were supplemented with 3.80 and 5.06% soy oil, respectively.

[2] H = two female piglets from each litter with the highest birth weight; L = two females with the lowest birth weight (but > 800 g birth weight)

[3] dUS-H = unsaturated-to-saturated fatty acids ratio of 2.88; dUS-L: unsaturated-to-saturated fatty acids ratio of 2.47

[4] M = effect of maternal gestation and lactation diet; BtW = effect of birth weight; F = effect of finisher diets

[5] SEM = pooled standard error of the mean

[6] BtW × F interaction is significant at ($P < 0.05$) (Figure 1)

This was mainly caused by the lower growth rate (P = 0.02) in the finisher period. In accordance, the gain-to-feed ratio was impaired (P ≤ 0.06) in the overall grower and finisher period. As a result of the slower growth rate, pigs of the CF sows stayed 7.1 d longer on feed (P = 0.05), tended to be 8.7 d older (P = 0.09) on the day of slaughter and tended to ingest in total 6 kg more feed (P = 0.09). One could hypothesize that the effect on growth in the finisher period was related to the two finisher diets differing in the dUS. There are two arguments against this hypothesis. First, the dUS of the finisher diet had no effect on the growth performance of the pigs. Second, the lack of significant interactions between the two experimental factors (M [maternal diet] × F [finisher diet's dUS]; P > 0.10) indicates that the observed differences in growth were solely due to the FA composition of the gestation and lactation diets. Therefore, we currently have no explanation for the negative impact of CF on the growth and feed efficiency of the progeny.

By design, the selected H pigs were heavier (P < 0.01) at birth but were also heavier (P < 0.01) at weaning than the L pigs. In the finisher period, but not in the grower period, ADG was greater (P ≤ 0.02) in H pigs, despite the fact that the ADFI and total feed intake in the same period did not differ between the two BtW groups. The gain-to-feed ratio was greater (P ≤ 0.03) in the grower and overall grower and finisher periods in H pigs. In the finisher period, the BtW × F interaction (P < 0.05) showed that, regardless of the maternal diet, the gain-to-feed ratios of H pigs offered the dUS-H or dUS-L diets were similar, whereas the L pigs fed the dUS-H diet were less efficient than the dUS-L pigs. The lower efficiency of the L pigs of the dUS-H group can be explained by the numerically lower ADG compared to the other groups. Due to the faster growth (P < 0.01) from birth to slaughter, H pigs were almost 14 d younger (P < 0.01) at slaughter. The impaired growth performance could be expected, as it has previously been shown that BtW often causes intra-uterine growth retardation and has lasting negative effects on growth performance traits *(Bee, 2004; Foxcroft et al., 2006).*

Carcass characteristics and organ weights

Maternal diets did not affect the carcass characteristics of the offspring

Table 5. Carcass characteristics and organ weights of high (H) and low (L) birth weight progenies born from sows fed gestation and lactation diets supplemented with coconut fat (CF) or soy oil (SO) and offered, in the finisher period, a diet with high (dUS-H) or low (dUS-L) degree of fatty acid unsaturation

	Maternal nutrition[1]		Birth weight[2]		Finisher diet[3]		*P*-value[4]			
	CF	SO	H	L	dUS-H	dUS-L	SEM[5]	M	BtW	F
Hot carcass weight, kg	87.1	86.6	87.1	86.7	86.9	86.6	0.51	0.49	0.38	0.94
Carcass yield, %	81.9	81.5	81.6	81.6	82.0	81.4	0.24	0.31	0.29	0.02
Cold carcass weight, kg	84.8	84.3	84.7	84.4	84.6	84.5	0.49	0.51	0.49	0.95
Cold loss, %	2.69	2.63	2.70	2.62	2.65	2.67	0.029	0.18	0.09	0.72
Lean meat, %	59.0	58.7	59.0	58.8	58.5	59.3	0.32	0.58	0.61	0.09
Loin, %	26.2	26.1	26.2	26.0	26.0	26.3	0.19	0.66	0.39	0.24
Ham, %	19.8	19.9	20.0	19.8	19.8	20.0	0.15	0.84	0.45	0.41
Shoulder, %	13.0	12.8	12.8	12.9	12.7	13.0	0.08	0.11	0.56	0.01
Belly, %	17.9	17.7	17.7	17.9	17.8	17.7	0.12	0.29	0.50	0.66
Total subcutaneous fat, %	11.3	11.6	11.5	11.4	11.7	11.2	0.21	0.88	0.78	0.08
Omental fat, %	1.6	1.6	1.5	1.6	1.6	1.5	0.06	0.88	0.75	0.16
Backfat thickness, mm	13.2	14.1	14.3	13.0	13.8	13.5	0.57	0.26	0.09	0.65
Organ weights, g										
Heart	419	417	424	412	428	408	7.2	0.85	0.31	0.05
Liver	1507	1528	1518	1517	1483	1551	32.8	0.72	0.98	0.02
Kidney	292	293	300	284	300	285	5.9	0.96	0.04	0.05

[1] The CF gestation diets were supplemented with 3.80 and 5.06% coconut fat, respectively; the SO gestation diets were supplemented with 3.80 and 5.06% soy oil, respectively.

[2] H = two female piglets from each litter with the highest birth weight; L = two females with the lowest birth weight (but > 800 g birth weight)

[3] dUS-H = unsaturated-to-saturated fatty acids ratio of 2.88; dUS-L: unsaturated-to-saturated fatty acids ratio of 2.47

[4] M = effect of maternal gestation and lactation diets; BtW = effect of birth weight; F = effect of finisher diet

[5] SEM = pooled standard error of the mean

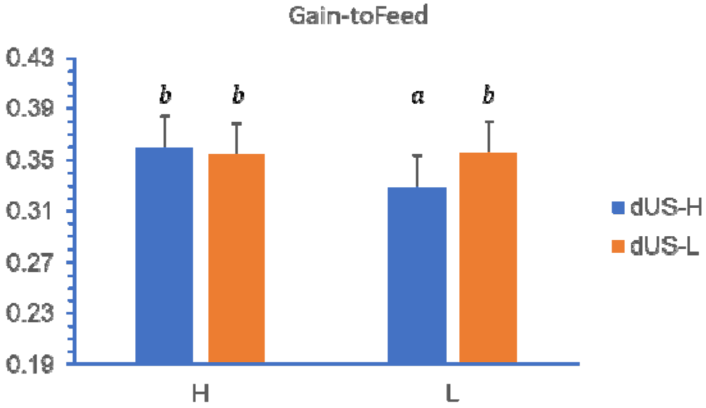

Figure 1. Gain-to-feed ratio of high (H) and low (L) birth weight progenies offered, in the finisher period, a diet with a high (dUS-H) or low (dUS-L) degree of fatty acid unsaturation.
^{ab} Bars with different superscript differ ($P < 0.05$)

Compared to L pigs, H pigs tended to have greater cold losses ($P = 0.09$) within the 24 h after slaughter and tended to have greater backfat thickness ($P = 0.09$) at the 10th-rib level. The latter did not coincide with a greater percentage of backfat. A possible explanation is that the fat tissue was redistributed within the carcass. The H pigs had heavier kidneys ($P = 0.04$) than the L pigs, whereas heart and liver weights were unaffected. Similarly, *Bérard et al. (2008)* have reported significantly lighter kidneys but also lighter livers in pigs slaughtered at market weight, whereas heart weights were not affected by the BtW. As BW at slaughter was similar for BtW categories in both studies, this effect seems to be independent of the slaughter weight. This is surprising, as at birth, except for the greater relative brain weight in low compared to high BtW pigs, relative organ weights were similar for the low and high BtW categories in the study by *Pardo et al. (2013)*.

The carcass yield of pigs fed the dUS-L diet was lower ($P = 0.02$) than that of the dUS-H group

. The percentage shoulder weight was greater ($P = 0.01$), and the overall lean meat percentage tended to be greater ($P = 0.09$) and that of the backfat tended to be lower ($P = 0.08$) in dUS-L than in dUS-H pigs. Pigs fed the more unsaturated finisher diet had lighter hearts and kidneys ($P = 0.05$) and heavier livers ($P = 0.02$). These differences were not expected, as the finisher diets had no effects on traits such as ADG, ADFI and gain-to-feed ratio, which could have impacted tissue development. The dUS-L and dUS-H diets differed mainly in the levels of corn and soybean meal, as previously mentioned. To our knowledge there is no evidence that different inclusion levels of these two ingredients can have a significantly different impact on the carcass characteristics and organ growth.

Fatty acids of adipose tissue

The current data indicate that, despite the large difference in the FA composition of the sow gestation and lactation diets, they had only negligible direct effects on the FA profiles of the AT of the progenies at slaughter (Table 6). The relative arachidic acid (20:0) and 20:1(n-9) levels were greater ($P \leq 0.09$) if offspring originated from sows fed the CF gestation and lactation diets. These differences were compensated by a tendency to a lower level ($P = 0.09$) of arachidonic acid (20:4(n-6)). Similarly, the impact of BtW on the FA profiles of the pigs at slaughter was small, ranging from 0.04 to 0.44% of units. The levels of 14:0, 16:1(n-7), 20:4(n-6), docosatetraenoic acid (22:4(n-6)) and eicosatrienoic acid (20:3(n-3)) were greater ($P \leq 0.09$), and the levels of 20:0, 18:1(n-9) and total MUFA were lower ($P \leq 0.07$) in H than in L pigs. The small differences can be explained by the fact that neither feed intake in the grower and finisher period nor carcass fat deposition differed between the BtW categories.

Out of the three experimental factors, the dUS of the finisher diets had the most distinct impact on the FA profile of the AT in the slaughter pigs (Table 6), which is in line with the well-established close relationship between the FA composition of the diet and that of the AT *(Wood et al., 2008)*. Pigs of the dUS-L group had an AT with a lower proportion ($P < 0.01$) of PUFA due to lower amounts ($P < 0.01$) of FA of the n-6 family. However, the total PUFA and 18:2(n-6) levels in the AT were similar in pigs fed the dUS-H diet and originating from CF and SO sows (Figure 2). By contrast, the levels of 18:2(n-6) and PUFA were lower when pigs fed the dUS-L diet originated from CF sows than from SO sows (M × F; $P < 0.05$). The lower content of PUFA was compensated by greater amounts ($P < 0.01$) of 16:0, SFA, 16:1(n-7), 18:1(n-9) and MUFA (Table 6). For the 18:1(n-9) and SFA tissue levels, significant M × F interactions ($P < 0.05$) were found (Table 6). As illustrated in (Figure 2), only in the AT of pigs originating from CF sows, the 16:0 and SFA levels were lower ($P < 0.05$) when the pigs were fed the dUS-H diet. These findings are interesting, as they show that the seemingly small effect of the dietary FA composition of the gestation and lactation diets on the AT's FA composition of the progeny impacts FA metabolism. Deposition of dietary PUFA from the dUS-L diet was lower if pigs originated from a sow fed a highly saturated diet and ingested more saturated milk during lactation. Conversely, the greater PUFA level of the dUS-H diet lowered the *de novo* fat synthesis and SFA desaturation when pigs originated from CF sows. These effects were less evident in the offspring of the SO sow. It is difficult to explain these results, as the activity of key enzymes of the FA metabolism *(Kouba and Sellier, 2011)*, such as FA synthase, acetyl-CoA-carboxylase, malic enzyme, glucose-6-phosphate-dehydrogenase and stearoyl-CoA-desaturase activity were not determined.

In conclusion, this study demonstrates that the fats included in the maternal diet have a long-lasting impact on the FA composition of slaughtered pigs, and this effect furthermore depends on the dietary FA composition of the post-weaning diet.

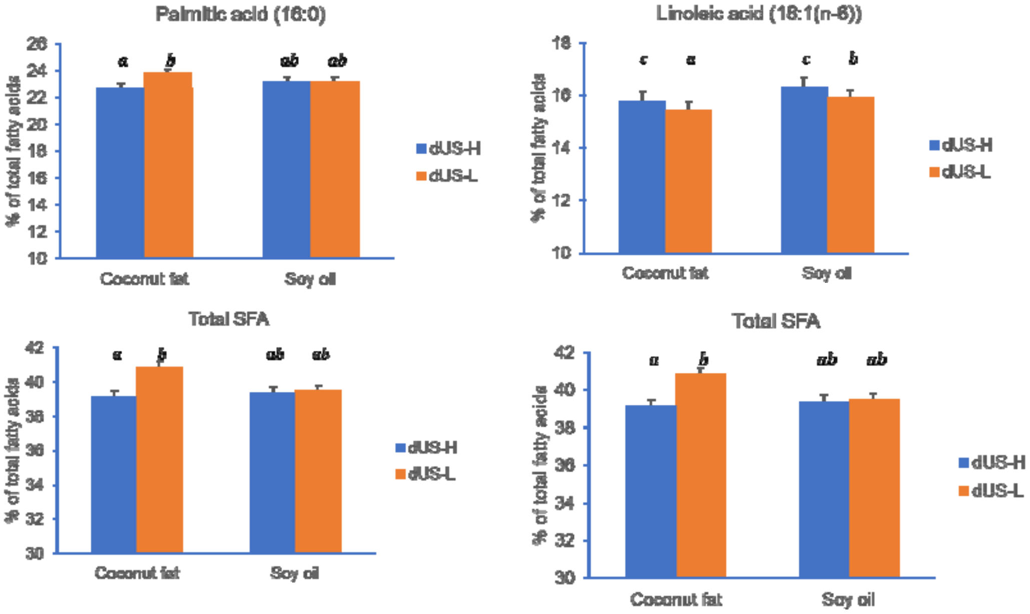

Figure 2. **Palmitic, linoleic, total saturated (SFA) and polyunsaturated (PUFA) fatty acid levels in the adipose tissue of progenies born from sows fed gestation and lactation diets supplemented with coconut fat or soy oil and offered, in the finisher period, a diet with a high (dUS-H) or low (dUS-L) degree of fatty acid unsaturation. ^{abc} Bars with different superscript differ ($P < 0.05$).**

Table 6. Fatty acid composition of the adipose tissue of high (H) and low (L) birth weight progenies born from sows fed gestation and lactation diets supplemented with coconut fat (CF) or soy oil (SO) and offered, in the finisher period, a diet with a high (dUS-H) or low (dUS-L) degree of fatty acid unsaturation

	Maternal nutrition[1]		**Birth weight[2]**		**Finisher diet[3]**			**P-value[4]**		
	CF	**SO**	**H**	**L**	**dUS-H**	**dUS-L**	**SEM[5]**	**M**	**BtW**	**F**
Total FA, %	**87.43**	**88.01**	**87.47**	**87.97**	**87.96**	**87.48**	**0.312**	**0.23**	**0.26**	**0.25**
Total SFA$^{\text{Error!}}$ Bookmark not defined.	**40.01**	**39.43**	**39.63**	**39.81**	**39.27**	**40.17**	**0.360**	**0.38**	**0.65**	**0.01**
14:0	**1.34**	**1.28**	**1.33**	**1.29**	**1.30**	**1.32**	**0.018**	**0.10**	**0.04**	**0.17**
16:0	**23.27**	**23.21**	**23.28**	**23.20**	**22.95**	**23.53**	**0.189**	**0.86**	**0.67**	**<0.01**
18:0	**14.47**	**14.07**	**14.13**	**14.40**	**14.19**	**14.35**	**0.239**	**0.33**	**0.36**	**0.56**
20:0	**0.22**	**0.20**	**0.21**	**0.22**	**0.21**	**0.21**	**0.005**	**0.05**	**0.09**	**0.62**
Total MUFA	**41.82**	**41.92**	**41.71**	**42.03**	**41.11**	**42.63**	**0.201**	**0.77**	**0.07**	**<0.01**
16:1(n-7)	**1.87**	**1.92**	**1.96**	**1.82**	**1.78**	**2.00**	**0.055**	**0.60**	**0.02**	**<0.01**
18:1(n-9)	**36.57**	**38.76**	**38.44**	**38.88**	**38.06**	**39.26**	**0.191**	**0.54**	**0.02**	**<0.01**
20:1(n-9)	**0.97**	**0.83**	**0.89**	**0.90**	**0.90**	**0.89**	**0.021**	**<0.01**	**0.79**	**0.79**
Total PUFA$^{6\text{Error!}}$ Bookmark not defined.	**18.16**	**18.68**	**18.66**	**18.19**	**19.67**	**17.17**	**0.270**	**0.26**	**0.21**	**<0.01**
18:2(n-6)[6]	**15.61**	**16.11**	**16.05**	**15.68**	**17.08**	**14.64**	**0.239**	**0.24**	**0.26**	**<0.01**
20:2(n-6)	**0.77**	**0.73**	**0.76**	**0.74**	**0.80**	**0.70**	**0.017**	**0.15**	**0.16**	**<0.01**
20:4(n-6)	**0.31**	**0.33**	**0.33**	**0.32**	**0.33**	**0.32**	**0.007**	**0.09**	**0.09**	**0.09**
22:4(n-6)	**0.11**	**0.11**	**0.12**	**0.11**	**0.12**	**0.11**	**0.002**	**0.19**	**0.04**	**<0.01**
18:3(n-3)	**1.04**	**1.09**	**1.08**	**1.05**	**1.03**	**1.10**	**0.017**	**0.17**	**0.12**	**<0.01**
20:3(n-3)	**0.19**	**0.19**	**0.20**	**0.19**	**0.19**	**0.20**	**0.004**	**0.48**	**0.02**	**0.01**
22:5(n-3)	**0.12**	**0.12**	**0.12**	**0.12**	**0.12**	**0.12**	**0.003**	**0.25**	**0.18**	**0.61**
n3:n6 ratio	**12.48**	**12.43**	**12.38**	**12.53**	**13.74**	**11.17**	**0.074**	**0.67**	**0.15**	**<0.01**
dUS	**0.43**	**0.45**	**0.44**	**0.43**	**0.47**	**0.40**	**0.010**	**0.31**	**0.39**	**<0.01**

[1] The CF gestation diets were supplemented with 3.80 and 5.06% coconut fat, respectively; the SO gestation diets were supplemented with 3.80 and 5.06% soy oil, respectively.

[2] H = two female piglets from each litter with the highest birth weight; L = two females with the lowest birth weight (but > 800 g birth weight)

[3] dUS-H = unsaturated-to-saturated fatty acids ratio of 2.88; dUS-L: unsaturated-to-saturated fatty acids ratio of 2.47

[4] M = effect of maternal gestation and lactation diet; BtW = effect of birth weight; F = effect of finisher diet

[5] SEM = pooled standard error of the mean

[6] M x F interaction is significant at ($P < 0.05$) (Figure 1).

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BALANCING AMINO ACID LEVELS IN PIGLET DIETS

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Invited paper

Abstract: To maintain optimal performance with low crude protein diets, good knowledge of the amino acid profile within these low crude protein diets is crucial. Amino acid levels will affect piglets' feed intake, growth and feed efficiency. Not only absolute levels but especially the digestibility and balance between amino acids is crucial for optimal health. In this overview, we first discuss the factors and methodology affecting the dietary amino acid requirement of piglets and then we give an overview of amino acid research that has been performed at ILVO. We consider the interaction between lysine and crude protein level, showing that piglets may be fed below their lysine requirement shortly after weaning. Next, we discuss the effect of dietary amino acid level on feed intake and the interaction between amino acids. In particular the branched chain amino acids (leucine, valine, isoleucine) and tryptophan have been of interest in our research. Last, we discuss the relationship between health status of a farm and amino acid requirements.

Key words: amino acid, pig, requirement, health, performance

Introduction

In contemporary pig production, piglets are weaned at an age of 28 days, or sometimes younger. This is a challenging age, since they are weaned from liquid feed and protective factors in the sow's milk at a moment that they are still immature. To minimise the risk of disease, careful management -amongst others appropriate feeding- is necessary. Formulating diets with low crude protein (CP) levels is one way to decrease the risk for weaning diarrhoea *(Nyachoti et al., 2006; Wellock et al., 2008; Wellock et al., 2007)*. It is a way to reduce the dietary acid-binding capacity of the feed, which should be kept as low as possible, since the capacity of the piglet to decrease the stomach's pH is limited *(Lawlor et al., 2005)*. A low gastric pH serves as a barrier to pathogens attempting to enter the small intestine *(Hansen et al., 2007)* and is important for protein digestion.

To maintain optimal performance with low CP diets, good knowledge of the amino acid (AA) profile within these low CP diets is crucial. Amino acid levels will affect piglets' feed intake, growth and feed efficiency. Not only absolute levels but especially the digestibility and balance between AA is crucial for optimal health.

Determining the optimal amino acid level in piglet diets

A lot of empirical trials are carried out to find the optimal AA level in piglet diets. Still, large differences in requirements are reported which could be due to the response parameter, the study design and diet formulation, or on the model used to deduct requirement levels.

Response parameter

Traditionally, AA requirements are determined through empirical dose-response studies to identify the minimum AA supply that maximizes performance. Feed efficiency, daily gain, and daily feed intake are typically measured and used as response parameters in those performance trials. Optimum supply of AA maximizes feed efficiency and daily gain, and any level below or above this optimum leads to an inefficiency of AA utilization. Deficiency of certain AA means that these are not present in sufficient amounts to support optimal protein synthesis. The excess dietary energy in AA that are not used for muscle growth, is used for fat deposition. Muscle growth is more efficient than fat deposition, primarily due to extra water retention: protein accretion is accompanied by water deposition in lean gain, in a ratio of about 4:1 *(Patience et al., 2015)*. Logically, feed efficiency is closely related to the amount of protein deposition. Daily gain increases as a direct result of increased feed intake, but it also increases with more muscle instead of fat deposition because of the increased efficiency. In pig production, feed intake is an essential parameter since adequate feed intake can help alleviate weaning symptoms. For some AA, increased feed intake is the first and major response to AA supplementation. This will be discussed later. Based on the response parameters of interest, the requirement may differ.

Study design and diet formulation

To determine the optimal AA levels, the first prerequisite is to ensure that the AA of interest is the primary factor limiting growth. If an unbalanced AA profile is used, another AA may limit growth before meeting the requirement of the AA of interest. Typically, an optimal AA pattern is used, where only the AA of interest deviates from the profile. It is crucial that even at the highest level of inclusion, the balance of AA compared to lysine is above the ideal AA profile. The ideal AA profile is the profile that exactly meets the animal's requirements, ensuring that all AA are equally limiting for performance (van Milgen and Dourmad, 2015). Standardised ileal digestible (SID) lysine is mostly used as reference AA. In practice, a minimum (but no maximum) ratio of SID AA to SID lysine is often set.

While this approach is practical, it is important to maintain the balance between AA and avoiding excesses, as AA imbalances may have a negative effect on performance. An example of ideal AA profile is given in Table 1.

Table 1. Optimal ratio of amino acids relative to lysine (in %), according to CVB* (2023)

Methionine + Cysteine	Threonine	Tryptophan	Isoleucine	Valine	Leucine	Histidine	Phenyl-alanine	Tyrosine
60	65	20	52	67	100	34	54	40

*CVB= Centraal veevoederbureau,
https://www.cvbdiervoeding.nl/pagina/10021/home.aspx

The second prerequisite is testing sufficient levels in the appropriate range. In order to fit models (see further), at least 4 levels are needed. Moreover, there should be levels below and above the optimum, which may be challenging when designing the study. Therefore, we prefer using at least 5 and preferentially more levels.

A third prerequisite, when testing the optimal ratio of an AA relative to lysine, is ensuring that the SID lysine level is the second factor limiting growth. If the lysine level is above the requirement, or if another AA is limiting growth it will not be possible to accurately calculate the optimal ratio of the test AA relative to lysine. Usually, experimental diets in dose-response studies are formulated to contain 90% of the lysine requirement and all other AA, except the AA of interest, are supplied in a ratio to 100% Lys. As such, lysine is marginally limiting to avoid underestimation of the requirement *(Boisen, 2003)* and all AA other than the tested one are supplied at 10% above their requirements.

Model fitting

As the supply of the test AA increases, the response parameter will increase up to a certain point where the AA is no longer limiting for performance. Beyond this point, further increases in the AA supply will not increase the response parameter. This point at which the response parameter reaches its maximum value corresponds to the animal's requirement for that particular AA. However, different models can be used to determine the point of maximal response. The most commonly used models are the linear plateau model, quadratic model, and quadratic plateau model.

The linear plateau model assumes a linear increase in response parameter until a maximum is reached, while quadratic and quadratic plateau models assume that there are diminishing improvements in response with increasing AA levels. The requirement estimated by the quadratic or quadratic plateau model is greater than that estimated by the linear plateau model, as illustrated by an example in Figure 1.

While the linear plateau model may indicate the response of an individual pig to increasing AA levels up to it’s requirement, quadratic plateau models may best describe the observations using a group of animals *(Pomar et al., 2003).* This reflects the variation in requirements between individual animals of a group.

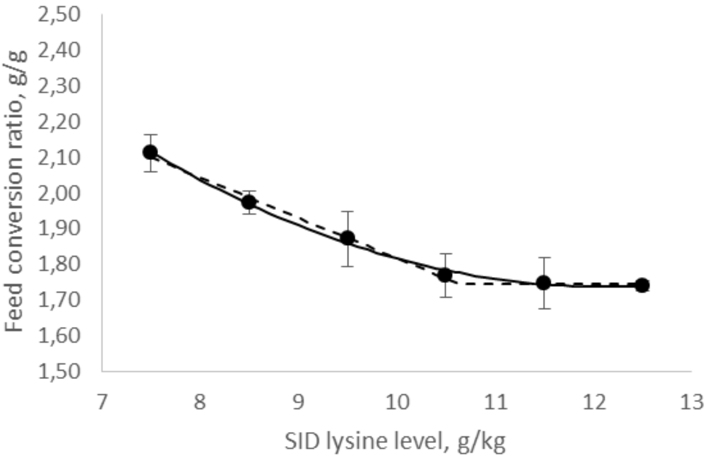

Figure 1. Effect of lysine level on feed conversion ratio of boars between 10 and 15 weeks of age. With a linear plateau model, the optimum is 10.65 g/kg, with a quadratic plateau model, the optimum is at 12.11 g/kg. Data from *Millet et al. (2018b)*

The optimal lysine level for health and performance

Since lysine is often considered the first limiting AA in piglet diets and requirements of other AA are expressed relative to lysine, good knowledge of the lysine requirement is essential. Several studies have been carried out with piglets to determine the lysine requirements. However, while most studies observe a clear linear increase in daily gain and feed efficiency with increasing lysine levels, several studies do not find an optimal level. For example, in two studies performed at ILVO *(Millet et al., 2020b),* we found an optimal lysine level when feeding the piglets a diet with low CP content, but not when feeding a higher CP level in the diet (Figure 2).

In this study, our results suggest that factors other than lysine levels may be limiting performance at the low CP level (18%).

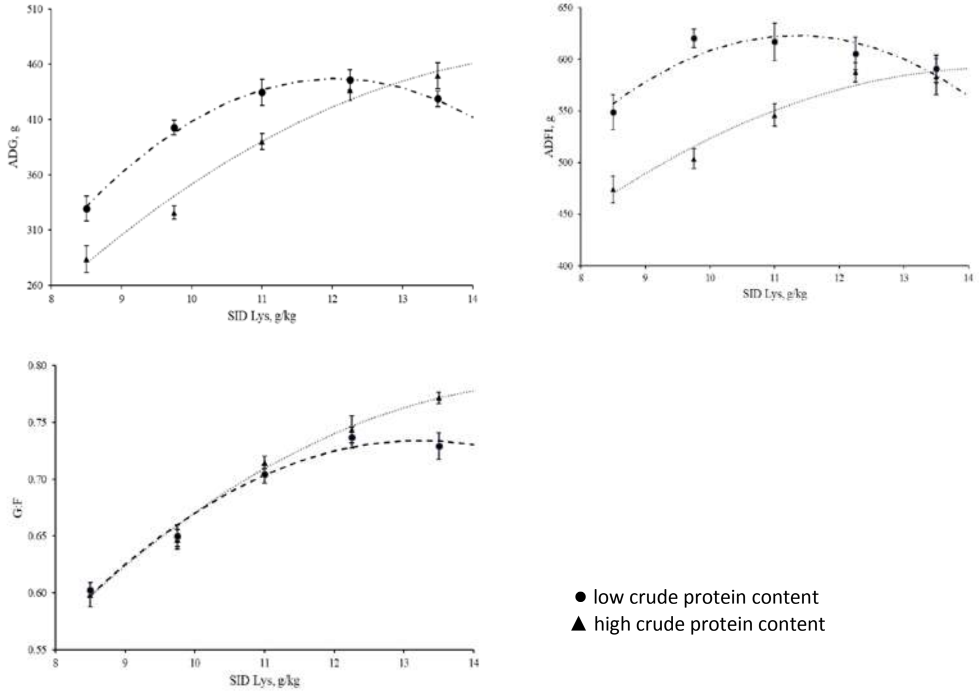

Figure 2. Effect of lysine level on daily gain, daily feed intake and feed efficiency in studies differing in crude protein content. Reprinted from Millet et al. *(2020b)*, with permission from Elsevier

This finding is consistent with our previous experiment *(Millet et al., 2018a)*, which demonstrated that CP, rather than lysine, was the limiting factor for performance at low CP levels. In that trial, we investigated the effects of CP reduction in pigs receiving either 10 or 11 g SID lysine/kg diet. As expected, pigs on the lower lysine levels showed reduced performance. We observed that reducing CP levels did not initially affect performance in either group, but after a certain point, performance was negatively impacted. This breakpoint occurred more quickly (and thus at higher CP levels) in the group receiving 11 g SID lysine/kg diet than in the group receiving 10 g SID lysine/kg. This interaction between lysine and crude protein level was also reflected when measuring the serum urea level in response to decreasing CP levels. Urea levels in the serum may give an indication of protein excess, since AA given in excess are deaminated, and the resulting urea is excreted in the urine *(van Milgen and Dourmad, 2015).* At both lysine levels, CP reduction led to a decrease in serum urea level, until a minimum was reached. This minimum was observed to lay at an SID lysine: CP ratio of 0.064, as illustrated in Figure 3.

A logical consequence is that low CP diets for piglets may contain AA levels below the requirement for maximal growth and efficiency. In this period, it may be a valid choice to feed low CP diets, with therefore AA levels below the requirements for optimal growth and as such decrease the risk for gastrointestinal disturbance *(Millet and Everaert, 2022).*

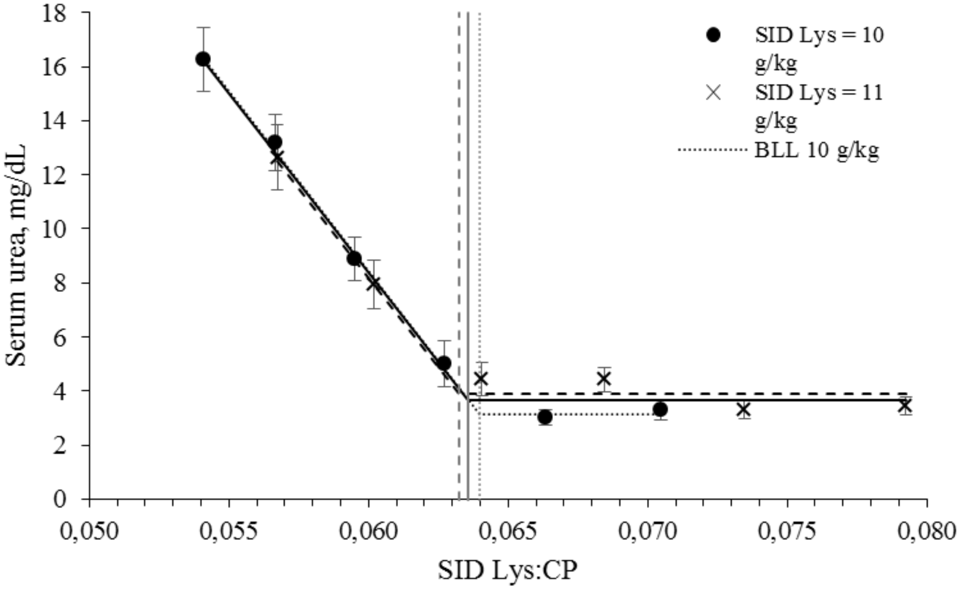

Figure 3. Figure from *Millet et al. (2018a)*, by permission of Oxford University Press. Effect of dietary standardized ileal digestible (SID) lysine (Lys) to crude protein (CP) ratio on serum urea levels in piglets at 7 weeks of age. Piglets receiving 10 or 11 g SID Lys/kg diet are presented, together with their corresponding and overall linear plateau (BLL) models. Vertical gray lines represent breakpoints. Urea level decreases with decreasing CP level (=increasing SID Lys:CP ratio) until a minimum is reached. This indicates the point of maximal nitrogen retention and minimal protein breakdown.

It is thus important to know the optimal lysine level, given a certain CP level. Therefore, the question may be: what is the optimal SID lysine:CP ratio, rather than the absolute SID lysine level. The answer to this question is not entirely clear and will depend on the methodology and models used. This is illustrated in

Figure 4, where data from 2 experiments *(Millet et al., 2018a; Millet et al., 2020b)* are combined.

In both experiments, linear plateau models were used to determine a breakpoint, representing the optimal SID lysine:CP ratio with gain:feed as response criteria. Decreasing the CP level while keeping the SID lysine level constant led to a lower breakpoint, than when the dietary SID lysine level was increased while the CP level was kept constant. If quadratic or quadratic plateau models were used, the difference in breakpoints using the two methodologies may be even bigger.

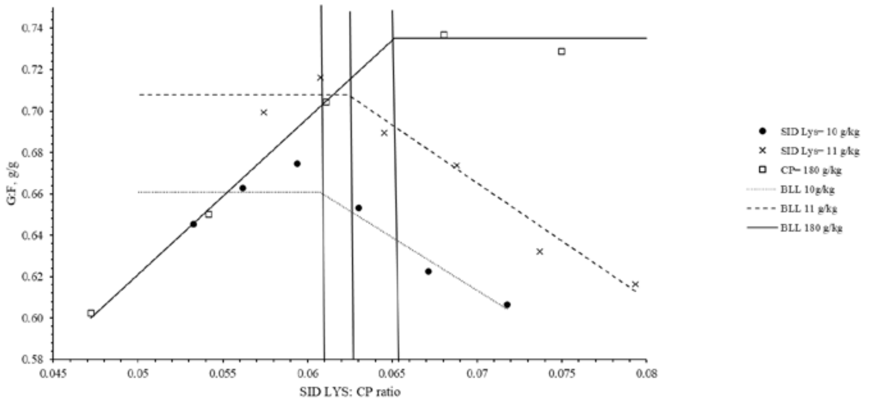

Figure 4. Determining the optimal SID lysine: CP ratio, based on feed efficiency measured in the studies *Millet et al. (2018a), Millet et al. (2020b)*. In two studies (SID lysine=10g/kg and SID Lys= 11 g/kg), the lysine level was kept constant, while decreasing the CP level, whereas in a third study, SID lysine was increased, while keeping the CP level constant at 18%.

Feed intake as a driver for growth

A reduction in feed intake is often the first reaction to a single AA deficiency, modulated via a chemosensor in the anterior piriform cortex (APC) of the brain *(Gietzen et al., 2007)*. Hence, adding amino acids to an unbalanced diet may first lead to increased feed intake. With some AA, this is even more clear. Dietary tryptophan in particular has been shown to increase daily feed intake. In 1992, *Henry et al. (1992)* reported a correlation between brain serotonin level and feed intake, and this has led to the interpretation that tryptophan stimulates feed intake by increasing dietary serotonin level. Still, we have challenged this interpretation *(Millet and Goethals, 2022)*, since no causal relationship between brain serotonin level and increased feed intake has been proven until now.

Different other hypotheses have been formulated to explain the effect of dietary tryptophan on feed intake. An interaction with dietary leucine may be one of the reasons. Especially excess leucine depresses feed intake and growth of piglets *(Cemin et al., 2019, Gloaguen et al., 2012)*. High brain leucine levels reduce feed intake by hypothalamic mTOR signalling *(Cota et al., 2006)*. Therefore, reducing brain leucine levels might increase feed intake. It has not been shown yet whether the positive correlation between dietary tryptophan and feed intake is a direct effect of tryptophan or rather an indirect effect, for example as a result of the competition with leucine, as leucine and tryptophan share a common transport system at the blood-brain barrier.

Also valine supplementation to a deficient diet has been shown to increase feed intake: increasing the dietary SID valine:lysine ratio from 0.58 to 0.67 increased daily feed intake between 5 and 9 weeks of age with approximately 20% (Millet, 2012). Similarly, in a dose-response trial *(Millet et al., 2020b)*, increasing the SID valine:lysine ratio between 0.58 and 0.82 increased feed intake from 400g/day to more than 500 g/day in piglets between 4 and 9 weeks of age. As a logical consequence, daily gain improved with increasing dietary valine levels. Other researchers saw similar effects of dietary valine on feed intake *(Barea et al., 2009, Gloaguen et al., 2011).*

Interaction between amino acids

As said before, the balance between AA may be more important than the absolute levels of AA. This is especially shown for the branched chain AA (BCAA: valine, isoleucine and leucine). They are structurally similar and share the first steps of their catabolism. Therefore, excess leucine may result in increased degradation of all three BCAA. Moreover, BCAA and large neutral amino acids (LNAA, including tryptophan) share common brain transporters *(Cemin et al., 2019)*, hence the optimal dietary level of one of these AA may depend on the concentration of the other AA. An overview on recent literature is given by *Cemin et al. (2019).*

In a study at ILVO with individually housed piglets, we varied dietary leucine, valine, isoleucine and tryptophan levels. Increasing leucine greatly decreased feed intake and growth, which was counteracted by adding more valine to the diet (P-value of the valine×leucine interaction = 0.008). The addition of isoleucine or tryptophan did not further improve growth performance. Thus, it seems that the dietary valine requirement may depend on dietary leucine level.

Interactions between AA may also explain differences in feed intake with decreasing lysine levels. In our study *(Millet et al., 2020b)*, feed intake was largely affected by decreasing lysine levels when piglets were fed a high protein diet, while this effect was less pronounced when they were fed a diet with a lower CP level and more balanced AA profile (Figure 2). In the high protein diet, the leucine:lysine ratio was 162, and this decreased with increasing dietary lysine levels.

Amino acids for gut health

In recent years, there is increasing attention to the effects of AA apart from their function as building block for tissue proteins. In particular, AA related to the immune response may be required at higher levels in piglets in low sanitary conditions since AA involved in the immune response cannot be utilized for protein accretion. On the other hand, low sanitary conditions may lead to slower growth and therefore lower daily AA requirements. Indeed, *Goodband et al. (2014)* stated that increases in AA requirements for immune function in times of disease are more than offset by the associated decrease in protein accretion and increased muscle protein degradation. This implies that there would be no need for increased dietary AA levels in low sanitary conditions. Still, the balance between AA may be altered. Also, conditionally essential AA may become limiting in challenging health conditions. With growing pressure on the use of antibiotics and ZnO, the search for diets improving the health status of piglets is ongoing. The effect of single AA are also investigated with his perspective. In a piglet trial, glutamine supplementation beneficially affected performance of early-weaning piglets in the first 2 weeks after weaning, especially when fed a low protein diet *(Millet et al., 2020a)*. More research is needed on the optimal AA balance to increase resilience and prevent disease. The amino acids arginine, glutamine, threonine, aspartate and glycine have been linked to intestinal health, but more work needs to be done to come to clear recommendations to improve piglet’s health.

Conclusion

Amino acids are important nutrients in piglet diets. As building blocks for protein synthesis, their levels are major determinants for observed performance results. Amino acid levels and the balance between AA within the diet may affect feed intake and consequently growth. Still, deducting requirements may be challenging. Also, feeding below the requirement for optimal growth may be a valid choice to decrease the risk for gastrointestinal disturbance. Under challenging conditions, the optimal balance between AA may be different, but further research is need to elucidate this.

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HEAT STRESS EFFECTS ON IBERIAN PIG GROWTH AND PRODUCTIVITY

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Invited paper

Abstract: Heat stress challenges pig production resulting in large economic losses because of growth depression, altered carcass and meat quality traits, and mortality. Pigs are extremely sensitive to elevated environmental temperatures because of high basal metabolic heat production, skin insulation due to subcutaneous fat depth and lack of functional sweat glands. Although Iberian pigs are considered adapted to its environment, the thick subcutaneous fat layer could make them more sensitive to elevated temperatures compared to lean breeds. When growing Iberian pigs were under chronic heat stress a reduced growth performance associated to a decreased voluntary feed intake was observed, although the magnitude of reduction was lower compared to values reported for cosmopolitan pigs. Dietary betaine or zinc addition did not improve detrimental effects on performance. Plasma biochemical parameters suggested an increased protein catabolism under heat stress conditions. Meat quality and oxidative status of pigs seemed to be not compromised by prolonged heat exposure. Overall, growing Iberian pigs seem resilient to heat exposure in terms of performance and meat quality.

Key words: Iberian pig, heat stress, meat quality, nutritional strategies, performance

Heat stress effects on pig production

In modern livestock production, pigs are essential contributors to the global food supply. However extreme weather events are becoming more frequent and the agricultural sector faces new challenges due to the adverse impact of heat stress on pigs. Climate is one of the main factors limiting production efficiency in the swine industry. Heat stress challenges pig production worldwide resulting in large economic losses because of detrimental effects on performance, carcass and meat quality among others *(Ross et al., 2015).* In homeotherms animals the balance between heat produced in metabolic activities and heat lost from the body is crucial to maintain core body temperature *(Berman, 2011).* Heat stress results when this balance is disturbed because the animal is not able to dissipate enough heat to the environment.

Pigs are very sensitive to high environmental temperatures due to high basal metabolic heat production and rapid growth. Additionally, skin insulation due to subcutaneous fat depth and lack of functional sweat glands *(Renaudeau et al., 2006)* make pigs particularly vulnerable to heat stress. High temperatures are associated to the reduction of productive and reproductive performance due to the decrease in feed intake -to lower the production of metabolic heat- and the partitioning of the metabolic energy associated to the heat dissipation *(Williams et al., 2013).* Heat dissipation associated to heat stress causes blood flow reduction at the internal organs *(Ooue et al., 2007)* which decreases the supply of oxygen and nutrients to the gastrointestinal tract *(Hinnebusch et al., 2002).* The reduced voluntary feed intake in heat stressed pigs may further reduce nutrient supply to the small intestine. Hypoxia and nutrient deficiency appear to damage the intestinal epithelia as evidenced by a reduced height of the intestinal villi *(Pearce et al., 2013)* and changes in cellular proliferation and membrane function *(Sonna et al., 2002)* observed in heat stressed pigs. A reduction in adipose tissue mobilization takes place, and as a consequence, protein and amino acids are catabolized to obtain energy *(Cottrel et al., 2015).* These processes, among others, give rise to effects on meat quality in animals subjected to this type of stress. For instance, a decrease in pH and negative effects on color and antioxidant capacity have been described in *longissimus* muscle associated with a decrease in carnosine concentration *(Yang et al., 2014)*. Despite advances in management, housing and cooling technologies *(Schütz et al., 2014),* environmental heat stress compromise animal welfare and decrease animal productivity leading to devastating economic consequences for animal agriculture across the globe *(Bernabucci et al., 2010).* These losses have been estimated equivalent to 1000 million USD/year for the swine industry in USA *(Mayorga et al., 2020)* and 16.5 million AUD/year in the livestock industry in Australia *(Chauhan et al., 2021).* Although the main pig producing areas in Spain are under hot summer Mediterranean climate and elevated ambient temperatures are common during summer with average maximum temperatures in July ranging 32–36 ºC, no similar calculations have been found for Spain or other Mediterranean countries.. This clearly highlights the need to develop strategies to reduce the impact of heat stress on livestock production.

The Iberian pig

The EU is the second largest pork meat producer and Spain one of the most relevant pig producers worldwide. After China and USA, Spain is the third producing country, and the first one at European level, representig nearly 24% of total pig meat produced and 25% of total pigs census (more than 34 million animals (*MAPA, 2023*)). Nevertheless, during 2022 pig production has dropped by 2.2% in tonnes and 2.9% in number of pigs, reversing the upward trends of the last years *(MAPA, 2023)*. The pig industry represents a meaningful sector for the country accounting for nearly 40% of the economic value derived from livestock production. Within a national context dominated by cosmopolitan breeds, there are also some native pig breeds from which the most relevant -both in number of animals and economic importance- is, by far, the Iberian. This native breed is derived from ancestral domestic pig populations of the Iberian Peninsula widely spread for centuries all over the territory *(López-Bote, 1998),* that can be found nowadays at the Southwest of the Peninsula where the Mediterranean-type forest (*dehesa*) still persists. Currently it represents 10% of total pigs produced in Spain -including purebred and crossbred animals- and this proportion has been fairly maintained over the last years *(MAPA, 2023).* Iberian meat products are very appreciated due to their outstanding high-quality *(Nieto et al., 2019).* 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).* Although Iberian pigs are considered to be perfectly adapted to the environment (Lopez-Bote, 1998), the thick subcutaneous fat layer *(Barea et al., 2006)* could make Iberian pigs sensitive to elevated temperatures compared to lean breeds.

Heat stress studies on growing Iberian pigs

There is a lack of information regarding the effects of heat stress on autochthonous pig breeds. Our research group has recently examined the effects on physiological, productive, carcass and meat quality traits of exposing growing Iberian pigs to heat stress. Potential nutritional strategies to increase heat tolerance have also been explored by adding betaine or supplemental zinc to pig diets. The ability of betaine to act as an osmolyte decreasing basal heat production and maintenance requirements *(Schrama et al., 2003)* may be useful in heat stressed pigs. In this sense, in a study in our lab in thermoneutral conditions heat production from the portal-drained viscera tended to be lower in pigs fed betaine *(Rojas-Cano et al., 2017).* Under mild heat stress conditions, pigs fed betaine had lower respiration rate and reduced intestinal permeability in the ileum compared with control pigs *(Gabler et al., 2013).* However, the effects of betaine on heat stressed pigs are not consistent. Regarding zinc, *Sanz Fernandez et al. (2014)* reported that dietary zinc improved intestinal functionality in pigs subjected to short-term heat stress. Using intestinal Caco-2 cells as a model, *Pardo and Seiquer (2021)* showed that supplemental zinc decreased heat-induced damage to the intestinal membrane alleviating the generation of reactive oxygen species and promoting the activity of different antioxidant enzymes, leading to improved integrity and functionality of the damaged intestinal monolayer.

The experimental protocol in studies in growing Iberian pigs is summarised as follows. The assays consisted of a 7 d adaptation period with all pigs at thermoneutral conditions and fed a control diet *ad libitum* followed by a 28 d experimental period. Forty pure Iberian barrows (Sánchez Romero Carvajal strain, 44 kg initial body weight (BW)) were allocated to one of the two environmentally controlled rooms. Pigs were blocked by initial BW and randomly assigned to one of the five environmental and dietary conditions: 1) thermoneutral (20 ºC) fed *ad libitum* a control diet (TN-CON); 2) heat stress (30 ºC) fed *ad libitum* a control diet (HS-CON); 3) thermoneutral fed the control diet pair-fed to HS-CON to eliminate possible confounding effects of dissimilar feed intake (TN–CON–PF); 4) heat stress fed *ad libitum* a betaine supplemented diet (HS-BET); and 5) heat stress fed *ad libitum* a zinc-supplemented diet (HS-ZN). The temperature was progressively raised for heat stressed pigs (from 20 to 30 ºC). Pigs had free access to water. Main results of the study are summarised below.

Effects on growth and metabolic parameters

The elevated constant temperature (30 ºC, 28 d) produced a noticeable hyperthermia independently of feed intake, as rectal temperature in HS-CON pigs were elevated compared to both TN-CON and TN–CON–PF pigs *(Pardo et al., 2022).* Similar results were reported in growing crossbred gilts during a constant thermal load (35 ºC, 7 d; *Pearce et al., 2013*). Betaine or zinc supplementation did not mitigate increased rectal temperature under heat stress. Heat stress decreased voluntary feed intake (20%) throughout the experiment. Similarly, a 27% reduction in feed intake was reported in 49 kg Creole pigs subjected to heat stress (31 ºC, 20 d) compared to control pigs (24 ºC; Renaudeau *et al*., 2007). The reduction of feed intake observed was of inferior magnitude than in growing gilts after 1 week at 35 ºC (46%; *Pearce et al., 2013),* which may be explained by a greater heat load and a shorter period of adaptation to elevated temperature in the latter experiment. In addition, different responses of Iberian pigs to heat compared with lean pigs due to genetic adaptation cannot be discarded. Chronic heat stress during the growing phase of Iberian pigs had negative effects on performance which were explained by the 20% decrease in feed intake *(Pardo et al., 2022).* Reduced feed intake of pigs under heat stress could explain the growth depression *(Collin et al., 2001; Hao et al., 2014),* since no differences with the pair-fed animals grown in thermoneutral conditions were found. Elevated temperature did not affect gain to feed ratio in accordance with previous reports *(Hao et al., 2014),* whereas *Nienaber et al. (1987)* reported a lower feed efficiency above 20 ºC. Levels of performance, genotype, or the marginal response of BW gain and its components to a change in feed supply may explain disparities in the response of feed efficiency to heat stress. No benefits in growth were observed when diets were supplemented with betaine or zinc.

Fasting plasma urea nitrogen and creatinine increased in Iberian pigs under heat stress conditions compared to pair-fed pigs in thermoneutral conditions, which may indicate increased protein catabolism (decreased efficiency of nitrogen utilization), which is usually increased during chronic heat stress *(Pearce et al., 2013).* Heat stress augmented fasting plasma glucose compared to thermoneutral conditions (both in *ad libitum* and pair fed pigs) with no differences on insulin concentrations, suggesting modifications in the mechanisms controlling glycemia *(Pardo et al., 2022).* The elevated glucose concentration under heat stress may be the consequence of a whole body shift in nutrient partitioning in order to spare glucose for an activated immune system *(Baumgard and Rhoads, 2013),* as it is well established that once activated, immune cells become obligate glucose utilizers *(Maciver et al., 2008).* Regarding betaine and zinc supplementation, although no positive effects on growth rate were detected, a beneficial effect on glucose homeostasis was observed (decreased plasma glucose and improved *β*-cell function) under heat stress conditions *(Pardo et al., 2022).* In addition, the study of the effect of heat stress on intestinal fermentation in Iberian pigs has shown that these animals adapt by increasing the production of volatile fatty acids improving intestinal health and increasing the availability of this source of energy for the animal *(Pardo et al., 2020).*

Effects on carcass and meat quality

Long-term heat stress decreased carcass weight and consequently the weight of most cuts. In the case of ham, loin and sirloin, this effect was independent of decreased feed intake *(Pardo et al., 2021a)* which may indicate fatter carcasses in Iberian pigs after a long term heat stress exposure. The effect of chronic exposure to high temperature on meat quality and antioxidant markers has been investigated in *longissimus lumborum* and *gluteus medius* muscles of growing Iberian pigs *(Pardo et al., 2021b).* Muscles of the heat stressed group had greater intramuscular fat content than the thermoneutral pair-fed group and higher zinc levels than thermoneutral *ad libitum* and pair-fed groups. Differences on fatty acid composition were negligible. Heat exposure did not affect pH, colour coordinates of redness (a*) and yellowness (b*) and lipid peroxidation values but had a positive influence on lightness and drip losses. Moreover, chronic heat stress stimulated the activity of antioxidant defences. The zinc addition improved the water retention capacity of *longissimus lumborum,* increased the antioxidant properties and the glutathione peroxidase activity, and reduced lipid peroxidation levels. No significant effects associated to the betaine diet were observed in quality traits and muscle antioxidant markers.These findings support an adaptive response of the Iberian pig to high temperatures and show the high meat quality even under adverse climate situations *(Pardo et al., 2021b; Pardo et al., 2023).*

Future studies on Iberian pigs

Lactating sows are particularly susceptible to heat stress *(Williams et al., 2013)* due to the high generation of metabolic heat associated with milk production. Heat stress may result in reduced feed intake, milk yield, reproductive performance, and growth rate of the piglets *(Bjerg et al., 2020).* The decreased feed intake and the direct impacts of heat stress both contribute to compromised lactational performance. When lactating sows are subjected to heat, physiological and behavioural changes occur to sustain homeothermia. These processes are all metabolically expensive, which can further increase the mobilisation of sow body reserves and compromise the energy and nutrients supply for milk production. Nevertheless, no information on physiological and productive effects of heat stress on lactating Iberian sows is available. Therefore, a new set of studies are underway in order to investigate possible detrimental effects of heat stress on Iberian lactating sows´ performance during the summer months along with nutritional strategies to improve heat tolerance.

Conclusions

In growing Iberian pigs chronic heat stress causes a reduction in growth performance associated to decreased voluntary feed intake, although the magnitude of reduction is lower compared to reports on cosmopolitan pigs. Dietary betaine or zinc addition do not ameliorate detrimental effects on performance. Plasma parameters suggest an increased protein catabolism under heat stress conditions. Meat quality and oxidative status of pigs seem to be not compromised by prolonged heat exposure. Overall, growing Iberian pigs seem to be somewhat resilient to heat exposure. However, further studies are needed to explore mitigation strategies to improve heat tolerance, and expand the investigation to other productive phases of Iberian pigs more susceptible to detrimental effects of heat. This information is necessary to improve the production efficiency of this native breed of outstanding economic importance, in particular for rural areas of the Iberian Peninsula in which high temperatures and heat waves during summer months are common.

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ENHANCING PIGLET GROWTH WITH LIVE YEAST: A NUTRITIONAL SUPPLEMENT STUDY

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Abstract: The study encompassed 135 piglets of the Large White breed and aimed to evaluate the impact of a live yeast feed supplement on key production parameters—namely, feed intake, average daily gain, and feed conversion—among growing piglets. The trial spanned a total of 39 days, divided into two distinct periods. The initial phase, lasting 28 days (from day 22 to day 49), was followed by a shorter 11-day second phase (day 50 to day 60). Throughout the observation periods, two distinct feed mixtures were employed, as outlined in Table 1. The control groups were provided standard farm mixtures, while the trial groups received the same mixtures supplemented with varying concentrations (3% and 4%) of the commercial live yeast dietary supplement, Biokvas-45. During the initial period, statistical analysis indicated no significant differences ($p>0.05$) across all three observed parameters between the groups. However, notable disparities emerged during the second period. Specifically, feed intake (FI), average daily gain (ADG), and feed conversion ratio (FCR) displayed variation between the T_1 and T_2 groups. The T_2 group exhibited particularly favorable performance, achieving an impressive average daily gain of 563.24 g/d and a commendable feed conversion ratio of 1.92 g/g. Considering the entirety of the trial, the T_2 group consistently outperformed the other trial groups in terms of average daily gain, achieving a rate of 348.26 g/d. The results collectively indicate that the incorporation of the dietary supplement Biokvas-45 holds promise as a beneficial addition to the nutrition of growing piglets. However, further research is warranted to comprehensively assess the supplement's effects on pigs during the fattening phase. These findings contribute to a growing body of knowledge that could potentially lead to more effective nutritional strategies for pig farming.

Key words: nutrition, production parameters, weaning

Introduction

Weaning stands as a pivotal juncture within modern pig production systems. Piglets, during the weaning phase, confront an array of challenges including reduced feed intake, acute diarrhea, and body weight loss. These issues are chiefly rooted in nutritional, immunological, and psychological disruptions *(Lallès et al., 2007).* Addressing the aftermath of weaning, piglet diets have seen the integration of antibiotic growth promoters for alleviation *(Yin et al., 2001).* Nonetheless, the incorporation of antibiotics for growth promotion in pig farming raises potential concerns for public health, as it could contribute to an escalation in bacterial resistance among humans *(van den Bogaard and Stobberingh, 2000).* Consequently, various nations have imposed limitations or outright bans on antibiotic use in swine diets, underscoring the need for viable alternatives to antibiotics *(Gong et al., 2014; Si et al., 2006).*

One avenue that has garnered attention is the utilization of probiotics, specifically through dietary supplementation, to bolster intestinal development and immunity in weaned piglets *(Shen et al., 2009).* Among these probiotics, yeast emerges as a prevalent choice within pig production. Notably, augmenting piglet diets with yeast products has exhibited enhancements in immune function and intestinal development during the weaning period *(Broadway et al., 2015).* The incorporation of live yeast into diets has showcased positive impacts on both performance and health in weaned piglets by invigorating the immune system and cultivating an optimal intestinal milieu *(van Heugten et al., 2003; Rozeboom et al., 2005; Li et al., 2006)*. Furthermore, yeast additives have demonstrated efficacy in bolstering gut integrity among weaned piglets *(van der Peet-Schwering et al., 2007),* mitigating the deleterious effects of mycotoxins on pig growth and well-being *(Weaver et al., 2014),* and amplifying host immune responses across the intestinal tract *(Pontier-Bres et al., 2014).*

Notably, the probiotic yeast *Saccharomyces cerevisiae*, renowned for its therapeutic applications in addressing gastrointestinal disorders and diarrhea in both humans and animals, has gained prominence *(Gallois et al., 2009; Kelesidis and Pothoulakis, 2012; Hancox et al., 2015; Palma et al., 2015).* A recent study has highlighted the potential of various forms of *Saccharomyces cerevisiae* to enhance feed conversion rates, bolster immunity, and foster intestinal development among weaned piglets *(Jiang et al., 2015).*

With these insights in mind, the primary objective of this study is to ascertain the impact of incorporating live yeast feed supplements on key production parameters, namely feed intake, average daily gain, and feed conversion, in the context of growing piglets.

Materials and Methods

The study encompassed 135 piglets of the Large White breed, thoughtfully divided into three distinct treatment categories: the control group (C) and two experimental trial groups (T_1 and T_2). Each treatment cluster comprised three replications, or pens, housing a total of 15 piglets per pen. Commencing on day 22, the weaning event marked the initiation of the trial, and all piglets were situated within equivalent environmental conditions, encompassing temperature, humidity, and lighting.

Spanning a comprehensive duration of 39 days, the trial was segmented into two distinctive periods. The initial trial phase, spanning from day 22 to day 49, spanned 28 days, while the subsequent phase extended from day 50 to day 60, encompassing an 11-day interval. Throughout these meticulously observed periods, a duo of feed mixtures were meticulously administered (refer to Table 1 for specifics). The control groups were administered conventional farm-standard feed mixtures, whereas the trial groups were provided identical mixtures infused with Biokvas-45, a commercial live yeast dietary supplement, at varying concentrations (3% and 4% respectively).

Biokvas-45 is a specialized product crafted from an amalgamation of soybean flour, soya protein isolate, livestock leaven enriched with active *Saccharomyces cerevisiae 1026* cells, alongside components derived from food grains. This formulation is further fortified with a fusion of essential minerals, enzymes, vitamins, and antioxidants, presenting a comprehensive nutritional profile.

Piglets were fed *ad libitum.* Average daily feed intake (FI) was calculated by subtracting unconsumed feed at the end of trial from the preweighed amount and splited by the days. Body mass were weighted at start and at the end of trial. Piglets were weighed at the beginning and at the end of the experiment and the average daily gain (ADG) were calculated with the following equation:

$$\text{ADG} = \frac{\text{Final weight - Initial weight}}{\text{Duration of the experiment (days)}}$$

Feed conversion (FCR) was also calculated:

$$\text{FCR} = \frac{\text{Daily feed intake}}{\text{Daily weight gain}}$$

Table 1. Ingredient and nutrient composition of mixtures used in experiment

	Mixture 1 Day 22-49			Mixture 2 Day 50-60		
Group	C	T_1	T_2	C	T_1	T_2
Ingridients g/kg						
Corn	546.5	546.5	546.5	588.8	588.8	588.8
Wheat flour	-	-	-	25.0	25.0	25.0
Soybean meal	205.0	175.0	165.0	182.0	152.0	142.0
Sunflower meal	-	-	-	20.0	20.0	20.0
Ecofish meal	50.0	50.0	50.0	45.0	45.0	45.0
Extruded full-fat soybean semolina	120.0	120.0	120.0	100.0	100.0	100.0
Milk replacer	40.0	40.0	40.0	-	-	-
Calcium carbonate	14.0	14.0	14.0	16.0	16.0	16.0
Monocalcium phosphate	12.0	12.0	12.0	10.0	10.0	10.0
Sodium chloride	2.5	2.5	2.5	3.2	3.2	3.2
Premix*	10.0	10.0	10.0	10.0	10.0	10.0
Biokvas-45**	-	30.0	40.0	-	30.0	40.0
Calculated nutrient composition, g/kg of feed***						
Crude protein	220.50			200.30		
Lysine	13.00			11.10		
Methionine	4.10			3.60		
Cysteine	3.50			3.30		
Threonine	8.70			7.80		
Tryptophan	2.60			2.30		
Crude fiber	35.70			37.90		
Crude fat	50.40			47.60		
Calcium	11.25			11.00		
Phosphorus	7.70			6.92		
DE content, MJ/kg	14.07			13.88		

*Added per kg diet: 15,000 IU Vitamin A, 1500 IU Vitamin D3, 40 IU Vitamin E, 1.0 mg Vitamin K3, 2.0 mg Vitamin B1, 4 mg Vitamin B2, 10 mg d-Pantothenic acid, 18 mg Niacin, 70 mg Biotin, 18 mg Vitamin C, 0.03 mg Vitamin B12, 4 mg Vitamin B6, 170 mg Fe: Fe(II) sulphate, 4 mg Cu: Cu(II) sulphate, 16 mg Zn: Zn(II) oxide, 50 mg Mn: Mn(II) oxide, 0.304mg KI, 0.3 mg Se: Se-selenite.

**Biokvas-45containg min. 45% protein, 5.5% fat, max 3% fiber

***Difference between groups within one mixture is irrelevant

All statistical analyses were performed using R-project software. For the purpose of production performance analysis one way ANOVA was used while the

Tukey test served to determine the statistical significance of the differences between individual means values.

Results and Discussion

The production performances are comprehensively presented in Table 2. Throughout the initial period, no statistically significant distinctions ($p>0.05$) emerged across all three monitored parameters between the assorted groups. However, during the subsequent period, notable differentiations in Feed Intake (FI), Average Daily Gain (ADG), and Feed Conversion Ratio (FCR) emerged between the T_1 and T_2 groups. Specifically, the T_2 group exhibited the most favorable Average Daily Gain at an impressive 563.24 g/d, along with a commendable Feed Conversion Ratio of 1.92 g/g. Impressively, over the course of the entire trial, the T_2 group maintained superior results in terms of Average Daily Gain, achieving 348.26 g/d, in comparison to the remaining trial groups. It's also important to highlight that there were no mortalities recorded throughout the duration of the trial, underscoring the overall robustness of the study subjects.

Table 2. Production performance (mean ± SE) of post-weaning piglets fed with Biokvas-45 supplement (T_1 and T_2) or without (C)

	Treatments			p
	C	T_1 3%	T_2 4%	
First period (22-49d)				
FI, g/d	518.36±0.016	521.42±0.013	514.89±0.021	0.546
ADG, g/d	270.14±0.028	275.46±0.028	264.33±0.036	0.227
FCR, g/g	1.92±0.035	1.89±0.025	1.94±0.015	0.662
Second period (50-60d)				
FI, g/d	1026.69 ± 0.042^{ab}	971.69 ± 0.051^{b}	1081.63 ± 0.041^{a}	$p<0.05$
ADG, g/d	527.42 ± 0.025^{ab}	491.55 ± 0.024^{b}	563.24 ± 0.034^{a}	$p<0.05$
FCR, g/g	1.95±0.039	1.98±0.089	1.92±0.056	0.259
Whole trial (22-60d)				
FI, g/d	661.26 ± 0.044^{ab}	648.56 ± 0.069^{b}	673.48 ± 0.035^{a}	$p<0.05$
ADG, g/d	342.89 ± 0.051^{ab}	335.22 ± 0.023^{b}	348.26 ± 0.026^{a}	$p<0.05$
FCR, g/g	1.93±0.019	1.93±0.021	1.93±0.023	0.846
Mortality, %	-	-	-	

SEM, Standard error of the means; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate; $^{a,\,b,}$ in a row, the least squares means with a different superscript differ significantly ($p<0.05$)

The noteworthy improvement observed in the feed conversion ratio of piglets within our study resonates with earlier documented findings regarding the benefits of live yeasts *(van Heugten et al., 2003; Bontempo et al., 2006; Shen et al., 2009)*. However, contrasting results have surfaced in other investigations, where no discernible positive impacts were identified *(White et al., 2002).* Indeed, the application of probiotics has consistently demonstrated a contributory role in bolstering gut health among weaning piglets, thereby augmenting the integrity of the intestinal epithelial barrier *(Cheesman et al., 2011)*. Interestingly, our study revealed an absence of gut-related issues, such as diarrhea or colitis, which could be attributed to the favorable effects of the yeast supplement. Yeast and its derivatives have long held prominence as viable candidates for incorporation as probiotics or prebiotics within swine diets. A thorough scrutiny of the effects of yeast on the immune system and enteric microbiota has been undertaken across numerous studies *(Broadway et al., 2015)*. Nonetheless, the body of literature yields incongruous outcomes, possibly attributable to the distinctive attributes of specific yeast strains, the physiological stage of the animals, or the environmental conditions in which they are reared *(Monroy-Salazar et al., 2012)*. This inherent variability hampers direct inter-experimental comparisons. Encouragingly, our findings underscore the efficacy of yeast probiotics in assisting piglets to navigate the stress associated with the weaning phase during the early stages of production. Notably, research *(Jiang et al., 2015)* underscores the pivotal role of the administered form of yeast probiotics, hinting at its critical influence. Numerous additional investigations corroborate the notion that supplementing with live yeast can enhance disease resistance and performance by promoting a conducive intestinal environment for pigs *(Davis et al., 2004; Volman et al., 2008; Lessard et al., 2009).* The appeal of yeast-based supplements as a compelling antibiotic alternative for managing post-weaning diarrhea in weaned piglets is on the rise. Mounting evidence underscores that the administration of live yeast, particularly *Saccharomyces cerevisiae*, not only escalates antibody levels within the colostrum and milk of sows *(Zanello et al., 2013; Trckova et al., 2014)*, but also amplifies IgA levels within the serum of piglets *(Trckova et al., 2014).*

Conclusion

The outcomes of our study prominently reveal that the incorporation of the studied dietary supplement, Biokvas-45, into the feed mixtures wielded positive impacts, elucidated as follows:

- Enhanced Feed Intake: Remarkably, the second trial group exhibited superior Feed Intake (FI) in comparison to both the control group and the other trial group.
- Improved Average Daily Gain (ADG): Animals within the experimental groups, which were fed diets containing Biokvas-45, demonstrated a notable elevation in Average Daily Gain (ADG) when juxtaposed with the control groups.
- Stable Feed Conversion Ratio (FCR): While investigating the Feed Conversion Ratio (FCR) across the entire trial, the examined supplement exhibited consistency, demonstrating no discernible differences in relation to FCR among the various groups.

In light of these findings, it becomes evident that the inclusion of the dietary supplement Biokvas-45 holds substantial promise within the nutrition regimen for growing piglets. The implications suggest that this supplement could serve as a beneficial component in the diets of piglets at this stage of development. However, a prudent course of action entails further investigation to delve deeper into the potential effects of this supplement on fatteners, broadening our comprehension of its overall impact. Such expanded research endeavors would provide a more comprehensive understanding of the supplement's applicability and efficacy across varying stages of pig development.

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VARIATION IN CARCASS, MEAT AND FAT QUALITY OF AUTOCHTHONOUS BREED IN CONVENTIONAL AND ORGANIC PRODUCTION SYSTEM

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Abstract: The Krškopolje pig, a local Slovenian pig breed, is raised in various housing conditions (indoor, outdoor or combined) and often in organic farming. The aim of the present study was to compare carcass, meat and fat quality of surgically castrated Krškopolje male pigs reared in conventional (CON, n=108) and organic (ECO, n=136) production systems on data continuously collected from 2015 till present. Krškopolje pigs in ECO system were on average older and heavier at slaughter than CON pigs (355 vs. 299 days; 162 vs. 151 kg, respectively). Considering carcass characteristics, ECO pigs exhibited greater carcass length, thicker backfat (at the level of the last rib and at withers) and larger loin eye area ($P<0.01$). There was a significant difference in meat quality regarding colour parameters (L*, a*, b* and hue) and shear force, indicating that ECO pigs had darker, redder and less yellow meat colour, and more tender meat than CON pigs ($P<0.05$). The ECO group had also lower saturated and larger n-6 and n-3 polyunsaturated fatty acid contents of backfat than CON group ($P<0.001$). *Longissimus dorsi* muscle of ECO pigs exhibited lower vitamin E and larger magnesium contents ($P<0.05$). To conclude, the present study showed that ECO Krškopolje male pigs deposit more fat, which is further reflected in a different fatty acid composition. Although the ECO pigs were older and there was no significant difference in IMF content, their meat was less tough than the meat of CON pigs.

Key words: Krškopolje pig, production systems, carcass traits, meat quality, fatty acid composition

Introduction

Krškopolje pig is the only indigenous Slovenian pig breed with a specific white belt across the shoulders and forelegs *(Batorek Lukač et al., 2019)*.

Krškopolje pig is considered to be more robust and better adapted to local feed resources and to more extensive rearing conditions. This pig breed is also reputed for its high-quality meat and meat products, including salami, sausages, dry-cured hams and pancetta compared to modern breeds such as Large White, Landrace and Pietrain.

The production systems can be divided in two main groups: conventional and organic. The organic system differs from the conventional system in a holistic paradigm that combines best environmental and climate action practices, a high level of biodiversity, preservation of natural resources and application of high animal welfare standards and high production standards *(Regulation (EU) 2018/848)*. Livestock should be fed materials produced in accordance with the rules of organic production, preferably originating from the farmer's own holding. In the choice of breed, high degree of genetic diversity, the capacity to adapt to local conditions and disease resistance, should be encouraged. The organic pig production thus often uses local pig breeds. Housing conditions and husbandry practices should ensure a high level of animal welfare respecting species-specific needs and animals should have permanent access to open-air areas for exercise. Any suffering, pain or distress should be avoided. Feed should be of organic origin, without feed additives and processing aids and obligatory additional feeding with voluminous feed on an ad libitum basis *(Regulation (EU) 2018/848)*. Furthermore, genetically modified organisms, animal by-products, and chemical fertilizers should be strictly avoided.

As for Krškopolje pigs, they are raised in numerous production systems of conventional and organic nature *(Batorek Lukač et al., 2019),* and generally in more welfare-friendly housing conditions *(Čandek-Potokar et al.,* 2022*)*. The variability in farming practices in both organic and conventional systems leads to a wide range of obtained results *(Prache et al., 2022)*. Other than organic production standards, the pork quality attributes depend more on the on-farm factors (pig genotype, feeding, housing conditions, etc.) that farmers use to meet the specification, as stated by *Lebret and Čandek-Potokar (2022).* Also, the quality attributes of organic animal products generally show greater variability compared to conventional products *(Lebret and Čandek-Potokar., 2022).* Therefore, the objective of this study was twofold, to evaluate the variability of carcass, meat and fat pork quality of Krškopolje pigs in conventional and organic production systems and to evaluate if overall important differences in meat quality exist with respect to these two production systems.

Materials and Methods

Data (on 244 surgically castrated male Krškopolje pigs) were collected continuously from 2015 to the present in conventional and organic farms. Pigs in the conventional system (CON) were reared either indoors on deep litter or in indoor pens with solid floor, straw bedding and outdoor access area. Pigs from the organic production systems (ECO) for which data were collected were reared either completely outdoors with a shelter, or indoors with outdoor access area. The feeds and/or diets used varied from farm to farm and, apart from the general status (organic or conventional), were not specifically monitored and/or registered and also varied according to the season and year.

The pigs involved in the study were slaughtered in different slaughterhouses in Slovenia. The left half of the carcass was used for measurements. Carcass length was measured as a distance from *atlas* to *os pubis.* The backfat thickness was evaluated on split carcasses at the level of withers, the level of last rib and above the cranial edge of *gluteus medius* muscle. Loin eye area was assessed on a picture of a carcass cross section taken at the level of the last rib.

The pH was assessed in *longissimus dorsi* muscle (LD) using a pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) at two central locations in the central part of the LD muscle. Instrumental color parameters (CIE L*, a*, b*) were measured in triplicate with a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan). Hue angle (h°) and chroma (C*) parameters were calculated according to the formulas described by *Beltrán-Cotta et al. (2023).* Drip loss was determined by the EZ method *(Christensen, 2003)* and expressed as the difference (%) from the initial sample weight. To determine cooking loss and shear force, the LD samples (4 x 5 x 8 cm) were thawed, weighed and cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany). Shear force was measured on 3-4 half inch thick cylindrical cores excised from cooked LD sample using a TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK).

The intramuscular fat (IMF) content of LD muscle and fatty acid composition of backfat were estimated by near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using in-house calibrations developed in our laboratory.

The vitamin and mineral content of LD muscle was determined by ISO 17025 method in an accredited laboratory (Nutricontrol, Veghel, The Netherlands) using the LC-MS/MS (liquid chromatography combined mass spectrometry) for vitamin and ICP-OES and ICP-MS (Inductively Coupled Plasma Optical Emission Spectrometry) for mineral determinations.

The statistical analysis was conducted with the SPPS Version 23.0 (IBM Corp., Armonk, NY, USA). Carcass traits, meat quality and fatty acids measurements were analysed using linear mixed model with the fixed effects of treatment group and farm (nested within the treatment group):

$$X_{ij} = \mu + \alpha i + \beta j_{(i)} + \varepsilon k_{(ij)}$$

µ - represents the overall mean, αi - represents the effect of factor A (treatment group, i.e. rearing system), βj(i) - represents the effect of factor B within the factor A (farm nested within treatment group), ε k(ij) - the random error.

Since samples used for the analysis of vitamins and minerals were not individually chemically analysed (analyses made on pooled samples per farm), one-way ANOVA with production system as fixed effect was used for the analysis.

Partial eta squared was calculated to measure the effect size of the treatment (organic vs. conventional). Levels for significant differences were set at $P < 0.05$. All results were expressed as mean (estimated marginal means) ± S.E. (standard error).

Results and Discussion

A total of 244 surgically castrated male Krškopolje pigs (conventionally reared, n=108; organically reared, n=136) were slaughtered at an average age of 355 and 299 days for ECO and CON pigs, respectively. The obtained values showed no significant difference between the two production systems in live weight, carcass weight, average daily gain, *gluteus medius* muscle thickness and backfat thickness above the *gluteus medius* muscle (P>0.05). However, it should be noted that slaughter weight and consequently carcass weight were a bit higher in organic system (11.4 kg; P=0.07 and 7.5 kg; P=0.13, respectively).

Other carcass quality traits (Table 1) showed that pigs in the ECO groups had significantly greater carcass length (P<0.001), thicker backfat at withers and at the level of the last rib (P<0.05), and greater loin eye area (P<0.01). The differences in carcass length can be explained with the fact that ECO pigs were older at slaughter (i.e. 56 days on average), while growth rate was similar in ECO and CON pigs. As it was shown and concluded in *Pugliese et al. (2003),* greater length of outdoor animals was related to their greater ages. The differences in backfat thickness (at the level of the last rib and at withers) may be attributable to higher age and weight of ECO pigs *(Schinckel et al., 2008).* In line with our result, larger loin eye area of outdoor raised pigs was reported by *Gentry et al. (2002)* and *Maiorano et al. (2013),* while the opposite was observed for backfat thickness and loin eye area in a study comparing organic and conventional Krškopolje pigs *(Tomažin et al., 2019).*

Table 1. Carcass traits of conventionally (CON) and organically (ECO) reared Krškopolje pigs

	CON (n=108)	ECO (n=136)	*P*-value
	Mean ± S.E.		
Live weight (kg)	150.5±5.3	161.9±3.6	0.076
Average daily gain (g/day)	462±0.01	465±0.01	0.838
Carcass weight (kg)	124.7±4.1	132.2±2.8	0.130
Carcass length (cm)	101.2±1.0	107.2±0.7	<0.001
Backfat thickness above *GM* muscle (mm)	41.7±1.4	40.7±1.0	0.562
Backfat thickness at the level of the last rib (mm)	41.0±1.4	44.7±1.0	<0.05
Backfat thickness at withers (mm)	57.4±1.9	64.2±1.4	<0.01
BF (mm)	47.4±1.4	49.9±1.0	0.148
Gluteus medius muscle thickness (mm)	70.5±1.6	71.1±1.1	0.763
Loin eye area (cm^2)	39.6±1.7	46.7±1.2	<0.01

Mean: Estimated marginal means; S.E.=standard error; GM= *gluteus medius* muscle; BF=mean of the backfat thickness above *GM* muscle, at the level of the last rib and at withers.

The meat quality characteristics of the LD muscle are summarized in Table 2 and show significant differences between ECO and CON pigs in colour parameters (L*, a*, b* and hue), marbling score and shear force. The ECO pigs had lower L* (*P*<0.05), b* and hue (*P*<0.001), and higher a* values (*P*<0.05), denoting darker and redder muscle. The results on colour can be associated with two factors. On one side, the redder and darker colour could be due to increased muscle oxidative metabolism, which is in line with our previous study indicating more oxidative metabolism of organic Krškopolje pigs *(Fazarinc et al., 2020)*. The oxidative metabolism of muscle fibres is associated with more myoglobin (Mb) that gives a red meat colour *(Listrat et al., 2016)*. On the other hand, darker and redder colour of ECO pigs could also be due to animal’s age; the older animals have more myoglobin (deoxymyoglobin - DMb and metmyoglobin – MMb) *(Yu et al., 2017)*. However, with more oxidative metabolism of ECO pigs, higher intramuscular fat would be expected *(Lebret et al., 1999)* which was not the case in the present study. With regard to water holding capacity traits, there were no difference in pH 45 min, pH 24 h, drip, thawing and cooking loss between two systems (*P*>0.05). Interestingly, the organic meat was significantly more tender than conventional one (*P*<0.05), despite the fact that ECO pigs were older and had slightly less marbling (*P*<0.05) and IMF (*P*=0.13). Nevertheless, the shear force is not necessarily related to the IMF. The explanation could be in the muscle fibres (type and size) which we did not measure, but can be related to our previous study *(Fazarinc et al., 2020)* showing that organic production system influenced the composition of the LD myofibre type (smaller myofibre cross-sectional area, a shift toward oxidative myofibre types). More oxidative muscle fibres have smaller cross-sectional area which is easier to cut through i.e. giving less resistance to cutting.

Table 2. Meat quality traits of conventionally (CON) and organically (ECO) reared Krškopolje pigs

	CON (n=108)	ECO (n=136)	*P*-value
	Mean ± S.E.		
pH 45 min LD	6.34±0.04	6.41±0.03	0.147
pH 24 h LD	5.48±0.02	5.46±0.02	0.366
CIE L*	54.9±0.7	52.9±0.5	<0.05
CIE a*	9.8±0.3	10.7±0.2	<0.05
CIE b*	6.1±0.4	4.5±0.3	<0.001
Hue	29.9±1.6	22.3±1.1	<0.001
Chroma	11.9±0.4	11.8±0.3	0.699
Drip loss after 24 h (%)	4.7±0.3	4.9±0.3	0.526
Thawing loss (%)	11.8±0.7	12.9±0.4	0.161
Cooking loss (%)	26.4±0.9	26.6±0.5	0.851
Marbling score (1 to 7)[1]	3.5±0.2	3.0±0.1	<0.05
IMF, %	5.1±0.3	4.6±0.2	0.138
WBSF (N)	54.9±2.2	48.7±1.3	<0.05

Mean=Estimated marginal means; S.E.=standard error; LD=*Longissimus dorsi* muscle; CIE= International Commission on Illumination; L*= lightness; a*=red/green coordinate; b*=yellow/blue coordinate; [1] visual assessment on a freshly cut LD muscle using a scale from 1 (extremely lean) to 7 (extremely marbled sample); IMF=intramuscular fat; WBSF = Warner–Bratzler shear force.

The results of fatty acid composition (Table 3) showed no differences in MUFA between the ECO and CON groups. Polyunsaturated fatty acids (PUFA), PUFA/SFA, n-6 and n-3 polyunsaturated fatty acids were significantly higher in the ECO group (*P*<0.001).

Table 3. Fatty acid composition* of conventionally (CON) and organically (ECO) reared Krškopolje pigs

	CON (n=108)	ECO (n=136)	*P*-value
	Mean ± S.E		
SFA	43.1±0.3	40.5±0.2	<0.001
MUFA	46.6±0.4	47.3±0.2	0.100
PUFA	10.4±0.4	12.4±0.3	<0.001
PUFA/SFA	0.24±0.01	0.31±0.01	<0.001
n-3 PUFA	0.7±0.03	0.9±0.02	<0.001
n-6 PUFA	9.5±0.3	11.2±0.2	<0.001
n6/n3 PUFA	13.7±0.4	12.7±0.3	<0.05

Mean=Estimated marginal means; S.E.=standard error, SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids. *=fatty acids are presented as g per 100g of fatty acids.

The fatty acid profile of a diet can have a huge impact on the final fatty acid composition *(Wood et al., 2008)*. The higher PUFA content in ECO pigs can be attributed to the differences in the diet (the obligatory supplementation of roughage e.g. hay, which is rich in polyunsaturated fats), because PUFAs are obtained directly from the consumed food *(Wood and Enser, 1997)*. On the other hand, MUFAs could be deposited either from feed or by desaturation of saturated fatty acids (SFA) (obtained by de novo SFA synthesis) *(Wood et al., 2008)*. The SFA content was significantly lower in organic pork (P<0.001). The finding of lower SFA content of organic pork has also been reported by *Kim et al. (2009)* and *Wójciak et al. (2021)*. Some authors found that higher physical activity in free-range pigs positively correlated with n-3 PUFA content *(Daza et al. 2009; Škrlep et al., 2019)*.

When we consider the effect of farm (results not shown), the effect was significant for practically all measurements (except for CIE L* and drip loss). This reflects the effect of different conditions on farms with regard to the feeding and diets applied.

Analysis of the vitamins of the LD muscle (Table 4) showed that CON pigs had higher concentration of vitamin E (P<0.001), whereas all other vitamins showed no statistical difference. *Wójciak et al. (2021)* also showed higher vitamin E content in meat samples of pigs raised in conventional production system. The opposite was reported by *Högberg et al. (2002*) who found higher vitamin E content in organically raised castrated pigs. The possible reason for the differences in vitamin E content between the two groups is likely the diet *(Echenique, 2007)*, however it is also possible that ECO pigs deposit less vitamin E due to its higher depletion for maintaining oxidative stability. Physical activity is associated with increased oxidative stress and consequently affects the need for antioxidants such as vitamin E *(Packer, 1984)*.

Table 4. Vitamins composition of *LD* of conventionally (CON) and organically (ECO) reared Krškopolje pigs

	CON (N=10)	ECO (N=12)	μ^2_p	*P*-value
	Mean ± S.E.			
Vitamin A (mg/kg)	<0.1	<0.1	-	-
Vitamin A (IU/kg)	<333	<333	-	-
Vitamin D3 (µg/kg)	<20	<20	-	-
Vitamin D3 (IU/kg)	<800	<800	-	-
Vitamin E (mg/kg)	4.36±0.17	2.13±0.16	0.82	<0.001

LD= *Longissimus dorsi* muscle; Mean=Estimated Marginal Means; S.E. = standard error; η_p^2 – partial eta squared denotes effect size; 0.01 is considered a “small” effect size, 0.06 represents a “medium” effect size and 0.14 a “large” effect size.

The analysis of minerals (Table 5) showed that ECO pigs had higher Mg content (*P*<0.05). This could be explained by the fact that organic pigs have free access to soil and small stones, which are rich in Mg and Ca reserves *(Zhao et al., 2016)*. However, Ca content was higher in the CON system than in the ECO system (*P*<0.05). Commercial concentrate usually contains (is supplemented with) calcium phosphate and limestone, which could be a reason why the Ca concentration was higher in the conventional system. On the other hand, some authors argue that Ca content is stable despite different feeding regimes *(Zhu et al., 2007)*.

Table 5. Minerals composition of *LD* of conventionally (CON) and organically (ECO) reared Krškopolje pigs

	CON (N=12)	ECO (N=12)	μ^2_p	*P*-value
Mean ± S.E.				
Ca (g/100g)	0.004±0.001	0.003±0.001	0.176	<0.05
P (g/100g)	0.190±0.001	0.191±0.001	0.012	0.609
Fe (mg/kg)	5.542±0.223	5.667±0.223	0.007	0.695
K (g/100g)	0.371±0.004	0.368±0.004	0.013	0.598
Cu (mg/kg)	<5	<5	-	-
Mg (g/100g)	0.025±0.000	0.027±0.000	0.328	<0.05
Mn (mg/kg)	<5	<5	-	-
Na (g/100g)	0.038±0.001	0.040±0.001	0.053	0.281
Zn (mg/kg)	16.250±0.534	16.417±0.534	0.002	0.827

LD=*Longissimus dorsi* muscle; Mean= Estimated Marginal Means; S.E. = standard error; η_p^2 – partial eta squared denotes effect size; 0.01 is considered a “small” effect size, 0.06 represents a “medium” effect size and 0.14 a “large” effect size.

Conclusion

Krškopolje pigs in the organic system were older, heavier and exhibited greater *longissimus* muscle and backfat thickness. They also produced meat that was of darker and redder colour, with lower marbling, but higher tenderness, had lower vitamin E, lower Ca and higher Mg content. Their fat tissue was more unsaturated (higher PUFA, n-3, n-6 PUFA, PUFA/SFA ratio, and lower n-6/n-3 PUFA). It can be concluded that organic and conventional production systems affect the results which could be related to differences in the nutrition (e.g. amino acids balance, roughage), however the diets used on the farms were not monitored to be able to draw cause-effect conclusions.

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ATTITUDES OF CONSUMERS TOWARDS ANIMAL WELFARE IN UKRAINE AND AZERBAIJAN

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Invited paper

Abstract: The aim of this study was to investigate the views and opinions of consumers in Ukraine and Azerbaijan regarding the importance of animal welfare. A sample of 147 respondents in Ukraine and 105 in Azerbaijan was included in the study. Ukrainian consumers associated animal welfare with good housing and good feeding, whereas Azerbaijani consumers mainly considered the importance of good feeding, and ranked housing as less important. Only a small proportion of the consumers from both countries considered natural behaviour, happiness and other positive emotions as important. Older consumers expressed higher concerns about animal welfare compared to younger consumers. The majority of consumers from both countries (93% in Ukraine and 85% in Azerbaijan, p=0.058) believed that the welfare of livestock animals in their country should be improved. The major issues identified by the consumers included animal housing, feeding quality and lack of governmental support. Our results highlighted the importance of animal welfare among consumers in Ukraine and Azerbaijan. However, the understanding of animal welfare and the factors affecting it varied among consumers, which underlines the necessity of education regarding the conditions for livestock, animal welfare and animal rights.

Key words: farm animal, animal production, food ethics, animal welfare, animal protection

Introduction

Animal welfare is an important part of livestock operations, continuously attracting growing interest from society. Currently a growing number of consumers are demanding higher standards for animal welfare and food safety (*Pulina et al., 2022*). One of the current challenges in sustainable food production and consumption is to ensure and promote appropriate animal welfare and respect for animals. Governments and academia address animal welfare aspects at numerous points in the agricultural supply chain. The promotion and implementation of higher welfare standards can add value to agricultural products and promote competitiveness in world market. Consumers worldwide have many concerns currently about how farmers are raising their animals, including their housing, feeding, handling and slaughter. For example, organic production is perceived by many consumers as a more sustainable approach for meat production (*Schulze et al., 2021*). Consideration of consumers' views and attitudes regarding animal welfare is essential for a well-functioning production chain. Harmonization and standardization of procedures related to animal welfare and meat production are needed for connections between the countries, including marketing and trade.

There is a high discrepancy in consumer interest in livestock welfare and ethical issues across the world. It has been shown that 61% of consumers in Germany and only 34% of consumers in Poland agreed with the importance of animal welfare (*Bozzo et al., 2019*). There is limited knowledge also on the welfare aspects of animal production, which are important for consumers. Recently, the number of survey-based studies on consumer attitudes to animal welfare has increased, but information about animal welfare in Eastern Europe is limited (*Tomasevic et al., 2020*).

The aim of this study was to investigate the views and opinions of consumers in Ukraine and Azerbaijan regarding the importance of animal welfare.

Material and Methods

Data collection and sample characteristics

A cross-national survey was conducted in Ukraine and Azerbaijan in 2022. A sample of 147 respondents in Ukraine and 105 in Azerbaijan was included in the study. The questionnaire consisted of the following four parts:

- Socio-economic data: age, sex, geographic area (urban or rural)

- Meat consumption: meat eater or vegan/vegetarian
- Animal welfare: descriptions and concerns about animal welfare, the importance of animal welfare, the need to improve animal welfare and willingness to pay more for the products from animals with high welfare standards.
- Food product: desired quality of animal food products and desire of consumers in Ukraine and Azerbaijan to purchase "animal welfare-labelled" meat, dairy products and eggs in the supermarket.

In this study, questions on ethnicity and education were not included.

The questionnaire contained one open-ended question "What are the three most important welfare issues of farmed animals in your country?" The answers to this question were grouped into the following categories: related to 1) knowledge; 2) feed quality; 3) food quality; 4) housing conditions and 5) economic issues. The most common answers in each category are presented in Table 1.

Table 1. Categorization of the answers to the open-ended question "What are the three most important welfare issues of farmed animals in your country?"

Category	Examples of answers
Knowledge	Increasing knowledge in the field of animal husbandry: Lack of understanding of animal welfare; Farmers should be more informed Low understanding of animal welfare by consumers
Feed quality	Inadequate feed quality; Lack of different types of feed Use of antibiotics in feed
Food quality	Lack of quality food; Safety of final products; Effective use of animal products;
Housing conditions	Unfavourable conditions of livestock keeping; Poor natural conditions; Inadequate treatment of animals; Poor hygiene; Need for organic production
Economic issues	Livestock support from the government; Economic crisis; Strengthening of state support for animal husbandry; Consumers are not ready to pay high prices for high quality products

Ethical permission

No ethical permission was required for the study because the study did not involve any sensitive information.

Statistical analysis

Data were evaluated using SAS version 9.4 (SAS Institute, Cary, NC, USA). Associations of attitudes towards animal welfare (percentages) with the country, area (urban *vs* rural) and sex (male *vs* female) were estimated using a series of chi-square tests. The effect of age on animal welfare attitudes was estimated using regression analysis. Differences were considered significant at $p < 0.05$.

Word clouds were created using the WordArt.com online generator.

Results and Discussion

Characteristics of the study population

This study included a total of 252 individuals aged from 17 to 84 years, including 71 men and 180 women. The characteristics of the studied populations per country are presented in Table 2. The percentages of female respondents were higher in both countries and the mean age was higher in the respondents from Ukraine. The differences between the percentages of participating male and female respondents were in line with other studies, and the fact that women are more likely to participate in surveys compared to men.

Table 2. Basic characteristics of the studied populations

	Ukraine	Azerbaijan
Total	147	105
Male	43	28
Female	104	76
Rural area	62	28
Urban area	85	77
Vegans/vegetarians	1	4
Age, years	35 (17 – 84)	27 (18 – 63)

Attitudes towards animal welfare

Animal welfare is steadily becoming one of the key factors for consumers when purchasing meat and meat products. However, the importance of factors related to animal welfare is ranked differently in different regions. A summary of understandings of definitions of animal welfare by the consumers in Ukraine and Azerbaijan is presented in Figure 1. Ukrainian consumers associated animal welfare with good housing and good feeding, whereas Azerbaijani consumers mainly considered the importance of good feeding, and ranked housing as less important. Only a small portion of the consumers from both countries considered natural behaviour, happiness and other positive emotions as important.

Figure 1. Clouds of the words associated with high animal welfare standards according to the opinions of consumers in Ukraine (A) and Azerbaijan (B).

Despite differences in defining animal welfare, the majority of consumers from both countries expressed high concern about the welfare of farm animals. On a scale from 1 to 5, where 5 indicated the highest concern, 63% of the respondents from Ukraine and 71% from Azerbaijan ranked the concern about animal welfare as 5. These minor differences between Ukraine and Azerbaijan were not statistically significant (p=0.389). The percentages of respondents with high concerns in the present study are comparable with the percentage in Germany, and approximately two times higher than that in Poland (*Bozzo et al., 2019*). No sex- and area-related differences in the concerns about animal welfare were observed (p= 0.457 and p=0.602, respectively). We observed a weak, but significant, effect of age on the concern about animal welfare (p=0.006), with older consumers being more concerned about animal welfare compared to younger consumers. The results from the studies on age-related attitudes toward animal welfare are generally contradictory and vary from no association to negative or positive associations (*Randler et al., 2021; Carnovale et al., 2022*). The differences between the results might have been due to different experimental designs in these studies and selected population samples with variable cultural traditions and attitudes.

Concerns regarding animal welfare are one of the key drivers for a reduction in meat consumption, or complete avoidance of consumption of animal products (veganism). The reported concern about animal welfare, however, was not reflected in the avoidance of eating meat in the present study. Among the respondents, 1 person from Ukraine and 4 persons from Azerbaijan reported that they do not consume meat.

The majority of consumers from both countries (93% in Ukraine and 85% in Azerbaijan, p=0.058) believed that the welfare of livestock animals in their country should be improved. No differences between male and female respondents, nor between the respondents from urban and rural areas, were observed (p=0.827 and p=0.179, respectively). In contrast, other studies have highlighted sex as an important factor in the perception of animal welfare, and in the willingness to pay more for the products derived from animals with high welfare standards (*Miranda-de la Lama et al., 2019; Estévez-Moreno et al., 2021; Carnovale et al., 2022*). The factors which the respondents listed as requiring improvements are listed in Figure 2. Among other factors, violence towards the animals, poor veterinary care and inappropriate slaughter conditions were mentioned. Economic factors and lack of governmental support were listed as issues in both countries. However, improvement in animal welfare has been proven to results in many economic benefits long-term (*Fernandes et al., 2021*).

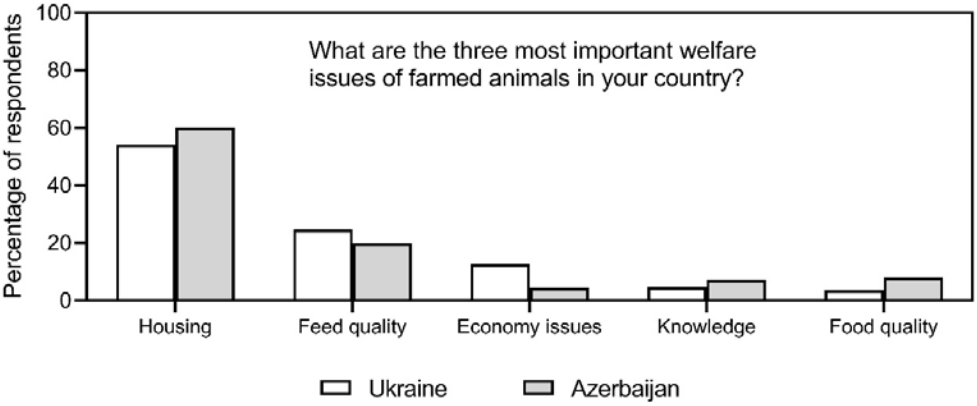

Figure 2. Overview of the most important welfare issues according to the opinions of consumers in Ukraine and Azerbaijan.

Many welfare issues might have an adverse effect on animal production and meat quality. From the list of characteristics regarding animal-based food, Ukrainian consumers highlighted safety as the most important followed by organic production and low use of antibiotics, while Azerbaijani consumers underlined safety, local and organic production as equally important (Figure 3). Interestingly, taste and the nutritional value of food were not priority factors in either Ukraine or Azerbaijan.

Figure 3. **Clouds of the words associated with desired quality of animal food products according to the opinions of consumers in Ukraine (A) and Azerbaijan (B).**

The consumers mainly get information regarding meat products from the product labels. Approximately 65% of the respondents from Ukraine and 70% from Azerbaijan stated that it is important to be able to purchase “animal welfare-labelled” meat, dairy products and eggs in the supermarket (Figure 4). There were no differences between countries, sex or geographic area ($p>0.05$). Similarly, *Sonoda et al.* (2018) demonstrated that 90% of Japanese consumers showed a positive attitude towards animal welfare labelling of beef. In the EU, all table eggs must be labelled with a code of the production method and the origin of eggs. However, apart from eggs, most animal welfare labelling are voluntary, which makes it difficult for consumers to understand how the animals producing them were kept. The labelling of food products regarding animal welfare would help consumers to make their purchasing decisions, and might also be relevant for producers, processors and retailers in the market.

Figure 4. Desire of consumers in Ukraine and Azerbaijan to purchase “animal welfare-labelled” meat, dairy products and eggs in the supermarket.

Conclusion

These results highlight the importance of animal welfare among consumers in Ukraine and Azerbaijan. However, the understanding of animal welfare and the factors affecting it, varied among consumers. Ukrainian consumers associated animal welfare with good housing and good feeding, whereas Azerbaijani consumers mainly considered the importance of good feeding, and ranked housing as less important. The factors affecting consumer attitudes towards animal-based food also differed between Ukraine and Azerbaijan. Ukrainian consumers highlighted safety as the most important followed by organic production and low use of antibiotics, while Azerbaijani consumers underlined safety, local and organic production as equally important. Thus, this further underlines the necessity of education for consumers regarding the conditions for livestock, animal welfare and animal rights.

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ORGANIZATION OF CATTLE PRODUCTION IN CONDITIONS OF CLIMATE CHANGE

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Invited paper

Abstract: The greenhouse effect has led to a changed precipitation regime, an increase in the average temperature, the occurrence of extreme meteorological events (droughts and floods) and the like. All this harms the yield and quality of feed, the incidence of mycotoxins in food, reduced productivity of animals, the incidence of new and non-specific pathogens, the development of diseases, etc. According to the FAO and the World Bank, the countries most affected by climate change are the countries of Central Europe and the Mediterranean. The population of people living in rural areas and engaged in agriculture will be particularly at risk. With difficult working and living conditions and reduced incomes, there will be an increased migration of the population to the cities. The simultaneous decline in agricultural production and increased food demand will lead to a food supply crisis (FAO predicts that by 2050 the global population will increase to 9.6 billion people, resulting in a demand for 70% more food than in 2013).Cattle production is affected by the climate in different ways, directly and indirectly. Livestock condition, production level, reproductive performance, morbidity and mortality are correlated with climatic conditions. The most pronounced negative impact on the health and welfare of animals is a phenomenon called heat stress. Exposure to microclimatic conditions characterized by a combination of high temperature and air humidity overcomes the ability of animals to maintain normal thermoregulation and constant body temperature, leading to an increase in body temperature that exceeds physiological limits. In addition to the direct effects of heat stress on the productivity, reproduction and health status of animals, global warming also affects animals indirectly through reduced soil fertility, water availability, crop yields, quality of plant nutrients and the circulation of pathogenic agents. Finding a solution to mitigate and prevent the consequences of unfavourable climatic and microclimatic conditions is a challenge for the entire scientific and professional community, but also for every cattle breeder. Solutions can be biological and technological. Biological ones refer to the animals themselves (breed selection,

selection for functional traits, selection for adaptability to heat stress, use of genomic selection, artificial insemination programs, herd size). Technological solutions relate to how animals are kept (free system, in outlets with canopies, use of grazing), facilities (new materials and technical solutions in the construction of facilities, use of insulating materials, regulation of microclimate conditions in facilities - ventilation and air humidity), nutrition and feeding (feeding method, feeding time, use of new types of feed, continuous water supply, etc.).The goal of agricultural production is to ensure food security in the face of climate change, and it is one of the most demanding tasks facing humanity.

Key words: greenhouse effect, climate change, cattle production

Introduction

Eradicating hunger, securing food supply and improving nutrition are important goals of sustainable development. At the same time, climate change, which negatively affects agriculture and food security, makes this task difficult. That is why the international community has been making efforts for many years to prevent climate change and its negative impact by adopting a series of legal acts, to which the Republic of Serbia is a signatory. The Intergovernmental Panel on Climate Change was created in 1988 at the request of the United Nations, the World Meteorological Organization and the UN Environment Program to assess the risk of climate change caused by human activity. The UN Framework Convention on Climate Change was adopted in 1992. It was followed by the Kyoto Protocol in 2007, the Paris Agreement in 2015, and the Glasgow Agreement in 2021.

And yet, according to the FAO report, between 691 and 783 million people have faced hunger in 2022, an increase of 122 million people compared to 2019. At the same time, more than 3.1 billion people worldwide cannot provide healthy nutrition, according to a joint report of the Food and Agriculture Organization (FAO), the International Fund for Agricultural Development (IFAD), the United Nations Children's Fund (UNICEF), the World Health Organization (WHO), the World Meteorological Organisation (WMO) and World Food Program (WFP). It was estimated that more than 250 million people in 2023 were exposed to acute food shortages. Of these, almost 57 million people in 12 countries found themselves in this unfavourable situation solely due to extreme climatic phenomena (droughts, floods, tornadoes, tropical storms, etc.). Compared to 2021, the increase in the number of people at risk is multiple (23.5 million people in 8 countries were affected by extreme conditions). By 2030, according to the

International Fund for Agricultural Development (IFAD), 670 million people will face acute hunger. Populations living in rural areas and engaged in agriculture are particularly affected. And such, according to FAO data, makeup almost half of the world's population. About 1.23 billion people were employed in 2019 in agri-food systems (857 million people in primary agricultural production and 375 million in food processing and distribution systems). Three times more, or almost half of the world's population, live in households whose source of financial income is related to agricultural production.

The results of the increase in global temperature are irregular precipitation, floods, tornadoes and storms, melting of glaciers, which is a threat to biodiversity. Estimates are that an increase in global average surface air temperature between 1.8^0C and 4.0^0C by 2100 will lead to the extinction of approximately 20 to 30% of plant and animal species, with severe consequences for food security in developing countries.

Climate change affects all branches of agricultural production, both crop and livestock production, given that they are closely related (*Hatfield et al., 2018*). The trend of continuous warming with the appearance of changed intensity and the seasonal nature of precipitation increases the sensitivity of agricultural systems, thereby endangering food production (*Gornall et al., 2019*).

In crop production, stagnation of grain yield is observed, as well as great yield variability. In addition, the nutritional and technological quality of the obtained products decreases. Diseases and pests further reduce the yield (*Fróna et al., 2021*). At the same time, due to desertification and soil degradation, the areas suitable for growing crops are also decreasing (*Arora, 2019*).

Livestock, and primarily, cattle production, are affected directly and indirectly by adverse climatic conditions (*Hempel et al., 2019, Angel et al., 2018, Nardone et al., 2010*). Directly, changed climatic conditions cause heat stress in animals, affecting growth reduction, reduction of milk and meat production, the occurrence of diseases, disruption of the reproductive cycle, etc. All of the above are indicated by the results of numerous studies (*Mićić et al., 2022, Samolovac et al., 2022, Samolovac et al., 2020, Samolovac et al., 2019, Beskorovajni et al., 2015, Beskorovajni et al., 2012, Nardone et al., 2010, Dickman and Hansen, 2009, Chase, 2000*).The indirect impact is reflected in the water and food deficiencies. An increase in air temperature requires higher consumption of water on the one hand and leads to a decrease in the amount of available water on the other. Lack of water affects the yields of plant crops that are used for food, both for animals and for humans. In addition to obtaining a smaller amount of food, its quality is significantly diminished. The reason for this is the changed chemical composition, digestibility and nutritional value of nutrients of plant origin.

The fact that agricultural production, especially cattle breeding, harms climate change through the emission of greenhouse gases (primarily methane and carbon dioxide) cannot be ignored. Nevertheless, the emphasis in this paper is on cattle production and its survival in the conditions of climate change due to its importance in supplying food to a large part of the human population. Therefore, it is necessary to create cattle breeding development programs through which all the advantages of modern technologies in the selection and rearing of cattle would be used. They must enable, on the one hand, the survival of cattle breeding in the conditions of climate change, and on the other hand, reduce to a minimum the emission of greenhouse gases whose sources are related to cattle breeding (enteric processes of ruminants, production and manipulation of manure, production of animal feed, processing and transport, etc.).

Impact of climate change on cattle production

Climate change has far-reaching consequences for all branches of agricultural production. Cattle production is one of the areas most susceptible to the negative consequences of climate change. It is estimated that livestock losses due to heat stress will be 40 billion dollars per year by the end of the 21st century, which is about 10% of the total value of milk and meat production in 2005. Exposure of animals to extreme temperatures can disrupt their capacity for high productivity despite their genetic potential. Economic losses due to the reduced production capacity of animals caused by heat stress exceed losses associated with livestock mortality by 5 to 10 times. Each year, environmental heat stress alone costs the dairy industry over $900 million and the meat industry over $300 million (*Hatfield et al., 2020*).

Heat stress caused by climate change negatively affects the production and welfare state of cattle directly and indirectly (*Angel et al., 2018*). Directly: reduced food intake, reduced growth, reduced milk production, disorders in reproduction, the occurrence of various diseases, increased mortality, reduced ability to adapt. The most reliable indicator of the occurrence or risk of occurrence of heat stress is the THI index, the ratio of temperature and air humidity. A THI of 72 is the threshold for the occurrence of stress in dairy cattle (*Beskorovajni et al., 2015; Beskorovajni et al., 2012*), and it has this value at a temperature of 24 ^{0}C and 66% air humidity. In Serbia, it is most pronounced in the period June-August, although the risk of heat stress exists from April to October.

According to *Dikman and Hansen (2009),* heat stress is the sum of all environmental influences acting on the animal, which causes an increase in its body temperature, causing a physiological response. Environmental factors that contribute to heat stress are high temperature, high humidity and radiant energy (sunlight). Heat stress can be simply defined as the point at which the cow cannot release an adequate amount of heat and maintain body heat balance (*Chase, 2000*). An increase in temperature and moisture content in the air impairs production (individual growth, yield and quality of meat and milk) and reproductive performance of animals, metabolic and health status and immune response (*Nardone et al., 2010).*

The intensity of the impact of climate change on animal health depends on several factors: genotype, specific and non-specific resistance of animals to diseases, cultivation area, as well as pathogen characteristics. Symptoms of heat stress can range from mild changes in metabolism and milk production to potential cow mortality. The severity of heat stress depends on numerous factors such as temperature and humidity, duration of exposure to heat stress, ventilation and airflow, degree of night cooling, keeping of animals, availability of water, etc. (*Chakrabarti et al., 2022; Samolovac et al., 2020*). Some factors that affect the severity of heat stress depend on the animal itself: breed, body size, body weight, level of milk production, intake of dry matter before heat stress, colour and density of hair. Black-coated cows absorb more radiation than light-coated cows and face more heat stress. A high-yielding cow will be more affected by heat stress than a low-yielding or dry cow (*Kumar et al., 2018; Collier et al., 2015; Chase, 2000*). As stated by *Chakrabarti et al. (2022),* at ambient temperatures above 23^0C and 80% air humidity cows show heat-induced depression such as lower feed intake and lower productivity. At high relative humidity, normal evaporation decreases and prevents heat loss through breathing and sweating. Also, direct solar radiation increases the body temperature of animals and causes heat stress. Due to the action of stress factors, there are disturbances in the normal biological function of the organism and energy that should be used for growth or reproduction is consumed.

High-yielding animals are more sensitive than low-yielding animals. Also, animals reared in an intensive production system due to limited ability to move, inability to cool down adequately, exposure to heat radiation from surfaces in housing facilities, feeding on high-energy nutrients, etc. In such production systems, it is necessary to ensure optimal microclimatic conditions in the facilities. (*Samolovac, 2019; Samolovac et al., 2016*). Dairy cows with THI $\geq$ 72 react with reduced intake and poorer feed conversion. This leads to a decrease in milk yield, a lower percentage of milk fat and protein, poor condition and body weight, an increase in the number of somatic cells in milk, etc. (*Mićić et al., 2022; Beskorovajni et al., 2015; Lambertz et al., 2014; Beskorovajni et al., 2012*).

The effect of heat stress on reproduction is great. In females, heat stress leads to lower fertility due to shortened estrus duration, frequent occurrence of anestrus, poorer ovulation and embryonic death. In males, it causes disruption of spermatogenesis, poorer sperm quality, a decrease in testicular volume, decrease in the amount of fertile semen. Altogether it leads to a reduction in the conception rate by 10-20%. Heat stress in the last 50 days of pregnancy endangers udder development and reduces colostrum and milk production due to altered placental hormone production and reduced dry matter intake. Negative effects of heat stress have been identified from 42 days before to 40 days after insemination (*Jordan, 2003*). Increased temperature and air humidity affect the reduced expression of estrus. Plasma progesterone levels may increase or decrease depending on whether the heat stress is acute or chronic, as well as the metabolic state of the animal. All changes in hormone secretion reduce the activity of follicles and change the ovulatory mechanism, which leads to a decrease in the quality of oocytes and embryos. It also affects the change in the environment in the uterus and reduces the possibility of embryo implantation. Appetite and dry matter intake are reduced due to heat stress, which prolongs the postpartum period of negative energy balance and increases the duration of the service period, especially in highly productive dairy cows *(De Rensis and Scaramuzzi, 2003; Jordan, 2003)*.

The indirect impact of heat stress on animals is manifested through a reduced yield and poorer quality of food, lack of water, the presence of mycotoxins in food, and the appearance of pathogenic agents and pests. Agriculture is the single largest global consumer of water, with a share of 69%. Global warming is causing problems in the quantity and quality of available water. An increase in temperature leads to increased water consumption per head and hectare of arable land. With water shortages, there is increased competition between livestock, crops and non-agricultural water use, further exacerbating the problem. Water deficiency with high air temperature leads to premature ageing of plant tissue and lignification, which contributes to a lower quality of plant nutrients (lower digestibility and chemical composition of plants). Pasture systems are particularly at risk because higher temperatures and less precipitation reduce plant mass yields and increase soil degradation. Also, frequent droughts and extremely high temperatures lead to the dominance of more resistant plant species with lower nutritional value (weeds and lower-quality species). Climate change at the global level leads to seasonal variations and the occurrence of extreme climatic events, even in areas for which they are not typical. This is why climate zones change. High temperatures and changes in the precipitation regime promote the development and spread of pathogens, the modification of microorganisms, the emergence of new diseases, and increase the susceptibility of livestock to diseases, leading to increased morbidity and mortality of animals. The number of disease-carrying vectors (rodents, insects) is also increasing. The immune system has the greatest impact on the quality of health, but the problem is that heat stress has an immunosuppressive effect.

Nevertheless, it is encouraging that over time, animals have developed, to varying degrees, different adaptation mechanisms that include morphological, behavioural, physiological, neuroendocrine, biochemical and cellular responses that enable their survival in a given environment (*Angel et al., 2018; Summer et al., 2018*).

Response to climate change

The consequences of unfavourable climatic conditions can be mitigated in different ways. Some mechanisms are related to the animals themselves (adaptability, genetic diversity of breeds, raising animals to obtain different types of products) and to the organization of production that depends on the breeder (herd size, mobility, balance between productivity and welfare of the herd, technological equipment). These mechanisms can be used individually or in combination, depending on the goal of production, means, intensity of climate change action, environment, etc. (*Nozieres et al., 2011*). Some of the most important measures to mitigate climate change in cattle breeding include biological adaptation and technological optimization of cattle production (*Samolovac et al., 2022*).

Biological adaptation refers to animals and includes several processes, such as:

- selection of genotypes for heat tolerance, resistance to diseases and more efficient food utilization,
- use of genomic selection,
- use in the breeding of individuals whose offspring are resistant to heat stress (heat stress tolerance),
- introduction of new breeds tolerant to high temperatures and air humidity and
- breeding of domestic breeds adapted to the conditions characteristic of a specific breeding area.

Some of the effects of climate change are rising temperatures, water shortages, and rising grain prices due to increased demand for human and animal feed. Consequently, future dairy systems must rely more on grazing instead of using cereals in the diet of cows, so as not to represent "competition" with human nutrition. It is also very important to carry out selection in the herd in the direction of the efficiency of food utilization. However, the efficiency of selection in cattle breeding is reduced by a long generation interval. This requires the development of new selection methods to select superior heads suitable for future production systems. One solution is to find different genetic markers that can be used to detect cows resistant to heat stress, which would efficiently utilize poorer quality feed with high milk yield (*Hayes et al., 2009*). Identification of such animals presents a challenge to scientists due to the complexity of the response to heat stress and the negative correlation between heat tolerance and productivity. The development of new technologies provides an opportunity to solve this problem. After finding highly productive individuals resistant to the negative impact of climatic factors, the information obtained about their genome should be used for comparison with the genome of individuals sensitive to heat stress to identify the polymorphism of certain genes and perform selection in that direction. Various physiological indicators, such as rectal temperature, breathing frequency and salivation, can be used to genetically identify animals with more efficient adaptation mechanisms to the presence of heat stress. However, heritability for these physiological traits is low. That is why it is necessary to develop methods for evaluating the reproductive value of the head, which will include indicators for improving heat tolerance in dairy cattle.

Negative genetic relationships observed between heat tolerance and other economically important traits should be included in the selection indices to enable the selection of cattle that have resistance to heat stress while simultaneously maintaining or increasing productivity *(Luo et al., 2021).*

Thanks to the application of intensive selection and modern technological methods (artificial insemination, embryo transfer, genomic selection, etc.), enormous progress has been made in livestock production in the past few decades. The level of milk production has increased many times while the number of cows has decreased. However, it also has its drawbacks. Genetic diversity has been reduced, with negative consequences for health, longevity, fertility and resistance of animals to unfavourable conditions of keeping. In the future, dairy cattle breeders should place greater emphasis on traits related to animal welfare, health, longevity, environmental efficiency (e.g. methane emissions and feed utilization efficiency) and overall resilience. To achieve the set goal, it is necessary to define criteria (traits) that (a) represent the biological mechanisms underlying the corresponding phenotypes, (b) are hereditary and (c) can be measured in a large number of animals in the early period of life. Also, it is necessary to preserve and use the gene pool of domestic breeds whose resistance and ability to adapt should be integrated into modern breeding goals. This would avoid further losses of genetic diversity in the populations of modern breeds of cattle and the reduction of production potential while improving the health and welfare of the animals *(Brito et al., 2021*).

One of the methods of biological adaptation refers to the reproduction of animals. Techniques that have been investigated to reduce the negative effects of heat stress on reproduction include embryo transfer, ovulation induction and a combination of different procedures. (*Jordan, 2003; Rensis and Scaramuzzi, 2003).*

Breeding strategies for heat-tolerant heads depend on the production system. Systems that can provide sufficient resources for high productivity with the use of high technology and farm equipment will benefit more from the cultivation of highly selected noble breeds. In contrast, production systems with scarce resources in terms of housing and nutritional conditions will benefit more from crossbreeding with domestic breeds (*Carabaño et al., 2019*).

Technological optimization implies corrections in the method and time of feeding:

- Increasing the share of energy in the diet, because animals that are exposed to heat stress use part of their energy to maintain homeostasis;
- Avoiding feeding animals during the warmer part of the day;
- Increasing the quality of nutrients used in nutrition;
- Use of additives, such as probiotics and supplements that increase resistance to heat stress;
- Provision of sufficient quantities of fresh water;
- Control of temperature and air humidity in buildings
- Ventilation, cooling and airflowin buildings;
- Use of THI control applications;
- Thermal insulation of roofs;
- Natural shading of buildings, provision of ranges and canopies on ranges;
- Direct cooling of animals (dew or pouring water);
- Avoiding relocation, unnecessary movement or transport of animals;
- Control of insects (primarily flies) and
- Screening for the emergence and spread of new animal diseases.

The use of new materials and technical solutions that are now used in a certain form in construction and that would be adapted to the principles of building facilities for housing animals and enable a more effective fight against the consequences of heat stress in cattle. These are various technical solutions related to the shape and position of the barn, new insulation materials, such as cold materials or cross-laminated wood, as well as polyurethane insulation or shading systems, etc.

Grazing cattle on grasslands and natural pastures of satisfactory quality has significant advantages, which are reflected in a reduction of feeding costs, diminished risk of nutritional deficits and infectious diseases, i.e. increasing general resistance due to the possibility of movement, being in the fresh air and exposure to the sun. In principle, the quality of food largely depends on the way it is produced and preserved. In addition to ensuring the quality of feed used in cattle nutrition, great attention must be paid to the optimization of meals concerning the specific needs of animals in nutrients according to breed, sex, head category and stage of the production cycle.

Adaptation of the diet involves changes in the time and frequency of feeding (cooler time of day, more feedings per day), adequate feed, enough space for all the cows to eat together, plenty of cold water and adequate airflow. Increasing the number of feedings per day has two advantages. First, the food is fresher, which encourages consumption and reduces the number of insects that come into the food. Second, cows are curious by nature, so they will be stimulated to come and get food more often. Feeding time is also important. Increasing the amount of food available during the cooler part of the day, early in the morning or late in the evening, maybe another alternative. During hot days, the cow will eat mostly at night and after milking (*Kumar et al., 2018*).

In milk production, the occurrence of heat stress leads to huge economic losses every year. To better manage the heat regime in dairy cows, its early detection must be improved to apply certain cooling measures in time. With the development of sensor and wireless transmission technologies, body surface temperature and breathing rate can be automatically measured via portable devices. This will enable rapid, automatic regulation of microclimatic conditions in facilities and areas for housing animals using different methods of cooling animals (*Shu et al., 2021*).

Cattle production is directly affected by climate change, as it uses resources whose seasonality and capacity depend on climatic conditions. Farmers are responding to the negative impact of climate change by applying different adaptation strategies. The choice of strategy depends on many factors. The main factors that, according to research, influence the choice of adaptation strategy of farmers are experience, herd size, membership in agricultural organizations and associations, level of education and climate zone (*Idrissou et al., 2020*).

Conclusion

If it is necessary to react to unfavourable conditions when they are present, it is usually too late and the damage has already been done. In order to avoid such a situation, it is necessary to define long-term strategies to minimize the risk of climate change.

Improving the genetic potential for higher milk production per head can lead to an increase in total milk production without increasing the total number of heads. The necessity of improving milk production and increasing the efficiency and competitiveness of milk producers was also recognized by the Government of the Republic of Serbia with the adoption of the Action Plan for the implementation of the Government Program 2023 - 2026, the adoption of the Agriculture and Rural Development Strategy (2014-2024), as well as the IPARD (Instrument for Pre-Accession Assistance in Rural Development) projects.

The measures that should enable the achievement of the planned goals in the field of work of breeding organizations are the application of modern technologies in the assessment of the breeding value of animals, including resistance to heat stress (genomic selection, determination of genetic polymorphism, etc.), selection and breeding of animals with the best breeding values, both for production and functional traits (application of artificial insemination, embryo transfer), education of breeders, up-to-date forwarding of information about breeding animals (bull catalogue, public presentation, publications, online accessible information, etc.), sale of breeding animals, provision of a sufficient number of doses of semens of suitable bulls, prevention of infectious diseases, etc. It is of strategic importance to optimize forage production (mainly by improving water and soil management) and to improve the ability of animals to cope with heat stress. In order to achieve this, a fast flow of information and application in agriculture, that is, livestock production, is necessary (*Nardone et al., 2010).* Rapid changes in climatic conditions require the advisory and professional work on farms with breeders. For each breeder, one should analyze the possibilities and look at individual problems. In line with that, each of them should be helped to find a sustainable solution for their farm. When it comes to production technology, solutions can be related to improving microclimate conditions in buildings (use of fans, sprinklers, etc.), changing the way of rearing (ranges, grazing) and feeding the cows (various nutrients, additives, change of feeding time). In terms of selection, individual insemination plans should be developed that will include bulls that have been tested for tolerance to heat stress. Also, breeders must be familiar with the progress of modern methods in selection and the possibilities of application in their farm. If there is a possibility, it should be introduced into the production of milk and milk of other breeds or strains of the same breed, which are more resistant to unfavourable microclimatic conditions. However, the first prerequisite for professional and advisory work is the education of agricultural producers. This is the only way to achieve success because “scientia potentia est”!

The world can still be a nice place to live if agricultural production is maintained in a planned, conscientious and efficient manner in the conditions of increasingly pronounced climate changes.

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GOATS AND CLIMATE RESILIENCE

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Abstract: Climate change poses a major global concern and is therefore an ongoing topic. World’s population is expected to reach 9.7 billion people by 2050 and 10.4 billion by 2100, which means that the food resources will become crucial. In that respect, animal protein is considered a vital nutrient for growing human population. However, in the light of ever-changing climate events food and water sources for both animals and humans can become scarce in certain areas. The impacts of higher temperatures, changes in precipitation and extreme weather events pose the most risk on agricultural systems such as livestock. Direct and indirect influence of heat and drought caused by global warming is harmful to livestock. Small ruminants and particularly goats are considered more resilient and better adapted to hot and dry environments compared to other livestock. These animals require less in terms of feed, water and labor than large ruminants and are also more thermo-tolerant. They have certain physiological, behavioral and anatomical advantages aiding their survival during heat and drought. Goats are less of a competition to humans in terms of available food as they can thrive on plants unusable for human nutrition. The review discusses advantages of goats as species in terms of adaptation to changing climate.

Key words: goats, climate, heat, adaptation, thermotolerance

Introduction

The climate has been constantly changing and consequently impacting agricultural systems, both plant and livestock. These changes, which include higher temperatures, changes in precipitation and extreme weather events, have direct as well as indirect impact on animal breeding. These effects, which can vary by region, animal species, level of adaptability and production type, may be favourable or unfavourable *(Sejian et al., 2017)*. In northern Europe, warmer and wetter climate might favour crop and pasture growth, as well as higher yields of forage in central Europe, although quality will vary according to water availability and soil characteristics *(Gauly et al., 2013; Stagge et al., 2017)*. However, negative effects are most likely to be felt in tropical and subtropical areas *(Henry et al., 2018)*. Animals are directly impacted by heat stress caused by increased air temperatures and indirectly by reduced water and feed availability and quality, as well as increased exposure to pests and pathogens *(Gauly et al., 2013)* and the end effects are the same for both impacts, as they lead to impaired welfare, health and productivity.

Goats are regarded as the perfect domestic animal model for climate change due to their great resistance to disease, high resilience to heat and drought, and capacity to subsist on scarce pastures *(Reshma Nair et al., 2021)*. Compared to other domestic animal species, they have an advantage in thriving in harsh environments, as evident from African countries. As the climate change unfolds, environmental conditions globally are becoming more heat and drought prone. Even countries with temperate climate are experiencing more heat and drought during summer months as well as milder and drier winters. In some areas of Central Europe, such as Hungary, the percentage of days that cause heat stress, defined as days with a temperature humidity index (THI) value above a specific comfort threshold, increased by 4.1% between 1973 and 2008 *(Solymosi et al., 2010)*. Goats require less care compared to other livestock and don't need a lot of initial housing or equipment investment. Additionally, goats are tough and much more likely to withstand a prolonged dry spell and they even consume failed crops. They can walk longer distances and harsher terrains in search for forages and are disease resistant and drought tolerant, especially those of native breeds. When given low quality feed goats are better at digesting dry matermatter, utilizing nutrients and converting them into milk and meat compared to cattle or sheep.

Differences between species, as well as breeds, in adaptation to heat stress can be attributed to three main factors: ability of adaptation to elevated temperature and radiation, ability of adaptation to water restriction and ability of adaptation to periods of feed scarcity, all of which are found in goats through their efficient behavioural and physiological thermoregulation, capacity to withstand severe dehydration, low metabolic requirements supported by small body size, efficient digestive capacity and skilful grazing behaviour *(Nyamushamba et al., 2017)*.

In this review, we present what is known about the adaptation and production in goats as species under harsh environments caused by climate change.

Goats as the most climate-adapted animal model

Through the years population of goats increased in hot and dry areas whilst suppressing other ruminant species, especially in tropical continents such as Asia and Africa *(Scherf et al., 2009; FAO, 2018)*. Goats have distinctive morphological, behavioural, biochemical, cellular, and molecular characteristics which allow them to survive in various tropical environments *(Reshma Nair et al., 2021).*

Such behavioural, physiological and anatomical advantages aid their survival during drought. During the periods of water shortage, the voluntary intake of dry matter, i.e., feeding, decreased in an effort to maintain equilibrium with the available body water *(Maloiy et al., 2008; Alamer 2009).* Also, feeding frequency can be modified in terms of more frequent and shorter meals to reduce heat production caused by rumen fermentation or nocturnal feeding can be implemented to avoid high temperatures during the daytime *(Morand-Fehr, 2005).* Goats also have the ability to desiccate their faeces and can concentrate the urine thereby reducing water loss through waste matter discharge when faced with limited water availability *(Jaber et al., 2013; Kaliber et al., 2016).* As a part of physiological response, the heart rate was found to decline in water-restricted goats, therefore reducing their metabolism to conserve water and to compensate for the reduction in feed intake. This ability to reduce metabolism allows goats to survive even when faced with prolonged periods of severe limited food availability *(Silankove, 2000; Brosh, 2007).* They also have the ability to repress the evaporative water loss pathways both through the respiratory tract and skin surface, by reducing the water turnover when faced with water deficiency and this is considered as one of the key aspects of goat adaptation as compared to other farm animals *(Robertshaw and Dmi'el, 1983).* Rumen, which can serve as water tank, also contributes to ability of goats to withstand drought. Some studies found that the rumen has the capacity to store water for some time, thus preventing hemolysis and osmotic shock to tissues, and also allowing animals to walk longer distances in search of feed (*Silankove, 2000; Jaber et al., 2013).* Rumen of goats allows the intake of large volumes of water upon rehydration which is then temporarily stored in the rumen and this is more pronounced in goats as opposed to sheep *(Giger-Reverdin and Gihad, 1991).*

Goats are well adaptable and opportunistic feeders, as they can consume almost anything in order to survive. There is no specific feed requirement for goats and they can utilize forages not consumed by other ruminants. Goats can survive even on thorns and spines *(Decandia et al., 2008).* Goats also have some anatomical distinctions that contribute to their specific feeding behaviour such as the ability to assume a bipedal position, which makes them capable of browsing higher vegetation that is beyond the reach of sheep, as well as specific anatomy of the mouth with mobile upper lip, which enables them to make the maximum use of attainable vegetation, as opposed to sheep which have a cleft upper lip. In addition to this browsing behaviour goats can also travel long distances in search of food and water.

Morphological attributes such as body shape and size help in reducing heat loads and minimizing water losses *(Silankove, 2000).* When compared to other ruminants, goats are small with low body weight which helps them through periods of feed scarcity. Smaller animals also benefit from a relatively larger surface area which allows them to better dissipate heat from the body to the environment *(Jaber et al., 2013).*

Goats also have some digestive advantages making them more potent to survive on limited pastures. These animals are believed to have better digestive efficiency than other ruminant species as they have longer mean retention time of digesta in rumen and better feed conversion efficiency because of the microflora inhabited in rumen *(El-Tarabany et al., 2017).* It was found by *Daramola and Adeloye (2009)* that in goats fed with low-quality forages, lignin undergoes modification, degradation, and absorption from the gastrointestinal tract. This was recognized as one reason for the enhanced microbial activity in rumen.

In addition to their exceptional adaptation abilities to heat and drought stress goats also have high feed conversion efficiency resulting in lower enteric methane emission per unit of feed. Numerous studies demonstrated the least enteric CH_4 emission in goats as compared to other ruminant species *(Görgülü et al., 2009; Moeletsi et al., 2017; Darcan and Silanikove, 2018)* thus highlighting the advantage of goat rearing amidst the deteriorating environmental conditions *(Reshma Nair et al., 2021).*

Among the domestic ruminants, goats are considered to be the most disease resistant. In the review by *Daramola and Adeloye (2009)* it was reported that goats tend to have more lymphocytes than neutrophils in their circulation suggesting a well-developed immune system in this species. Disease resilience under thermal stress conditions depends, among other things, on physiological adaptation and responses on cellular and molecular level of animals, so the thermal-stress adapted animals will be in better health in general.

Variations in heat tolerance among breeds

Goats are often praised as heat resistant species, but just like other species they too have temperature comfort zone in which their homeostasis is best regulated. Outside this comfort zone goats also undergo changes in their energy and basal metabolism even though they are generally more resistant to the effects of temperature variations compared to other ruminant species *(Gupta and Mondal, 2019).* The resilience of goats to heat and drought varies according to genotype, genetic potential, life stage, management or production system and nutritional status.

Indigenous breeds of goats, as well as of other species, are considered to be more able to cope with high heat load and water and feed deficiency than their exotic counterparts in dry rangelands *(Alamer, 2003; Silanikove, 2000; Cooke et* *al., 2020)*. Selection for higher productivity in animal breeding often leads to reduced fitness of animals, disease resistance and tolerance caused by climate changes *(McManus et al., 2020)*. On the other hand, indigenous breeds have few unique adaptive mechanisms that help them to survive in a specific and often times harsh environment *(Wheelock et al., 2010)* and are more easily adapted to sudden environmental fluctuations and disease outbreaks than other exotic or crossbred breeds *(Alamer, 2003)*. Therefore, not all goats are created equal and breed contributes a lot to adaptive capacity. *Offoumon et al. (2019)* compares reproductive traits of Red Maradi, Saanen and crossbred Saanen x Red Maradi goats in sub-humid tropical Sudano-Guinean region and finds a rustic breed of Red Maradi to have better reproductive performances under constant heat load than pure Saanen or their crosses.

There are more than 600 goat breeds worldwide and the capability to adapt to the thermal challenges differs between the breeds *(Joy et al., 2020)*. *Gandhi and Arjava (2016)* state that tropical breeds can be comfortable in the environment with the temperatures as high as 38 ºC, while temperate breeds need temperatures in the range from 5 to 25 ºC for optimal performance. *Lallo et al. (2012)* note that tropical breeds have higher thermal tolerance thanks to low metabolic heat production and high heat dissipation.

However, even indigenous breeds that evolved in different regions of one country can also vary in their adaptive capacity to stressful conditions as shown in some previous studies *(Aleena et al., 2018; Pragna et al., 2018)*. An animal's ability to endure environmental challenges is determined by its genetic potential *(Silankove and Koluman, 2015)*. As a result of the interaction between numerous genes and traits, the genetic foundation of thermo-tolerance involves complex processes. Some researchers including *Yakubu et al. (2017)* and *Khan et al. (2019)* studied the SNP markers of MHC class II DRB gene and SOD3 gene and were able to identify association between certain number of SNPs and the heat stress response variables.

Recent studies *(Aleena et al., 2018; Madhusoodan et al., 2019)* report variations in goats' tolerance to heat stress and the effectiveness of their adaptation based on molecular alterations in terms of changes in heat shock protein 70 (HSP70), TLR2, TLR8. Heat shock proteins (HSPs) are a group of proteins synthesized by all living organisms as a response to heat stress. They help sustain cellular homeostasis and cellular adaptation to changing environment *(Hoffmann et al., 2003; Roti, 2008)*. One of the most prevalent and well-studied members of the HSP family, which is made up of highly conserved stress proteins, is heat shock protein 70 (HSP70), which is essential for environmental stress tolerance and adaptation *(Banerjee et al., 2014)*. Such research enables the identification and quantification of heat stress biomarkers, which may further support long-term breeding objectives to create breeds unique to agroecological zones *(Sejian et al., 2021).*

Climate change and milk production

Dairy industry is negatively impacted by elevated ambient temperatures, which has been well documented *(Salama et al., 2014; Silankove and Koulman, 2015; Contreras-Jodar et al., 2018).* Goats are generally considered more tolerant to heat stress compared to dairy cows because of their low body mass, and low metabolic requirements, as well as greater sweating rate and relatively larger surface area allowing greater heat dissipation. Their main advantage, unlike other ruminants, is their grazing strategy and efficient digestive system, which enable goats to attain maximal food intake and utilization in a given feeding situation. Because goats can consume browse (tree leaves and shrubs), there is a consistent and stable supply of food throughout the year. However, lactating dairy goats are not heatproof as they also exhibit several changes in performance due to thermal stress, including reductions in feed intake, milk yield and milk composition *(Hamzaoui et al., 2013). Contreras-Jodar et al. (2018)* report that heat stress not only negatively affects milk production in dairy goats, but also results in impairment in the functionality of immune cells, making the immune system of heat-stressed goats less capable of resisting diseases. Their research shows reduced feed intake, milk yield, protein, and fat content in heat stressed goats. Also, microarray analysis of blood reveals that 55 genes are up-regulated, whereas 88 are down-regulated by heat stress and bioinformatics analysis reveals that 31 biological pathways are impacted by heat stress as well. Research by *Sano et al. (1985)* as well as that of *Brasil et al. (2000)* demonstrates depression in milk yield by 3 to 13% in Saanen and 6% in Alpine goats, respectively, after being exposed to moderate or severe heat stress. *Silankove (2000)* and *West (2003)* state that under equivalent conditions of elevated THI, the reduction in milk yield in Holstein dairy cows would be much greater than in these Saanen and Alpine goats.

Lactating dairy animals generate substantial metabolic heat and also accumulate additional heat from radiant energy *(Coppock, 1985; West, 2003)*. High yielding cows are especially at risk of suffering from heat stress. As stated by *Coppock (1985)* the proportion of heat that gets produced through the metabolic processes of milk production in high yielding cows can exceed 50% of total heat generation. The built up heat load can cause disruptions in thermoregulation, which might result in increased body temperature and a general thermal stress *(Gauly et al., 2013)*, as well as cow mortality *(Vitali et al., 2009)*.

Hamzaoui et al. (2013) found heat stressed Murciano-Granadina dairy goats in late lactation to show dramatic physiological changes during the first week of exposure to elevated air temperature (climatic chamber treatment) and then to partially recover thereafter. They are able to sustain milk yield by losing body mass, but milk protein content and protein yield decrease.

So, no doubt that the heat stress causes depression in milk quantity and quality in dairy animals, goats included. But, even though goats are located in all types of ecology zones, their concentration is the highest in dry and tropic zones of developing countries *(Escareno et al., 2013)* and this is due to their higher resilience to heat and drought, as well as better adaptation to poor rearing conditions compared to other domestic animal species. Dairy industry in temperate zones hasn't been so far directly affected to the higher degree by elevated air temperature, however major concern is indirect impact through grain production. It is expected that there will be a conflict of interest between humans and animals as higher proportions of grains will be used for human instead livestock nutrition, as well as question of using high-quality forages that can be used as edible food for humans will arise *(Silankove and Koulman, 2015)*. In such scenarios, intensive production systems based on large ruminants like high producing dairy cows that rely on high concentrate diets, will no longer be possible.

Some previous studies demonstrated that milk production by local breeds of goats herding on natural pasture was not affected by heat stress *(Brown et al., 1988; Lallo et al., 2012; Di Rosa et al., 2013)* which suggests that milk yield in breeds adapted to hot environment are less affected by heat stress.

So, in the case of worsening of climate change the proportion of milk production from goats could be increased by exploiting the advantageous physiological traits of goats and thus lessening the overall impact of climate change on dairy production.

Mitigation strategies

When it comes to mitigation strategies, there are basically three available approaches to sustain survival and the productivity of animals in hot environment: through physical modifications of environment, nutritional management and genetic development of breeds less sensitive to heat stress *(Collier et al., 2006)*. These mitigation strategies can be used alone or in combination to assure better productive environment.

Physical modification of environment usually involves provision of shade and evaporative cooling techniques. Provision of shade is important when animals are kept outside, on pasture, as shade provides protection from direct solar radiation. Shades can be natural from trees or artificial (eaves/canopy). Trees are considered better option as they are cost-effective and also capture radiation by evaporation of humidity in the leaves. A good-designed shade structure can reduce heat output by 30 - 50% *(Muller et al., 1994). Habeeb et al. (2018)* suggests that the adequate surface area from shade for small ruminants should be 1.86 - 2.79 m^2 per animal in order for animals to be kept loose.

Provision of artificial ventilation in facilities is another strategy to reduce the heat load on animals. Preferably, buildings should be built from natural materials that ″breathe″ and airflow can be increased by fans or coolers if possible. Spraying the roof and walls of the barns helps in cooling of the surroundings and consequentially animals *(Brouk et al., 2003)*. Also, fully enclosed facilities are not recommended for hot climates because of the restricted natural air flow, therefore, partially enclosed shelters are preferred *(Sejian et al., 2015)*.

Nutritional management strategies include: feeding animals during the cooler periods of the day, providing enough fresh water, providing diet with high quality forages (i.e. grass–legume mixtures have higher crude protein concentration and lower fibre concentration than pure grass stands), increasing concentrate to forage ratio which improves the efficiency of nutrients utilization in animals under heat load, implementing fat supplementation which increases net energy intake in heat stressed animals due to its higher energy density and its lower metabolic heat, when compared to fibre or starch, supplementing with certain minerals, vitamins, amino acids and plant extracts *(Sivakumar et al., 2010; Hamzaoui et al., 2012; Hamzaoui et al., 2013; Kholif et al., 2016; Conte et al., 2018; De Lima et al., 2019)*.

Autochthonous goats are considered more capable of coping with harsh environments bringing on high heat load, water, and energy deficit compared to their exotic breeds. Goat production in such environments should be based either on rearing purebred autochthonous goats accustomed to specific environmental conditions, or using this locally adapted breeds in crossbreeding programmes. Also, selecting individual animals based on adaptive/fitness traits and/or heat stress biomarkers in breeding programs can be a good mitigation strategy. In small ruminants, genes with significant roles in metabolic, immune, and thermoregulatory pathways may serve as potential biomarkers for genetic selection. Identification and mapping of the individual genes responsible for better adaptation features is necessary for the incorporation of specific genes to improve thermo tolerance, which paves the way for an improved breeding program using marker-assisted selection and transgenics *(Henry et al., 2018)*. Prospective selection should balance productivity, health and thermal adaptability *(Joy, 2020)*. Keeping animals in good health and condition is also very important as healthy individuals will better resist heat than ailing animals.

Conclusion

Climatic changes will undoubtedly impact future supply of meat, milk, fibre and other products from ruminant animals. Increased temperatures, altered precipitation patterns, and extreme weather events will have varying effects on different geographic regions, animal species, and socioeconomic adaptation capability. Developing countries are by far the most vulnerable to climate change and elevated ambient temperatures since their agriculture is almost completely weather dependent. All ruminant livestock production systems will inevitably have to adapt to climate change. Some of the climate change negative outcomes could be mitigated through nutritional and breeding management, which will yield fast aid, but also through much slower genetic selection, which is more of a long-term approach.

All species of livestock will undoubtedly experience heat stress to some extent under the scenario of climate change, which will negatively influence their ability to produce and reproduce. However, goats are considered the most climate-adapted domestic animals and are expected to perform better than other livestock species, particularly in terms of feed and fodder shortage. Their physiological, anatomical and behavioural features allow them advantage in this demand. This is especially true for autochthonous and locally adapted breeds whose survival and protection should be imperative for all concerned. However, even though goats possess some advantages over other species in reducing the effects of climate change, they have consistently been a neglected species. Goats should gain far more attention in scientific community as they can be a go-to species when it comes to protecting economy of farmers through overcoming climate change–associated adversities.

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CARBON CAPTURE TECHNOLOGIES FOR LIVESTOCK FARMS

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Abstract: The Paris Climate Agreement, signed by 175 countries, has set the goal of limiting “net zero” carbon dioxide emissions and global temperature rise to 1.5°C compared to the pre-industrial level in the second half of the century in international law. The 1.5°C target was reiterated with the 26th United Nations Climate Change Conference held in Glasgow. In order to achieve this target, countries need to stay within a certain carbon budget. The use of renewable energy and the increase in energy efficiency are very important for combating climate change. However, these measures may not be sufficient to stay within the carbon budget and limit the temperature rise to 1.5°C. At this point, carbon capture, use and storage technologies will play an important role in achieving the "net zero" carbon dioxide emission target. The rapid rise in atmospheric carbon, or CO2, concentrations throughout the industrial period is of anthropogenic origin. Today, the most important sectors that cause greenhouse gas emissions are energy, transportation, industry and agriculture. Livestock practices have an important place in the production of greenhouse gas emissions originating from the agricultural sector. Conservation and restoration of farmland that already sequesters carbon is key to increasing carbon capture. Livestock is a unique sector in that it both sequesters carbon and releases it into the atmosphere. Therefore, through suitably managed farming systems and new technologies, the ability to enhance carbon capture can be achieved with the overall outcoreview, it is aimed to specify the technological methods used to capture emissions and carbon in livestock farming.

Key words: carbon capture, livestock, biomass, greenhous gas

Introduction

While agriculture is considered a net emitter of greenhouse gases (GHG), it also captures large amounts of carbon in soil, bioenergy substrates and food products (*Linderholm et al., 2020*). The agriculture sector alone accounts for 14% of global anthropogenic greenhouse gas emissions. This rate rises to 30% when we take into account other factors such as fertilizer production and deforestation. It has many applications and complements used in agriculture. Agricultural emissions are important, but the agricultural sector also raises the climate. This potential of agriculture includes the application of specific practices and technologies that enable: - reduction in emissions from agricultural activities, - increase in carbon storage, and - replacement of fossil fuels with biomass energy (*Foucherot and Bellassen, 2011*). Figure 1 shows the main greenhouse gas emission sources and processes in agricultural lands.

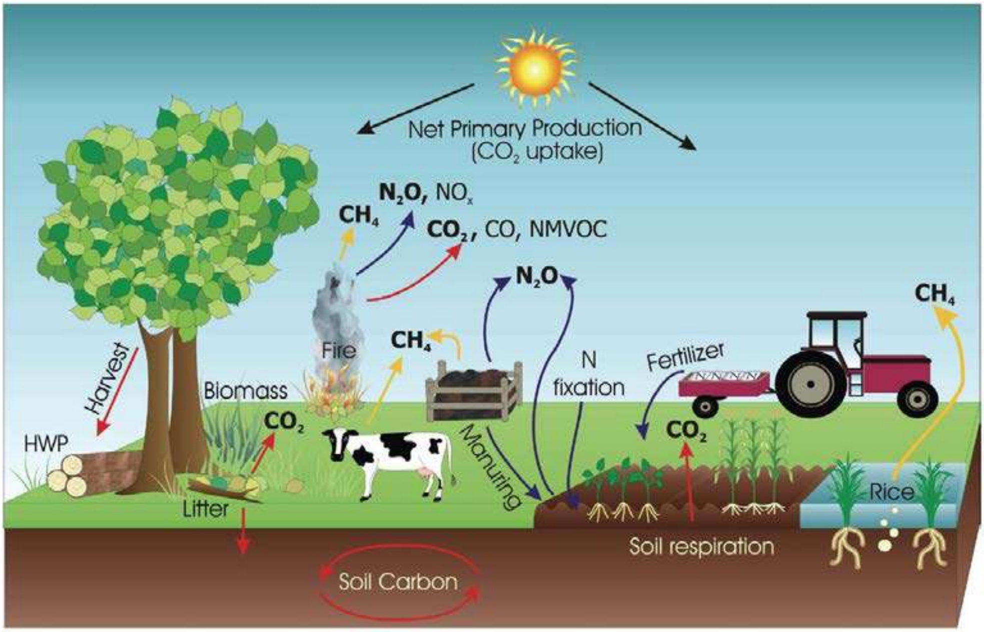

Figure 1. The main greenhouse gas emission sources/removals and processes in managed farmland (Source*: IPCC, 2006)*

The livestock sector has been a major focus of attention in the last decade all over the world. The livestock sector, the largest land use system in the world, accounts for 30% of the world's non-ice-covered surface. Agriculture contributes 40% of the global agricultural gross domestic product, providing a source of income for more than 1.3 billion people (*Herrero et al., 2013*). With the increasing interest in the livestock sector, the effects of animal husbandry on carbon emissions also attract increasing attention. Because excessive carbon emissions from livestock are an important issue that affects the sustainable development of livestock, as it exacerbates global warming, deteriorates environmental quality and adversely affects human life (*Shi et al., 2022*). Methane (CH4) emissions from enteric fermentation and both CH4 and nitrous oxide (N2O) emissions from manure management systems are the main emissions from livestock. Cattle are an important source of CH4 in many countries due to their large number and high CH4 emission rates due to their digestive systems. Methane emissions from manure management are also important, although less than enteric emissions. However, the most significant emissions are associated with closed animal management systems, where manure is processed in liquid-based systems. Nitrous oxide emissions from manure management vary considerably between the types of manure management systems used and may also result in indirect emissions due to other forms of nitrogen loss from the system (*Dong et al., 2006*). According to the FAO, livestock contributes approximately 9% of anthropogenic carbon emissions by emitting 37% of anthropogenic methane gas, 65% of anthropogenic nitrous oxide and 64% of anthropogenic nitrogen (*Shi et al., 2022*). Therefore, in this review, carbon capture in livestock farming and the approaches and technologies used in this field are discussed.

Livestock mitigation strategies to decrease emissions

According to climatologists, CH4, N2O and CO2 are considered as the main greenhouse gases emitted into the atmosphere, while for the livestock producer these emissions are losses of energy, nutrients and soil organic matter. These emissions generally reflect an inefficient use of initial inputs and resources, such as livestock feed. These losses also mean losses. Enteric CH4 emissions are a waste of energy in the production system. Nitrous oxide emissions from NH3 losses, directly or indirectly, are both forms of N loss and are associated with carbon dioxide emissions, fossil fuel consumption, and land use activities. Reducing the sector's emissions can be achieved primarily through a combination of reducing production and consumption, reducing emissions intensities from production, or modifying land use for production, and sequestering carbon in the soil. It is estimated that improved grazing management practices in rangelands can capture approximately 409 million tons of CO2-equivalent carbon per year globally (or 111.5 million tons C per year over a 20-year period) (*Gerber et al., 2013*).

The reduction of methane emissions during enteric fermentation can be offset by increased greenhouse gas emissions in the applied fertilizer. Reducing direct nitrous oxide emissions during storage can result in higher nitrate leaching and ammonia evaporation during field application. The reduction to be achieved in this way will reduce the amount of greenhouse gases directly emitted and increase the efficiency of production (*Grossi et al., 2019*).

The concept of 'Carbon Farming', which is frequently mentioned today, is a new trend that means capturing and storing carbon and/or reducing greenhouse gas emissions at farm level. Carbon farming mainly focuses on the management of farm-level carbon pools, streams, and greenhouse gas flows with the aim of mitigating climate change (*McDonald et al., 2021*). From a livestock perspective, carbon farming encompasses the management of both land and livestock, all carbon pools, materials and vegetation in the soil, as well as CO2, CH4 and N2O flows. It includes carbon sequestration (the capture and permanent storage of carbon in soil and biomass), avoided emissions (preventing the loss of already stored carbon) and emission reductions (i.e. reducing greenhouse gas emissions below current farm emission levels). The European Commission has also developed a regulatory framework to approve carbon sequestration, which aims to offer incentives to farmers to engage in carbon farming in EU countries, which published a "Carbon Farming Initiative" in December 2021. Carbon farming in agriculture and livestock offers significant potential in Europe to mitigate climate change and is therefore a European priority (*Grossi et al., 2019*). The European Commission expects carbon farming to contribute to the storage of 42Mt of CO2 by 2030, making agriculture a key strategic sector to achieve its 'Fit for 55' strategy.

As it is known, carbon is one of the most common elements on earth and is necessary for life. However, the increase in the concentrations of carbon dioxide and other greenhouse gases in the atmosphere is a significant negative contributor to global climate change and variability. Carbon sequestration refers to the long-term capture and storage of carbon from the atmosphere (typically CO2). This application is a promising method to increase biological carbon sequestration in soil and plants, reduce greenhouse gas emissions and combat climate change. Carbon sequestration is the long-term storage of atmospheric carbon in soil and plants. There are many different techniques to achieve carbon sequestration, especially methods such as reducing tillage and creating permanent grasslands. Beef cattle play an important role in increasing carbon sequestration through human food production from cultivated pastures and pastures, and through the integration of cattle grazing into "no-till" cropping systems (*Brookcks et al., 2020*).

Conservation and restoration of farmland that already sequesters carbon is key to increasing carbon sequestration. Livestock is a unique sector in terms of both capturing carbon and releasing it into the atmosphere. Therefore, through properly managed farming systems and new technologies, carbon sequestration capabilities and skills can be provided, with the overall result of negative emissions where carbon is actively removed from the atmosphere. Because it will be even more important in the future to aim to reduce on-farm emissions through the use of bio-energies to store and capture carbon and the use of on-farm carbon storage in increasing productivity in agriculture and livestock. The ways to achieve this are to increase energy efficiency, use the circular economy in which production-related by-products are converted into high value-added products, prevent soil compaction in grasslands, use new technological feed additives to reduce methane emissions, and precision farming methods (*Cureton, 2022*).

Carbon capture, storage, and utilization technologies

Since the beginning of the 19th century, the CO_2 concentration in the atmosphere and for average temperatures of the world have increased exponentially with a similar trend seen. The effects of high GHG concentration in the atmosphere of the world results in higher average temperatures on the planet and dramatic modifications of climate and the environment *(Lisbon et al., 2021).* The Paris Climate Agreement, signed by 175 countries, has set the goal of limiting “net zero” carbon dioxide emissions and global temperature rise to 1.5°C compared to the pre-industrial level in the second half of the century in international law *(Mikulcic et al., 2019).* The 1.5°C target was reiterated with the 26th United Nations Climate Change Conference (COP26) held in Glasgow. In order to achieve this target, countries need to stay within a certain carbon budget. The use of renewable energy and the increase in energy efficiency are very important for combating climate change. However, these measures may not be sufficient to stay within the carbon budget and limit the temperature rise to 1.5°C. At this point, carbon capture, use and storage technologies will play an important role in achieving the "net zero" carbon dioxide emission target *(Terlouw et al., 2021).*

The carbon formed in the world is examined in three classes as fugitive carbon, durable carbon and living carbon. Fugitive carbon includes CO_2 released into the atmosphere by burning fossil fuels, waste to energy plants, methane leaks, deforestation, and much industrial agriculture and urban development. it has ended up somewhere unwanted and it can be toxic. It ranges from reusable fibers such as durable carbon paper and fabric to building and infrastructure elements that can last for generations and then be reused. They are recyclable polymers that are locked in or used and reused in stable solids such as coal and limestone. Living carbon organic, growing in biological cycles, providing fresh food, healthy forests, and fertile soil. It is something we want to cultivate and grow. Soil includes living carbon in the form of fungi, microbes, humus, legumes, and grasses *(Akgün et al., 2023)*. It is seen that carbon forms on earth from Figure 2.

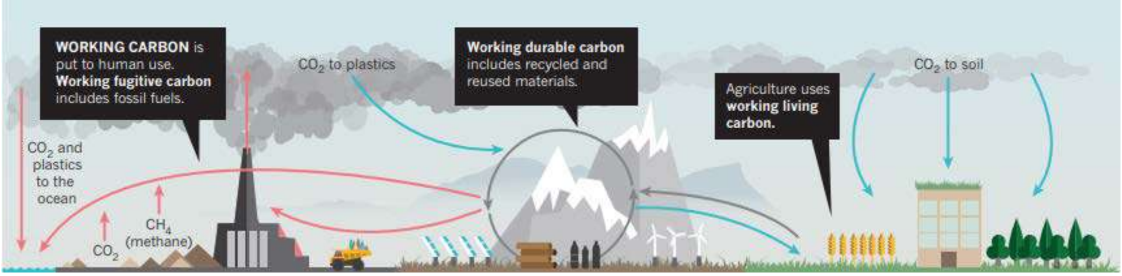

Figure 2. Carbon forms on earth (Source: https://www.nature.com/news/carbon-is-not-the-enemy-1.20976)

Carbon capture use and storage (CCUS)

The transport, energy, and industrial sectors are applying measures in order to reduce their GHG emissions to the atmosphere. The increasing use of renewable energy, especially in electric generation, reducing the consumption of fossil fuels, the use of biofuels, electric vehicles, or other alternatives in the transport sector; and energy efficiency improvement by the upgrades to equipment and machinery used in industry, are some of the measures to reduce their greenhouse gas emissions. But also the environmental policies of CO_2 capture technologies have also been promoted in order to help this energy transition. The technology of converting the carbon captured from GHG emissions in the atmosphere for another use is called carbon capture use and storage technologies (CCUS). There are currently 35 commercial CCUS facilities operating in the world. The annual total carbon capture capacity of these facilities is approximately 45 Mt CO_2. About two-thirds of these projects are concentrated in North America. CCUS facilities are necessary because it is difficult and expensive for heavy industries, namely fertilizer producers, steel mills, and cement producers, to adapt to working with cleaner energy *(International Energy Agency, 2022).*

There are three options for capturing CO_2, one of the greenhouse gas emissions, three different post-combustion pre-combustion options and oxy-fuel capture. The capture of CO_2 from the flue gas flow after combustion is called post-combustion capture. The most used CO_2 separation process capture is the -absorption using an amine as the absorbent. The low CO_2 concentrations in flue gas require powerful chemical solvents. when this system is applied, high energy amounts have to be expended to regenerate the solvents.

Obtaining the gas mixture (CO_2 and hydrogen gas mixture) after chemical methods such as gasification or reforming before combustion and then the capture of CO_2 from this mixture is called pre-combustion capture. In this system fuel is reformed by oxygen and/or steam to form a mixture of H_2 and CO_2. The CO_2 can then be separated from H_2 by adsorption or membranes, and the pure hydrogen is combusted with air in the power plant Biomass and natural gas can be used for pre-combustion capture technology. The aim of these systems is to convert the carbon fuel to carbonless fuel. The use of (nearly) pure oxygen to burn fuel is called oxyfuel capture. Oxy-fuel burning process is formed with pure oxygen produced using a cryogenic air separation or membranes. The combustion products are fundamentally CO_2 and H_2O, which are separated by condensing water. The combustion with pure oxygen is related to high temperatures when compared with the combustion with air. The nitrogen present in air is the major heat sink. In oxy-fuel process, cooled flue gases are used as heat sink instead of atmospheric nitrogen. The recycle of CO_2-rich flue gas reduces the temperature to usual values *(Porter et al., 2017; Pires et al., 2011).* The carbon must be converted into a form, liquid, for transportation. Liquid form carbon can injection into the earth for storage, deep in the underground chasms left vacant by the removal of oil and gas deposits, in deep saline formations, in unminable coal beds, or in the deep ocean *(Burns and Nicholsun, 2017).*

New technologıes for carbon capture in World ecosystems

Global ecosystems affect the atmospheric GHG composition and climate by contributing to the release and sequestration of CO_2, CH_4 and N_2O. In the last 50 years, about one-third of GHG emissions have been reduced by terrestrial ecosystems. Forest ecosystems and oceans are among the most important global carbon sinks. Forests absorb 45% of anthropogenic GHG emissions. The oceans absorb 22.7% of CO_2, one of the anthropogenic greenhouse gas emissions *(Wang et al., 2020).*

The food industry is one of the important greenhouse gas sources that affect greenhouse gas emissions in the world. The GHG formation caused by the food production industry accounts for more than one third of the world's GHG formation. 71% of the GHG formation caused by food technologies consists of agricultural products and livestock production systems and land use change activities *(Crippa et al., 2021).*

The use of multi-sensor drone technology, better crop systems, fertilization and irrigation practices, and the use of advanced digital farming technologies that allow farmers to manage crops, soil, fertilization and irrigation more effectively and precisely, can reduce nitrogen fertilizer input and reduce N_2O emissions. For example, intermittent irrigation in rice fields can significantly reduce CH_4 production and increase CH_4 oxidation *(Maresma et al., 2018; Hıya et al., 2020).* The development of manure management technologies, such as composting, reverse osmosis to concentrate and recover nitrogen from liquid manure for long-distance transport, maximizes the potential to recycle carbon and nitrogen from manure. In the livestock subsector, methane gas emission to the atmosphere is produced in the digestive systems of ruminant and non-ruminant animals. Carbon capture through improved pasture management could be another way to reduce GHG emissions. It is possible to reduce methane emissions in ruminant livestock production systems by controlling fermentation. Methane is a natural byproduct of hydrogen during fermentation. GHG emissions can be significantly reduced by optimizing grazing management, generating bioenergy on farms and using organic fertilizers with a low emission factor *(Harındıntwalı et al., 2021).*

Also aquaculture is a crucial food source producing 52% of the aquatic animal products consumed for people because world's population growing still. Marine aquaculture generates 37.5% of this production and 97% of the world’s seaweed harvest. Mariculture products often have lower GHG emission footprints than do the equivalent products which are farmed on land. For this reason, they can offer a climate-friendly, high-protein food source. However, sustaining low-emission seafood farming and intensifying production to meet production future demand is key to maintaining a low greenhouse gas footprint *(Jones et al., 2022).*Figure 3 shows carbon sinks and sources in marine aquaculture.

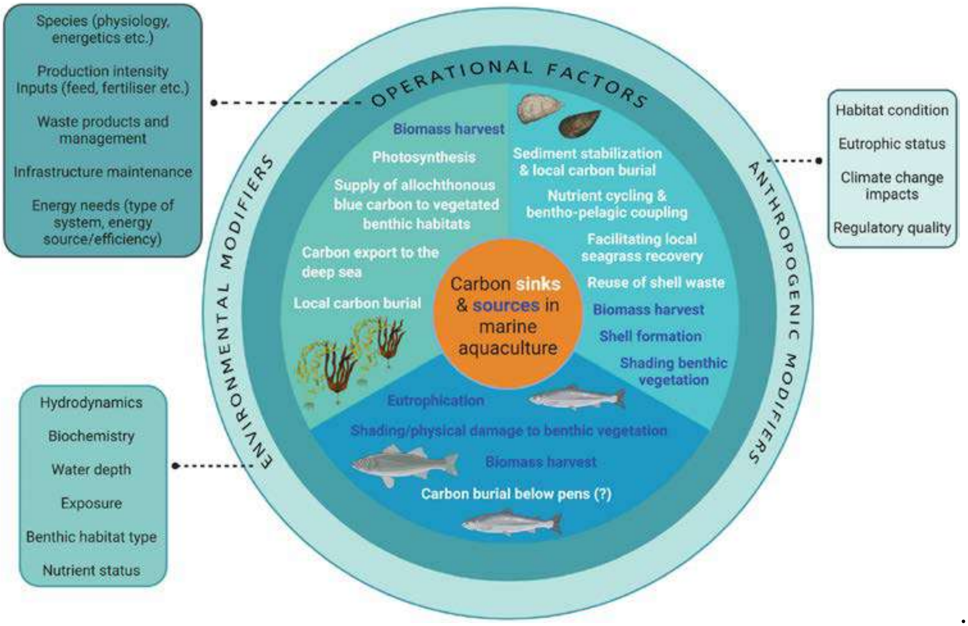

Figure 3. Carbon sinks and sources in marine aquaculture ***(Jones et al., 2022)***

Bioenergy and carbon capture with storage program

The combustible gas mixture obtained as a result of fermentation of various organic residues, especially farm manure, in an oxygen-free environment, is called biogas. Substances used to obtain biogas; grass and straw residues, urban garbage, field product residues, animal food residues, animal excrement and some plants grown for this purpose, and sea and land algae. By collecting a certain amount of them in an airless warehouse built in accordance with the technique, it is possible for chemical reactions to occur at certain stages and for the flammable gas mixture to be released. For this reason, such organic materials used to produce converted energy are called biomass in a general term and the energy obtained from them is called biomass energy. The most important of the biomass energy sources; animal manure. During the biomass application, organic substances, which are energy sources through photosynthesis, are synthesized and the oxygen necessary for the respiration of all living things is given to the atmosphere. Since the carbon dioxide produced as a result of the burning of the organic materials produced was previously taken from the atmosphere during the formation of these materials, the environment will be protected in terms of CO_2 emissions during the production of energy from biomass *(Herrero et al., 2013; Antar et al., 2021).*

As a result of the processing of biomass in biogas plants, basically 3 types of products are obtained: electricity, heat and organic fertilizer. Produced biogas; converted to electricity by means of gas engines, gas turbine, steam turbine. The electricity produced can be used by supplying it to the grid or using it for domestic consumption. Heat generation can be achieved by recovering heat during electricity generation or by burning it in gas-fired boilers for direct heat generation. Said heat can be used to provide the heat needed by the biogas plant and to dry the fertilizer to be produced. Solid and liquid fermented organic fertilizers will be produced after the materials to be digested in the biogas plant complete their economic life in the plant *(Herrero et al., 2013; Antar et al., 2021).*

Bioenergy and carbon capture with storage program would generate energy by converting vast amounts of biomass into liquid biofuels or directly burning biomass at appropriately equipped power stations. Feedstock including energy derived from woody biomass harvested from forests, including fuel wood, charcoal, and residues; energy crops, such as jatropha and palm; food crops, including corn, sweet sorghum, and annual crops such as switchgrass; agro-industrial and municipal solid wastes, other biological resources and also agro residues (animal manure and crop residues), are sources of bioenergy. İf the bioenergy system is paired with a carbon capture method, carbon emissions could be captured at the combustion source. After all process is finished carbon must be transported and then it is injected into the storage area which is thought suitable. Moreover, carbon dioxide is used for other purposes such as enhanced oil recovery, biochemical conversion into biofuels, or for producing hydrogen, and for energy storage technologies *(Tolun, 2023).*

Conclusion

Carbon capture, use and storage technologies will play an important role in achieving the "net zero" CO_2 emission target. Forest ecosystems and oceans are affect the atmospheric GHG composition and climate by contributing to the release and sequestration of CO_2, CH_4 and N_2O.

Agriculture is regarded as a net emitter of GHG, but sequesters huge amounts of carbon in soils, bioenergy substrates, and food products. Livestock production can result in CH_4 emissions from enteric fermentation and both CH_4 and N_2O emissions from livestock manure management systems. Agricultural emissions are therefore substantial, but the sector’s potential to play a role in mitigating climate change is just as significant. 71% of the GHG formation caused by food technologies consists of agricultural products and livestock production systems and land use change activities. The use of multi-sensor drone technology, better crop systems, fertilization and irrigation practices, and the use of advanced digital farming technologies that allow farmers to manage crops, soil, fertilization, and irrigation more effectively and precisely, can reduce nitrogen fertilizer input and reduce N_2O emissions. The development of manure management technologies, such as composting, reverse osmosis to concentrate and recover nitrogen from liquid manure for long-distance transport, maximizes the potential to recycle carbon and nitrogen from manure. In the livestock subsector, methane gas emission to the atmosphere is produced in the digestive systems of ruminant and non-ruminant animals. Carbon capture through improved pasture management could be another way to reduce GHG emissions. It is possible to reduce methane emissions in ruminant livestock production systems by controlling fermentation. GHG emissions can be significantly reduced by optimizing grazing management, generating bioenergy on farms, and using organic fertilizers with a low emission factor.

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DETERMINATION OF NEW WELFARE AND STRESS INDICATORS OF THE ANIMALS ON CATTLE AND PIG FARMS BASED ON DIFFERENT PUBLICATIONS

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Abstract: In recent years, numerous plans and programs, instructions, recommendations, scientific opinions, analyses, reports, best practices, regulations, codes of practices and assurance schemes have been published in publications which were not published in journals and symposiums proceedings that consider indicators of welfare and stress of the animals on cattle and pig farms intending to improve their health and productivity. These indicators were created mainly as results of research in numerous national and international projects. Mentioned projects consider key indicators and prescribe on-farm assessments of animal welfare and stress in cattle and pig farms. In the assessments of the welfare and stress of the animals in different systems of keeping and accommodation, the need to determine new welfare and stress indicators on cattle and pig farms was observed. The publications about plans and programs, instructions, recommendations, scientific opinions, analysis, reports, best practices, regulations, code of practices and assurance schemes related to animal welfare and stress contain numerous indicators. The analysis of these publications aims to determine the main characteristics of the existing and to generate ideas to define new welfare and stress indicators of the animals on cattle and pig farms.

Key words: cattle, pig, welfare indicators, stress indicators, publications

Introduction

Numerous scientific projects and publications such as monographs, textbooks and doctoral dissertations have considered the welfare and stress of cattle and pigs in different production systems. Based on the tangible results of those projects and detail descriptions in publications in recent years, numerous plans and programs, instructions, recommendations, scientific opinions, analyses, reports, best practices, regulations, codes of practices and assurance schemes for farmers, veterinary and animal science experts, as well as for policymakers were defined. Also, numerous regulations and guides have been published describing the abovementioned aspects of cattle and pig welfare and stress.

Mentioned projects and related publications consider key indicators and prescribe on-farm assessments of animal welfare and stress in cattle and pig farms. Although numerous indicators were identified in the assessments of the welfare and stress of the animals in different systems of rearing and accommodation, the need to determine new welfare and stress indicators on cattle and pig farms was observed. The paper analyses the most important publications, which were not published in journals and symposium proceedings, in which the main characteristics of the existing and generate ideas to define new welfare and stress indicators of the animals on cattle and pig farms are determined.

Cattle and pig welfare law regulations

In the publication by *Hild and Schweitzer (2019)* as editors of Animal Welfare: From Science to Law, detailed information on animal welfare and stress as taken into account by law around the world (acts, directives, regulations, etc.), recommendations, scientific opinions, plans and programs and other aspects of animal welfare and stress are presented in various topics. In the same publication, the European Union legislation on animal welfare by *Simonin and Gavinelli (2019)* is presented. An overview of laws and regulations on animal welfare in our country can be found in the publication "Ten Years of the Law on Animal Welfare of the Republic of Serbia" by *Stojanović (2019).* A prominent place in these publications in this regard is the mentioned aspects related to the welfare of cattle and the welfare of pigs. They contain the principles and concepts for defining regulations, as well as the provisions of welfare regulations that serve as a basis for defining reliable indicators.

In Council directives (1. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes were adopted for the welfare of pigs and cattle; 2. Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves; 3. Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs) and Council regulations (1. Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC, 2. Regulation (EC)

No 1255/976 and 3. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing) contain direct or indirect indicators of the welfare of different categories of cattle and pigs.

Projects related to the welfare and stress of cattle and pigs

In our country, two significant projects were realized in connection with the generation of ideas, principles and concepts for defining the new welfare indicators of cattle and pigs which the project managers were the authors of this paper. In the first project *(Projekt 26/13)*, in 2007 and 2008, the aspects of reviewing and implementing minimum standards of growing conditions and welfare on ruminant farms were considered in this regard. In the second project *(Projekt TR 20110)*, from 2008 to 2011), aspects of the development and implementation of welfare and biosecurity standards to improve the technology of cattle and pig production were discussed. In addition, as members of the MC, the authors of this paper participated in two COST actions. In the first *COST Action CA15134*, from 2016 to 2020, the relevant aspects of synergy for preventing damaging behaviour in group-housed pigs and chickens were considered, and in the second *COST Action CA21124*, which is ongoing (from 2022 to 2026), the aspects lifting farm animal lives – laying the foundations for positive animal welfare. At the international level, the Welfare Quality® project *(Canali and Keeling, 2009)* is of great importance for the consideration of welfare indicators. The numerous categories of indicators listed in these projects can mainly be divided into animal-based, resource-based and management-based.

Textbooks and monographs related to the welfare and stress of cattle and pigs

In the monograph on the stress of domestic animals *(Hristov and Bešlin, 1991)*, indicators of welfare and stress related to the health of animals, productivity, physiological changes in organisms and biochemical changes in the blood of animals, normal and abnormal behaviour, the relationship between the intensity of rearing and behaviour, experimental causing stress in animals, opportunities to choose the environment and behaviour of the animals in a specific environment are described. The genetics of stress syndrome is described in the monograph by *Stanković et al. (1992)*.

The chapters of the monographs listed as references in this paper describe the growing conditions, welfare and health care of animals in organic livestock production *(Hristov and Relić, 2005)*, microclimatic and hygienic conditions for raising cattle *(Hristov et al., 2006)* and the welfare of dairy cattle on farms *(Hristov* *and Stanković, 2016).* The textbooks listed as references in this paper describe farming conditions, stress and welfare of farm animals *(Hristov, 2002),* health care of domestic and farmed animals *(Hristov and Stanković, 2021)* and behaviour, welfare and protection of animals *(Vučinić, 2006).* The textbooks by *Webster (2005), Vučinić (2006)* and *Broom and Fraser (2015)* are particularly important, which discuss domestic animal behaviour, stress and welfare along with numerous aspects of determining welfare and stress indicators in different categories of cattle and pigs.

Doctoral dissertations related to the welfare and stress of cattle and pigs

The realized doctoral dissertations of the authors of the paper as candidates or mentors to doctoral students in our country represent a special contribution to deepening the understanding of indicators of welfare in cattle and pigs. In this sense, important are the doctoral dissertations on the influence of the way of keeping and some physiological conditions on the blood parameters of heifers and cows *(Hristov, 1992),* the assessment of the quality of welfare on dairy cow farms *(Ostojić - Andrić, 2013),* the influence of lameness on the milk yield of Simmental cows *(Zlatanović, 2015)* and the influence of rearing conditions and the season of birth on the welfare of calves in the first month of life *(Samolovac, 2016).* When it comes to the welfare of pigs, in the doctoral dissertation by *Živković (2023),* the influence of body weight, tryptophan levels and certain environmental factors on the behaviour and production results of piglets during the confinement period was considered.

Indicators of welfare and stress of cattle and pigs in scientific opinions and code of recommendations

It is considered that the abovementioned Council directives and regulations are out of date in certain aspects, and that is why the scientific opinion of the experts of the EFSA Panel on Animal Health and Welfare (AHAW) is needed. That is why methodological guidance for the development of animal welfare mandates in the context of the Farm to Fork Strategy was defined by the *EFSA AHAW Panel (2022).* In this methodology, the importance of animal-based measures (ABMs) is particularly emphasized.

In scientific opinion *(EFSA, 2022)* experts EFSA Panel on Animal Health and Welfare (AHAW) focused on the welfare of all categories of pigs on farms based on numerous literature data and expert opinions. The most relevant husbandry systems used in Europe were taken into account. For each system, highly relevant welfare consequences were identified, as well as related ABMs, and hazards leading to the welfare and stress consequences. In addition to ABMs, resource-based and management-based indicators were considered in terms of mutual conditionality.

The overall effects of farming systems on dairy cow welfare and disease, in which the indicators used for assessment of welfare and disease are listed by the experts of the AHAW panel *(EFSA, 2009a)* were described. In the same year *(EFSA, 2009b)* the scientific opinion on the risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on behaviour, fear and pain problems in dairy cows was discussed in detail with relevant indicators. This was followed by a scientific opinion *(EFSA, 2009c, 2009d)* on the risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on metabolic and reproductive problems in dairy cows and the risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on leg and locomotion problems in dairy cows with associated indicators. When it comes to indicators, the scientific opinion on the use of animal-based measures to assess the welfare of dairy cows *(EFSA, 2012)* is of particular importance.

In considering the welfare of calves, fifteen highly relevant welfare consequences were identified by the AHAW panel of *EFSA (2023)*, the most significant of which are respiratory disorders, inability to perform exploratory or foraging behaviour, gastro-enteric disorders and group stress. In this regard, adequate indicators for assessing the welfare and stress of calves are proposed. Also, recommendations are given to improve the welfare of calves, which include increasing space allowance, keeping calves in stable groups from an early age, ensuring good colostrum management and increasing the amount of milk fed to dairy calves.

The publication Code of Recommendations for the Welfare of Livestock: Cattle *(DEFRA, 2003)* lists numerous welfare and stress indicators concerning stockmanship, health, feed, water and other substances, accommodation, equipment, management, fire and other emergency precautions, pregnancy and calving, calf rearing, breeding animals and dairy cows, which are applied in England. Recommendations and indicators to the breeders of these animals are described in detail, which relate to procedures with animals, preservation of health, provision of water, food and other substances, housing and keeping, equipment, organization and production technology. In a similar principle, the indicators and recommendations for pigs are defined in the Code of Practice for the Welfare of Pigs *(DEFRA, 2020)*, which is applied in England, in two sections (section 1 – Recommendations applying to all pigs and Section 2 – Additional specific recommendations).

Welfare and stress monitoring systems

Different welfare monitoring systems have been developed in some European countries such as the animal welfare index TGI35L in Austria *(Bartussek, 2000),* TGI200 in Germany *(Sundrum, 1994)* for organic farms or specific tools for dairy cows, in France for assessing welfare in loose housed dairy cows *(Capdeville and Veissier, 2001)* and for assessing welfare quality in cattle, pigs and chickens *(Keeling and Veissier, 2005).*

In the guides concerning the welfare standards of cattle on farms *(Hristov et al., 2011a)* and the welfare standards of pigs on farms *(Hristov et al., 2011b)* in our country, questionnaires, as monitoring systems, containing indicators and criteria for their assessment were created. In essence, a total of 15 indicators were created with parameters and criteria for evaluation: 1. assessment of planning, organization and implementation of welfare protection, 2. assessment of employees concerning welfare protection, 3. competencies of employees concerning welfare protection, 4. the attitude of breeders according to the needs of animals, 5. assessment of monitoring and inspection of animals and equipment, 6. treatment of animals, 7. nutrition and feeding, 8. housing conditions, 9. microclimatic conditions, 10. hygienic conditions in the barn, 11. hygiene and body care of animals, 12. reproduction, 13. productivity, 14. behaviour and 15. health condition.

Analysis of the animal welfare and stress indicators in publications

In the mentioned publications in the paper, there are numerous physiological, behavioural and production indicators. When it comes to physiological indicators, it should be borne in mind that a change in the animal's welfare status causes physiological and psychological changes in the animal's organism, as well as changes in behaviour. That is why indicators of changes in the physiological status of the organism are taken into account as one of the groups of welfare and stress indicators. It is a well-known fact that when the animal's welfare status changes, its behaviour indicators also change. Behavioural indicators are indicators of the mental and emotional status of the animal. When the welfare status of the animal changes, there are also changes in its production characteristics. Therefore, changes in production characteristics can serve as an indicator of animal welfare and stress *(Hristov and Bešlin, 1991; Webster, 2005; Vučinić, 2006; Broom and Fraser, 2015).*

All the analysed animal welfare and stress indicators in this paper can be classified into two groups according to their impact on the animal welfare status, namely: 1. indicators originating from the external environment or the animal itself and 2. indicators of the animal organism that reflect the action of external and internal environmental factors.

The first group of indicators indicate the influence of the living environment on the welfare and stress of animals and includes the way the animal is housed, the degree of freedom of movement, the degree of contact with animals of the same species, the use of bedding materials, microclimatic and macroclimatic factors that affect the animal, the method and quality of nutrition and feeding, etc. In addition, the attitude of the breeder towards the animals is also included, as well as factors originating from the animals themselves. The breeder's attitude towards animals implies the expertise of the breeder for a certain type of animal exploitation or a certain type of livestock production, the breeder's feelings towards animals, the breeder's ability to recognize the welfare and change in the animal's welfare status, understanding and valuing the life of animals, etc. Factors originating from the animal itself include the genetic predisposition of the animal for a certain type of exploitation or production, the use of an appropriate breed of animal for a certain type of production or another way of exploitation, the use of animals of the appropriate age, etc *(Hristov and Bešlin, 1991; Hristov, 2002; Webster, 2005; Vučinić, 2006; Broom and Fraser, 2015; Hristov and Stanković, 2016).*

The second group of welfare and stress indicators is viewed through physiological, behavioural and production indicators. Physiological indicators include the physiological status of the organism, the presence or absence of clinically manifest diseases, the presence or absence of wounds and injuries, nutritional status, physical condition, etc. Behavioural indicators include physiological forms of behaviour (intake of food and water, body hygiene, exploratory behaviour, territorial behaviour, social interactions, reproductive behaviour, rest and sleep, etc.), changes in behaviour, behavioural disorders and pathological forms of behaviour. Production indicators mean the physiological level of production that corresponds to the species and breed standard or age and production category, as well as changes in the animal's productivity level *(Hristov and Bešlin, 1991; Hristov, 2002; Webster, 2005; Vučinić, 2006; Broom and Fraser, 2015; Hristov and Stanković, 2016).*

Conclusion

Based on the data presented in the paper on the determination of new welfare and stress indicators of the animals on cattle and pig farms contained in different publications, which were not published in journals and symposium proceedings, it can be concluded:

- In the mentioned publications, there are numerous new physiological, behavioural and production indicators related to stress and welfare of cattle and pigs.
- Analysis of publications, that were not published in journals and symposium proceedings, indicates that they contain numerous new direct and indirect welfare and stress indicators of cattle and pigs.
- All the analyzed new animal welfare and stress indicators of cattle and pigs can be classified into two groups according to their impact on the animal welfare and stress status: 1. indicators originating from the external environment or the animal itself and 2. indicators of the animal organism that reflects the action of external and internal environmental factors.
- In the mentioned publications in the paper, there are numerous new physiological, behavioural and production indicators which can be used to assess cattle and pigs welfare and stress.

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DETERMINATION OF NEW BIOSECURITY INDICATORS ON CATTLE AND PIG FARMS BASED ON DIFFERENT PUBLICATIONS

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Abstract: In recent years, numerous plans and programs, instructions, recommendations, scientific opinions, analysis, reports, best practices, regulations and other type of publications have been published in publications, other than in journals and symposiums proceedings that consider indicators of biosecurity on cattle and pig farms in order to improve their health and productivity. These indicators were created mainly as results of research in numerous national and international projects, which consider key indicators and prescribe on-farm assessments of biosecurity on cattle and pig farms. In the assessments of the biosecurity level in different systems of rearing and accommodation on cattle and pig farms, the need to determine indicators was observed. The publication about plans and programs, instructions, recommendations, scientific opinions, analysis, reports, best practices and regulations, related to biosecurity level contain numerous indicators. The analysis of these publications aims to determine the main characteristics of the existing and to generate ideas to define new biosecurity indicators of the animals on cattle and pig farms.

Key words: cattle, pig, biosecurity indicators, publications

Introduction

When considering farm level biosecurity, it is useful to think simultaneously about three related concepts: biosecurity planning, Hazard Analysis at Critical Control Points (HACCP), and risk management. Biosecurity plans are used to prevention certain negative events (*Uhlenhoop, 2007*), and undertaken biosecurity measures were presented by *Hristov et al. (2007)*, as well as the most significant failures in biosecurity by *Stanković and Hristov (2009*).

According to *Dickerson (2019)*, who was measuring success in biosafety and biosecurity activities in laboratories, there is a lack reliable data on the scope of community (number and types of laboratories, number of personnel "at risk”, activities conducted in labs, etc.), the effect of contemporary laboratory manipulations and technological innovations on biorisks, enable measurement of the effectiveness of control measures, on incidents and near-misses, understanding of the absolute and relative impacts of various engineering, administrative, and operational interventions on biosafety and biosecurity, and lack of standardized performance indicators and metrics, reliance on counting number of incidents to gauge "success". Finally, there are no data on the impact of human behaviours on the effectiveness of any biorisk management systems, including livestock farms. This is not only applicable to the laboratories, but in any of production plants, including livestock farms.

Definition of term indicator and its desirable traits

Generally, an indicator is a specific, observable and measurable characteristic that can be used to show changes or progress a programme is making toward achieving a specific outcome. There should be at least one indicator for each outcome. The indicator should be focused, clear and specific *(ANON, 2010)*. An indicator should be defined in precise, clear-cut terms that describe clearly and exactly what is being measured. The indicator should describe the data required and the population among whom the indicator is measured, but they do not specify a particular level of achievement – terms “improved”, “increased”, or “decreased” do not belong in an indicator. Indicators provide both qualitative and quantitative data which offers a simple and consistent approach to monitor, measure and determine performance and achieve accountability *(Kusek and Rist, 2004; Tengan et al., 2021)*. *Gudda (2011)* also outlines the CREAM criteria: all indicators should be Clear, Relevant, Economic, Adequate and Monitorable. Good indicators have to be *valid* (accurate measure of a behaviour, practice, task that is the expected after the intervention), *reliable* (consistently measurable over time, but not subjective), *precise* (defined in clear terms), *measurable* (quantifiable by available tools and methods), *timely* (provides a measurement at time intervals relevant), *programmatically important* (achieving the programme objective), according to *Gage and Dunn, (2009)*. In addition, indicators should be SMART, which means Specific, Measurable, Achievable, Relevant, and Time-bound. They should be clearly defined, measurable, and achievable within a reasonable timeframe, relevant to the goals and objectives of the program or project, and have a set timeframe for measurement *(ANON, 2023).*

Therefore, survey of biosecurity indicators has to be performed no less than periodically, in order to obtain more adequate, more reliable and more precise ones.

The types of indicators

Basically, there two types of indicator that may be applied in biosecurity level or biorisck level assessment *(ANON, 2010; ANON, 2023a)*. *Quantitative indicators* tell if the activities are taking place as it was planned, but do not provide any information on their effect or impact. *Qualitative indicators*, on the other hand, are usually concerned with outcome, providing information on changes caused by the undertaken activities. Unlike quantitative indicators which are in numeric forms, qualitative indicators are non-numeric and help determine the level of progress towards the achievement of objectives. It is, therefore, necessary to monitor both process and impact of undertaken activities.

In addition, according their traits, the indicators may be described as *(ANON, 2023b)*: *input indicators* (measuring the resources used, such as the amount of funding, staff time, or materials), *output indicators* (measuring the direct results or products of a program or project or set of measures undertaken), *outcome indicators* (measuring the changes or impacts that result from a activity, such as improvements in health or income), *process indicators* (measuring how well a set of measures is being implemented, such as the quality of services provided, the timeliness of delivery, or the level of stakeholder engagement), *impact indicators* (long-term, enduring effects of a programme or project on a population or environment can be measured, as specific sort of performance indicator), *efficiency indicators* (measuring the cost-effectiveness of a program or project, such as the ratio of resources invested to results achieved), *effectiveness indicators* (measuring the extent to which the set of measures is achieving its objectives), *quality indicators* (measuring the quality of program or project delivery, such as the satisfaction levels of beneficiaries), and *sustainability indicators* (measuring the potential for undertaken measures, program or project to continue after external support has ended).

Previously stated facts indicate what a good indicator of the level of biosecurity or biorisk on a livestock farm should be like. In summary, when measuring or assessing achieved biosecurity level in certain moment of time on certain farm, there should be at least one indicator for one trait or outcome or result, which has to be focused, clear and specific, and precisely and unambiguously defined.

Achievements in farm biosecurity assessment

When analyzing biosecurity plans and programs, instructions, recommendations, scientific opinions, analysis, reports, best practices, regulations and other type of publications, it is clear that farm biosecurity level assessment is based on certain components of biosecurity, such as isolation, traffic control and sanitation *(Buchman et al., 2007)*, or pillars of biosecurity, that is physical security, personnel management, material control and accountability, transport security and information security *(ANON, 2012)*, more or less defined in detail.

The inability to measure accurately and reproducibly the biosecurity and hygiene status of farms has long been one of the main obstacles in the pursuit of improvements in both. If farm managers need to be motivated to enhance the biosecurity or hygiene status of their farm, it is essential to provide them with quantitative goals and benchmarks, which can be used to position the farm with respect to its biosecurity and hygiene status, so that the measures required for improvements can be identified and their impact subsequently measured, if possible quantitatively *(Dewulf and Van Immerseel, 2018)*.

The systems that have been designed for making inventories of biosecurity measures taken in animal production are mostly developed as checklists or as manuals either by independent advisory organisations, or as support material for vaccines, such as COMBAT system (Boehringer Ingelheim) that helps to identify biosecurity hazards in relation to the PRRS infections in pig production, and many of these systems were developed with a view to controlling a specific disease, Wageningen University checklist developed for the risks factors and introduction and spread of *Streptococcus suis* in herds *(Wageningen University, 2008)* and PADRAP system designed by the *American Association of Swine Veterinarians (2007)* and Iowa State University (*Holtkamp et al., 2010*), that evaluates the biosecurity protocols for breeding or growing pig herds and identifying possible risk factors for PRRSV infection.

EFSA created specific documents for specific transmissible diseases, such as classical swine fever (*2009*) and avian influenza (*2016*). Experts identified and ranked a set of biosecurity measures against avian influenza that can be implemented in different areas of a farm that are classified as high or low risk – such as, respectively, a poultry house or places where feed is stored. These measures include preventing contact between wild birds and poultry, indoor housing of birds, and keeping geese and ducks separate from other poultry. EFSA recommends the development of biosecurity guidance tailored to the needs of individual farms, preferably before an outbreak. EFSA also scrutinized issues related to classical swine fever in wild boars, regarding detection, prevention, control and eradication, which can help to define certain usable indicators in establishing required biosecurity level on farms or hunting grounds. Taking into account nature of mentioned diseases, active surveillance and early detection is considered to be crucial in their successful control.

Terrestrial Animal Health Code (2022) created by OIE, may be useful in designing new biosecurity indicators; for instance, chapter 1.4. points out importance of wildlife as reservoir and indicator of risk for human population and domestic animals, and chapter 2.1. provides recommendations and principles for conducting transparent, objective and defensible risk analyses for international trade. The components of risk analysis are 1. hazard identification, 2. risk assessment, 3. risk management, and 4. risk communication, all using biosecurity indicators to recognize, evaluate and manage risks (Figure 1.).

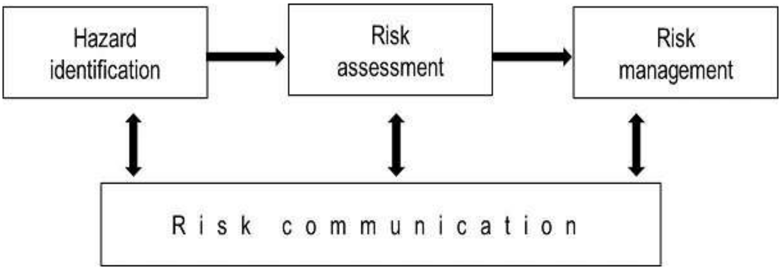

Figure 1. The four components of risk analysis

National veterinary authorities generally published fact sheets on the principles of biosecurity, in order to reduce the risk of introduction and spread of classical swine fever for instance *(DEFRA, 2007)*; in Australia, information on biosecurity is available for stakeholders *via* the *Australian Pork Industry (2003)*, and pig producers manuals for that include an internal audit system called APIQ√® which stands for Australian Pork Industry Quality Assurance Programme (www.apiq.com.au), for producers to adopt and apply good farming management, animal welfare, food safety, biosecurity and traceability practices.

The Biocheck.UGent™ biosecurity risk-based scoring system for quantification on-farm biosecurity was developed at Ghent University, available for use in pig, poultry, beef and veal farms *(Ghent University, 2015)*. It has general approach to biosecurity, focusing on paths of transmission of many types of transmissible diseases. Questionnaires for pig production include 109 (pig) mainly di- or trichotomous questions in several subcategories (2 to 19 questions each) for internal and external biosecurity, and weight factor for each subcategory and question *(Laanen et al., 2013)*. The final score for both internal and external biosecurity range from 0 to 100 points. Finally, the results are presented in a report and spider diagram, allowing evaluation of the strong and weak points of the biosecurity on a particular farm, providing guidelines for improvements.

Similar to mentioned Biocheck.UGent™, in 2011, the Ministry of Agriculture, Water Management and the Forestry of the Republic of Serbia financed the development of Guidances of Biosecurity Standards on cattle, pig and poultry farms (2011), and the Questionary for farm biosecurity assessment within, related to numerous indicators, which was developed in TR project 20110 "Welfare and Biosecurity Standards Development and Implementation in Improvement of Dairy and Pork Production" (2008-2011), and financed by Ministry of Science and Technology Development of Republic of Serbia *(Hristov and Stanković, 2009)*. Each indicator with different numbers of parameters within, is rated from grade 0 to 5: *Insufficient, without the potential to improve the biosecurity in the foreseeable future* – 0; *Insufficient, with the potential to improve the biosecurity in the foreseeable future* – 1; *Sufficient* – 2; *Good* – 3; *Very good* – 4 and *Excellent* – 5, and summarized. In addition, a SWOT analysis (Strengths, Weaknesses, Opportunities, and Threats) is applied for a more detailed overview of the possibilities for reducing the negative and improving the positive aspects of biosecurity on farms and completing the final audit. All of the 15 indicators have to be analyzed in order to find the biggest threat to biosecurity on the farm to overcome the disadvantages, risks that may hinder or prevent the overcoming disadvantages. The farm is then graded according to a rating scale: Group V 0-1.99 insufficient, Group IV 2.00-2.49 sufficient, Group III 2.5-3.49 good, Group II 3.5 - 4.49 very good and group 4.5 - 5.00 excellent. The indicators which are used are: 1. planning and monitoring the implementation of biosecurity measures, 2. farm isolation, 3. quarantine, 4. health status of the farm population, 5. movement and traffic control, 6. attitude towards visitors, 7. nutrition and water supply control, 8. manure management, 9. removal of dead animals, 10. presence of other species of animals on the farm, 11. rodent population control, 12. insect population control, 13. bird control, 14. sanitation, and 15. farm's attitude towards the environment.

Comparing to Biocheck Pigs questionnaire, part A. farm characteristics, in *Hristov and Stanković* questionnaire (*2009*), the size of the farm and categories of pigs are taken into account through different indicators, but the stuff size, their experience in keeping pigs, and the age of the facilities were not taken into account. In *Hristov and Stanković* questionnaire (*2009*) "stand down" period was investigated, comparing to "pig-free period (longer than 12 hours)" in Biocheck Pigs; part E. vermin and bird control are similar to indicators 11. Rodents control, 12. insects control and 13. birds control; indicator 2. farm isolation of presented questionnaire is similar to the part F. location of the farm of the Biocheck Pigs; differences are related to the wild boars presence; The indicator 4. heard health status of the farm population is similar to the part G. disease management of Biocheck Pigs. On the other hand, Biocheck Pigs parts H. farrowing and suckling period and I. nursery unit and J. finishing unit give more detail information regarding to this issue than in the presented questionnaire, which is covered by mentioned indicator; part K. measures between compartments, working lines and use of equipment of Biocheck Pigs is covered by different indicators of presented questionnaire (*Hristov et al., 2023*).

Suggested potential farm biosecurity level indicators and their traits

Analyzing available biosecurity plans and programs, instructions, recommendations, scientific opinions, analysis, reports, best practices, regulations and other type of publications, it may be noticed an almost identical or very similar point of view of the problem and therefore similar path of measuring or assessment of biological risk or biosecurity level on livestock farms. In addition, it has to be taken into account complexity of potential indicator. Some of them are easy to answer with yes or no or more or less; other consist of several parameters, each describing certain part of the issue.

For instance, it is common and correct to assess health status of the animals on the farm using veterinary data on clinical examinations and undertaken therapy, but, since the farm biosecurity level is being assessed in one particular moment in time, it would be correct (and definitely more simple) to include certain objective and easy to see clinical signs and their prevalence in different categories of animals on particular farm. In Canadian Dairy Farm Biosecurity General Risk Assessment Questionnaire (*ANON, 2018*) are suggested abortion, lameness, mastitis, diarrhoea, pneumonia, death and culling for milking cows, and diarrhoea, pneumonia and death for calves. For same species and other ones, it would be useful to include impaired breathing, coughing, constipation, persistent vomiting, skin rash, bruising or bleeding without previous injury, and nose, eye or genitalia discharge too. All of these clinical signs should be defined and explained in order to establish thresholds and minimize subjective differences between observers. Talking about mastitis, somatic cell count limit is defined as 500,000 cells/ml of milk; nevertheless, other signs have to be included, such as changes in milk, quarters and systemic signs.

Indicator of possibility to isolate farm or production unit and prevent physical breakthrough of vectors is often limited on perimeter and gate under control and should be supplemented with additional parameters. *Hristov and Stanković (2009)* suggest that location of the premise in respect to and required distance from risk sources is necessary, as well as separation of clean and dirty routes for movement and supply on the farm, knowledge of dominant winds directions, and protective belt of trees and shrubs which surrounds the premises. Explaining term ‘bioexclusion’, *Torremorell (2021)* in Merck Veterinary Manual point out that a systematic approach is required to prevent pathogen movement across protection zones, which are physical or imaginary barriers between farm sections, so as to eliminate or decrease the number of disease-causing organisms within the animal's environment. Sound epidemiologic principles should be used to establish zone boundaries while making use of existing physical/geographic barriers.

In Article 2.1.4. Risk assessment steps in Chapter 2.1. Import risk analysis of *Terrestrial Animal Health Code (OIE, 2022)* are presented all required steps of risk assessment regarding introducing new animals in the country, which can also be applied to the introduction of animals into an area, farm or pasture. These steps are 1. entry assessment, 2. exposure assessment, 3. consequence assessment, and 4. risk estimation, and any of these steps could be and are used separately for several other biosecurity issues, such as visitors policy or other animals on the premise, *e.g.* Nevertheless, risk of introducing newly acquired animals, even with any type of quarantine measures is serious risk which have to be estimated and taken into account.

Entry assessment consists of describing the biological pathways necessary for an importation activity to introduce pathogenic agents into a particular environment, and estimating the probability of that complete process occurring, either qualitatively as description or quantitatively as a numerical values It describes the probability of the “entry” of each of the hazards (the pathogenic agents) under each specified set of conditions with respect to amounts and timing, and how these might change as a result of various actions, events or measures. Examples of the kind of inputs that may be required in the entry assessment are: *biological factors* (species, age and breed of animals, agent predilection sites and vaccination, testing, treatment and quarantine), *country factors* (incidence or prevalence, evaluation of Veterinary Services, surveillance and control programmes and zoning and compartmentalisation systems of the exporting country), and *commodity factors* (quantity of commodity to be imported, ease of contamination, effect of processing and effect of storage and transport). If the entry assessment demonstrates no significant risk, the *risk* assessment does not need to continue.

Exposure assessment consists of describing the biological pathways necessary for exposure of animals and humans in the importing country to the hazards (in this case the pathogenic agents) from a given risk source, and estimating the probability of the exposures occurring, either qualitatively (in words) or quantitatively (as a numerical estimate). The probability of exposure to the identified hazards is estimated for specified exposure conditions with respect to amounts, timing, frequency, duration of exposure, routes of exposure, such as ingestion, inhalation or insect bite, and the number, species and other characteristics of the animal and human populations exposed. Some of inputs that may be required in the exposure assessment are: *biological factors* (properties of the agent), *country factors* (presence of potential vectors, human and animal demographics and spatial distribution, customs and cultural practices, and geographical and environmental characteristics), and *commodity factors* (quantity of commodity to be imported, intended use of the imported *animals* or products and disposal practices). If the exposure assessment demonstrates no significant risk, the risk assessment may conclude at this step.

Consequence assessment consists of describing the relationship between specified exposures to a biological agent and the consequences of those exposures. A causal process should exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socio-economic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring. This estimate may be either qualitative (in words) or quantitative (a numerical estimate). Examples of consequences include: a. direct consequences (animal infection, disease and production losses and public health consequences), and b. Indirect consequences (surveillance and control costs, compensation costs, potential trade losses, and adverse consequences to the environment).

Risk estimation is integrating the results from the entry assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset, taking into account the whole of the risk pathway from hazard identified to unwanted outcome. For a quantitative assessment, the final outputs may include: estimated numbers of herds, flocks, animals or people likely to experience health impacts of various degrees of severity over time, probability distributions, confidence intervals, and other means for expressing the uncertainties in these estimates, portrayal of the variance of all model inputs, a sensitivity analysis to rank the inputs as to their contribution to the variance of the risk estimation output, and analysis of the dependence and correlation between model inputs.

When using pasture and/or hay as forage, potential weeds and pasture pests have to be identified and prevented, by checking with regional council and the advisory service for information and advice, also whether feed sourced from off-farm doesn’t contain seeds of weeds new to farm (*Dairy NZ, 2023*). Presence of toxic plants, weeds and pasture pests should be included as part of indicator nutrition or separate indicator.

The sanitation and hygiene measures and on-farm biosecurity interventions in order to prevent and control infection to address antimicrobial resistance are primarily focused on human population protection, but they are also important to public health, as they can reduce the emergence and spread of resistant bacteria to consumers, farm workers, and the surrounding farm environment. Last but not least, biosecurity interventions (*OIE,* 2019) mainly focus on farmed animals, but their effect on protecting farm workers from animal infections (other than the known zoonoses) is not always measured or it is neglected. Recognising antimicrobial resistance as a development problem, the World Bank proposed the term antimicrobial resistance-sensitive to classify interventions that indirectly impact antimicrobial resistance by reducing multiple infections concurrently and the term antimicrobial resistance-specific for interventions aiming to curb antimicrobial resistance and antimicrobial use directly (*World Bank, 2019*). In this context, both hygiene and biosecurity procedures can be antimicrobial resistance-sensitive, *e.g.*, improving access to clean water and sanitation facilities or supporting farmers to implement biosecurity measures. Both intervention types can be implemented at a system level through standard operative procedures (SOP), from which point they could influence risk factors embedded in social structures and address socioeconomic vulnerabilities. Presence of increased therapy use of antibiotics, antimicrobial resistance, the correctness, timeliness and increased use of sanitation preparations, and presence of persistent infections in farm populations might be used as quality indicator for successful sanitation procedures, especially if related SOP are not clear or followed.

When Dr Jeroen Dewulf was asked, in personal communication, for his opinion: what is the most important in establishing required biosecurity level on farm, he said that the most important thing is a good biosecurity plan, and that is truth. Similarly, in assessing farm biosecurity, besides well chosen and designed indicators, one of the most important things is systematically created questionnaire. The questions can and should lean on each other and, if necessary, partially intersect, which gives a clear and detailed picture of the situation on the farm. It is very demanding to create modular and systematic questionnaire which would give precise description of biosecurity level of particular farm, but when achieved, mentioned traits enables adjustment and increased usability of such questionnaire.

Conclusion

Presented data of available biosecurity plans and programs, instructions, recommendations, scientific opinions, analysis, reports, best practices, regulations and other type of publications indicate what a good indicator of the level of biosecurity or biorisk on a livestock farm should be like.

It may be noticed complexity of potential indicators and high similarities point of view of the problem and therefore similar path of measuring or assessment of biological risk or biosecurity level on livestock farms.

Some of them are easy to answer with yes or no or more or less; other consist of several parameters, each describing certain part of the issue.

In summary, when measuring or assessing achieved biosecurity level in certain moment of time on certain farm, there should be at least one indicator for one trait or outcome or result, which has to be focused, clear and specific, and precisely and unambiguously defined.

Survey of biosecurity indicators is complex and has to be performed no less than periodically, in order to obtain more adequate, more reliable and more precise ones.

When assessing farm biosecurity, besides well chosen and designed indicators, systematically created questionnaire is of great importance. The questions can and should lean on each other and, if necessary, partially intersect, in order to give a clear and detailed picture of the situation on the farm. Modular and systematic questionnaire enables adjustment and increased usability of such questionnaire.

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ORGANIC POULTRY PRODUCTION: GENOTYPE CHOICE AND WELFARE

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Invited paper

Abstract: Organic poultry production has a continuous growth trend that is expected to continue in the following period. Today's consumers expect this method of production to ensure the quality of life and a high level of welfare for the reared animals, which will contribute to the quality of the products produced in this way. Since this is a relatively young and still insufficiently researched rearing system, there are a lot of unknowns and unresolved issues that slow down its faster development. A genotype that would provide optimal production results in this production system has not yet been selected, so either hybrids from conventional production or breeds with poor production characteristics are often used. In addition to the undoubted benefit that the use of the outlet brings, which is reflected in the better quality of the organic products and the greater degree of welfare and vitality of the reared poultry, the negative impact of extreme weather conditions still exists, as well as the increased risk of transmitting various diseases and parasites as well as predator attacks.

Key words: organic poultry, slow-growing genotypes, fast-growing genotypes, dual-purpose breeds, welfare, biosecurity

Introduction

The global organic poultry market will grow from $9.78 billion in 2022 to $10.34 billion in 2023 at a compound annual growth rate (CAGR) of 5.8%. The organic poultry market is expected to grow to $12.65 billion in 2027 at a CAGR of 5.2% *(The Business Research Company, 2023)*.

In European Union, 43.2% of hens are currently reared in cages, 36.2% in barns, 14% free range and 6.5% organic. Of the individual countries, Denmark (32.1%), Luxembourg (24.8%), Germany (14.1%), Austria (13.4%), Sweden

(11.7%) and France (11.2%) have the highest percentage of organic laying hens. By total number, Germany is the leading organic egg producer in Europe with 8.3 million layers, followed by France with 5.4 million layers and the Netherlands with 2.8 million organic layers *(European Commission, 2023)*.

On the other hand, 90% of broilers in the EU are reared in intensive indoor systems, around 4-5% in less-intensive indoor systems, up to 5% in free-range systems, and 1% in organic systems *(Augère-Granier, 2019)*. France leads organic chicken production in the EU, producing 14 million chickens per year, followed by Belgium with 3.6 million and Austria with 1.3 million chickens under the organic certification *(Maguregui, 2021).* The percentage of organic poultry in Serbia is less than 0.1% (*Simić, 2020*).

Genotype choice

Organic poultry production has a large number of its peculiarities and for this reason, it is significantly different from conventional. This implies that the animals selected and available for conventional rearing may not necessarily be well suited for organic, because different production systems may also require different animal traits, e.g. genes that are beneficial in one production system may not be beneficial in another *(Brunberg et al., 2014)*. *European Commission Regulation No 848/2018 (2018)* stated that "In the choice of breeds or strains, account must be taken of the capacity of animals to adapt to local conditions; their vitality, and their disease resistance. In addition, breeds or strains of animals shall be selected to avoid specific diseases or health problems associated with some breeds or strains used in intensive production. Preference is to be given to indigenous breeds and strains".

The poultry used in intensive production systems is mainly selected for high growth/laying capacity in strictly controlled environments, small opportunities to move and certain group sizes. Typical meat-type chicken grows to twice the size of a bird from 50 years ago in half the time while consuming less feed per kilo of gain. Similarly, egg-type chicken genotypes produce more eggs. This selection process resulted in birds that can provide a large quantity of meat and eggs to meet consumer demand with relatively low production costs *(Fisher, 2016)*. It is debated whether these animals are suitable for less intensive production systems, especially organic *(Rakonjac et al., 2021)*.

Choosing the appropriate genotype for organic egg production is less of a problem than for organic meat production. A large number of studies have confirmed that commercial hybrids can be reared quite successfully in an organic production system, regardless of the fact that they are selected for intensive production in closed facilities *(Sokołowicz et al., 2018; Rakonjac et al., 2021)*. Of course, a large number of challenges that are not represented in conventional production can occur here, related to reduced laying capacity, reduced egg weight, lack of methionine in the diet *(Rakonjac et al., 2018)*, as well as problems with diseases, parasites and predators *(Bonnefous et al., 2022)*. Due to these facts, dual-purpose breeds are the first choice for organic egg production. Implementing dual-purpose poultry in modern egg production is facing a challenge in exchanging the egg layer genotypes, which for many generations have been intensively bred for a high number of eggs, high feed efficiency, low bodyweight and high egg quality. These parameters are not at the same high levels in dual-purpose poultry. To implement dual-purpose genotypes in egg production, it is necessary to identify genotypes that among other production criteria have high egg qualities, which here are considered as shell strength, yolk-ratio, dry matter of egg albumen, and absence of blood and meat spots *(Hammershøj et al., 2021)*. Despite their lower productivity, these genotypes have several advantages: they are agile and can run fast, fly and roost in trees, so can escape predators, they are more resistant to bacterial and protozoan diseases and parasitic infestations than commercial layers are, their eggs are generally preferred to those from commercial birds *(Pym, 2013)*.

There are numerous differences between conventional and organic broiler farming technology, but two facts have the greatest effect on the choice of genotype for this production - the presence of an outlet (4m^2 per bird) and the minimum slaughter age (81 days). If the producers apply to these minimum slaughter ages, slow-growing genotypes must be used because commercial broilers grow too fast to be kept until 81 days. Fast-growing broilers at an older age (81 days) are very heavy and have an unbalanced body conformation as a result of intense genetic selection for additional breast muscle and body mass, which render kinetic activity more difficult and unusual. Also, active behaviours, immune responses, and thermotolerance were reduced *(Amato and Castellini, 2022)*. For this reason, many studies have recommended the use of slow-growing genotypes in organic poultry meat production. These genotypes are more adapted to "natural" environments, with a robustness that allows them to survive and reproduce constantly *(Perini et al., 2020)*. Also, slow-growing genotypes are generally preferred for their ability to cope with organic rules while maintaining successful health and welfare states *(Amato and Castellini, 2022)*. Generally, slow-growing chickens are a heterogeneous group of chickens made up of commercial strains, selected by poultry companies for outdoor farming and by local poultry breeds *(Dal Bosco et al., 2021)*. The same authors *(Dal Bosco et al., 2021)* introduce the concept of medium growing, also called the "slower growing" genotype, which emerged to indicate a subject with intermediate characteristics, both from the point of view of production and from the ability of the birds to adapt to organic production.

Slow-growing chickens currently make up only a small fraction (1-3%) of the commercially available chicken genetic stock around the world and many slow-growing genotypes are only available in Europe *(Fisher, 2016)*. In the EU, it is estimated that 2-5% of the broilers are slower-growing birds. Outside the EU, there is little demand for slower-growing birds. Three world's largest breeding companies (Broiler Breeders, Cobb-Vantress and Hubbard) indicate that slower-growing broiler products make up less than 1% of the company's turnover. For both organic and outdoor broiler production, it is expected that the market will only slightly increase *(Hiemstra and Napel, 2013*).

In Italy, big companies use both sexes of slow-growing genotypes and only the females of fast-growing genotypes in organic systems, while the males are used in intensive systems. The reason for this choice is due to the too-high body weight reached by the males at 81 days *(Mancinelli et al., 2020)*. Rearing of slow-growing genotypes, which include native breeds, is also important for maintaining biodiversity and genetic variability *(Mancinelli et al., 2021)* because some of these local breeds could have traits and genes relevant for adaptation to organic rearing system (i.e., resistance to heat stress, higher immune response, kinetic activity, and some meat characteristics) *(Dal Bosco et al., 2021)*.

However, the definition of genotype adapted to the organic system requires the measure of a wide panel of physiological and behavioural traits and not only daily weight gain. A multi-criteria analysis should be developed considering the economic, ecological, social and qualitative performance of different poultry genotypes for identifying which of them better fits with the organic system requirements *(Castellini et al., 2016).*

The welfare of reared animals

Conventional poultry production is one of the most intensive farming systems, and flocks often comprise several thousand birds reared in large compartments, with high stocking densities. Biosecurity is very high, and the indoor environment, including temperature, humidity and lighting, can be controlled meticulously *(Augère-Granier, 2019)*. From one point of view, this way of production satisfies the condition to be defined as high welfare because breeding companies consider the welfare as trouble-free production, absence of abnormalities that hamper production, low mortality and good performance in the range of customer production environments. They look at animal welfare in the context of the specific market, not on their own *(Hiemsta and Napel, 2013)*. On the other hand, organic poultry production exposes animals to natural light, natural climate conditions, and different temperatures. It is well-known that high temperatures are environmental stress factors that can badly compromise the welfare, health, and production of broilers *(Amato and Castellini, 2022)*. Also, *Gerzilov et al. (2022)* state that a higher risk of stress exists in the organic rearing system, associated with factors such as changing environmental conditions, parasitic, bacterial and viral infections, contamination, aggression from dominant birds, pecking and risk of cannibalism etc. especially, ambient temperature are being widely recognized as a main stress factor. However, consumers define the welfare of reared animals in a completely different way - priority has animals' quality of life, and positive experiences for animals *(Amato and Castellini, 2022)*. Consumers want to buy a product that is produced in a "natural way", and for such a product they are willing to pay a higher price. This contributed to the perception that the products of "happy animals" are better and healthier for human consumption *(Rakonjac et al., 2018)*. Public concerns about broiler welfare have resulted in the emergence of various ‘higher-welfare’ systems. Around 10% of the broilers in the EU are currently reared in alternative production systems *(Augère-Granier, 2019)*. These include loose housing indoor systems with e.g. lower stocking densities, slower growing hybrids and/or provision of environmental enrichment, as well as free-range systems. Organic broiler production comprises a relatively small share of the market, approximately 1%, in the EU *(Augère-Granier, 2019)*.

The main problems in free range and organic laying hen farms are uneven distribution of birds on pasture, where they use the area near the house heavily but the rest very little, feather pecking, cannibalism, high risk of infection, pod dermatitis, deformation of the keel bone, and amputated beaks. Moreover, dirty eggs and the loss of birds by predators are common *(Mahboub, 2004)*. The welfare of free-range birds may be poor during extreme winter weather as their egg production is substantially reduced. Furthermore, problems are associated with winter or windy weather conditions as free-range laying houses will cool down rapidly and relative humidity increases. Therefore the regulation of the climatic condition in the house is difficult. The low temperature in the house stimulates the bird to eat more. This will increase the feeding costs of egg production. In addition, wetting of litter in laying houses as a result of outdoor wetness by rain and snow is another problem *(Mahboub, 2004)*.

Mainly organic farming is well able to provide conditions which promote good animal welfare because this system more or less complies with the Five Freedoms (Freedom from hunger and thirst, Freedom from thermal and physical discomfort, Freedom from pain, injury and disease, Freedom to express normal behaviour and Freedom from fear and distress *(Spoolder, 2007*). Organically reared poultry have more space and the possibility to go outside. This gives the animals the possibility to perform more natural behaviour and move more, which generally should be positive for their health. The outdoor system also gives challenges. It is shown in many studies that different parasites are more common in free-range systems compared to cages *(Ferrante et al, 2009; Brunberg et al., 2014)*, as well as predation *(Ferrante et al, 2009; Bonnefous et al., 2022)*. In organic system, the environmental conditions, such as low density and access to large open spaces, should increase activity levels and improve the leg health of chickens *(Amato and Castellini, 2022)*. Generally, the good health of organic poultry should be maintained, preferably with preventive measures through the choice of breed, good management, and feed and flock sizes *(Brunberg et al., 2014)*.

From the results of numerous authors who have dealt with this topic, it can be said that the choice of genotype is crucial in terms of the welfare of organic-reared poultry. *Kalmendal and Bessei (2012)* and *Goransson (2022)* stated that slow-growing strains have lower mortality, less incidence of leg weakness and cardiovascular diseases, and generally show an improved welfare status in the organic rearing system. *Shim et al. (2012)* also reported higher mortality in fast-growing compared with slow-growing chickens, and 90% of this mortality was due to sudden death syndrome. Regarding broilers, there are clear differences in behaviour between fast and slow-growing genotypes. The slow-growing birds are more active, explorative and often spend more time outside *(Castellini et al., 2002; Fanatico et al., 2008)*. Using a GPS monitoring device to evaluate the outdoor activity of organic chickens, it was observed that the slow-growing birds performed more active behaviours, covering an average daily distance of 1 230 m, compared to the fast-growing birds which covered only 125 m *(Bokkers et al., 2004)*. Similar conclusions were also expressed by *Branciari et al. (2009)*.

Good pasture management is one of the most important measures in organic poultry production, and they provide birds' health and welfare. When outlets are not well managed from an environmental point of view, the pasture is scarcely taken care of (no grass and no shadow point presence) and the chickens' living space is unsafe. Several shelters should be made available to birds to make the outlet more attractive. Providing bushes and trees inside the pens could help chickens to feel safer from predators and more sheltered from the sun and bad weather; this would allow them to move further away from huts and eat and forage longer *(Sossidou et al., 2015)*.

Feather pecking and cannibalism are the two most important unwanted behaviours in poultry production. They are said to be redirected explorative/foraging behaviour and start when the animals cannot fulfil their behavioural needs *(Brunberg et al., 2014)*. Feather pecking and cannibalism may reduce the potential of organic husbandry to enhance the welfare of laying hens. Better feeding management, daily access to the free-range area and improved litter management may reduce the incidence of plumage damage and associated injurious pecking, hence enhancing the welfare of organic laying hens *(Bestman et* *al., 2017). Mahboub (2004)* established that feather pecking activity was decreased in the outside grass area compared to the outside roofed area. This may be attributed to the green food and its nutritive value that stimulate the birds to eat more grasses and perform foraging-related behaviours like scratching and pecking to be directed to the ground.

Outdoor access for broilers is associated with a lower incidence of foot pad dermatitis *(Dal Bosco et al., 2014)*. Contrary to this, *Sarica et al. (2014)* have demonstrated a negative effect on foot pad health in free-ranging chickens which is likely to be dependent on current weather and outdoor ground conditions. *Goransson (2022)* as a key effect in the occurrence of foot pad states the genotype used in organic production - fast-growing hybrids were much more prone to this occurrence. Similar conclusions are also stated by *Castellini et al. (2016)* - about 60% of the fast-growing genotype, when organically reared, had several body lesions and poor feather condition, whereas slow-growing chickens reared in the same conditions did not have footpad lesions or breast blisters. These studies suggest that the fast-growing genotypes are not suitable for organic-rearing systems. Indeed, these latter genotypes show an imbalance between skeletal and muscle mass that causes articular inflammations and metabolic disorders like myocardial infarcts and respiratory problems. The same conclusions are stated by *Meluzzi et al. (2009),* fast-growing chickens reared in the organic system exhibited many welfare problems such as a higher occurrence of footpad dermatitis and breast blisters, as well as an impaired immune response, compared with slow-growing birds.

Conclusion

Based on the results of numerous authors whose results are presented in this paper, it can be concluded:

- organic poultry production has a continuous growth trend that is expected to continue in the following period,
- an ideal organic bird that will achieve good production results in conditions that differ significantly from those of conventional production has not yet been defined and created,
- achieving welfare in organic poultry production is still a big challenge because it is not easy to harmonize the quality of life of reared birds with the problems that arise when it is not possible to control the environment.

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ASSESSMENT OF THE CURRENT STATE OF BIOSECURITY MEASURES ON BROILER CHICKEN FARMS WITH DIFFERENT CAPACITIES IN VOJVODINA

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Abstract: The broiler production faces many challenges, which can cause negative effects on their health and welfare. The great importance for farmers is to prevent disease outbreaks, and biosecurity measures are very significant. This study aimed to quantify the level of biosecurity measures in broiler farms of different capacities, using a standardized procedure, and to identify key aspects that would require improvements. The research was conducted from May to September 2022, and 15 randomly selected broiler farms participated. Five large-size (>30,000 chickens), five middle-size (10,000-30,000 chickens) and five small-size farms (<10,000 chickens) were analyzed. All farms are located in Vojvodina and farmers agreed to participate in the survey. The Biocheck.UGent scoring system (https://biocheckgent.com/en) was used to quantify biosecurity measures. The overall farm biosecurity is a weighted average of the external and internal biosecurity. Our results showed a low level of implementation of internal and external biosecurity measures on all farms (40-63%). The overall rating of biosecurity on farms was lower than the world and country's average. The results of this study suggest that the control of implemented biosecurity measures in broiler farms is very important. Most of the biosecurity risks for broiler farms originate from inappropriate site selection, purchase of day-old chicks of unknown quality, lack of procedures, and training of farm employees. This study should provide a good encouragement for the development of a biosecurity plan, identifying risks and the appropriate way to educate farm owners, as well as farm employees, on the implementation of biosecurity measures.

Key words: broilers, biosecurity measures, questionnaire, farm

Introduction

Poultry production is characterized by a huge variety of production systems with different production volumes, bird species, biosecurity measures, and production inputs and outputs *(Van Steenwinkel et al., 2011).* Broiler production is one of the main branches of animal husbandry and over 10 million tons of chicken meat are produced in the European Union in one year *(Luiken et al., 2019).* Such intensive production faces many challenges every day that can cause negative effects on the health and welfare of chickens and consequently lead to a decrease in production results, an extension of the production cycle, and poor profitability *(Butcher and Miles, 2012; De Paul Tatfo Keutchatang et al., 2021).* The risk of infectious diseases may be increased due to high population density, inadequate ventilation, and immunosuppression *(Tilli et al., 2022).* For this reason, it is of great importance for farmers to prevent disease outbreaks *(Gelaude et al., 2014),* especially if the disease is zoonotic or food-borne with potentially large consequences for public health *(Astill et al., 2018).* For many years, the prevention of the occurrence of the disease has been carried out by the preventive use of antibiotics, which led to the increasing development of antimicrobial resistance in veterinary and human medicine (*Gelaude et al., 2014; Luiken et al., 2019; Caekebeke et al., 2020; Dhaka et al., 2023).* Therefore, biosecurity measures are of key importance in the prevention of animal diseases on farms *(Gelaude et al., 2014).* Various factors are thought to play a role in a producer's decision to adopt different biosecurity measures, including farm and producer characteristics (flock size, years of experience, and risk awareness), local factors (prevalence of endemic diseases) and implementation-related factors (financial costs and ease of adoption) *(Greening et al., 2020).*

Assessment of biosecurity measures on broiler farms is carried out worldwide *(Greening et al., 2020; Ahmed et al., 2021; de Oliveira Sidinei et al., 2021; Dhaka et al., 2023).* Biosecurity measures are all those measures that aim to reduce the entry and spread of pathogens on the farm *(Ali et al., 2014).* The *World Health Organization (2010)* defined biosecurity measures as "an integrated approach for managing risks to human, animal and plant life and health". With increasing farm size and proximity to poultry houses, biosecurity has become an increasingly important measure *(Bernd et al., 2022).* The Food and Agriculture Organization of the United Nations recommends strict application of biosecurity measures as the most effective way to prevent and control the spread of the virus and prevent transmission to humans. Biosecurity is defined as all measures taken to prevent the introduction and spread of infectious agents on the farm and is a major factor influencing the occurrence of disease and the use of antimicrobial agents

(Cuc et al., 2020). Biosecurity measures are a key element in the control of endemic diseases *(Kouam et al., 2018).*

Activities in the form of restricted movement of people, quarantine and isolation of animals, animal transport protocols, fencing protocols, cleaning and disinfection of facilities are very effective in improving biosecurity. Biosecurity measures are divided into three components: isolation, traffic control, and sanitation. Isolation is a measure related to physical barriers (fences, showers, or disinfection barriers) and distance between farms, to prevent contact between sick animals and contaminated houses with the non-infected house. Traffic control refers to restrictions on the movement of feed, people, equipment, and animals on the farm *(FAO, 2008).* Sanitation refers to the cleaning and disinfection of poultry houses, agricultural machines, vehicles, and equipment *(Kouam et al., 2018).* Setting up hygienic barriers between the indoor and outdoor environment, controlling the entry of personnel, strict hygiene rules (hand washing and disinfection), and changing boots and coveralls before entering the farm have proven to be very effective measures *(Sibanda et al., 2018).* By preventing contact between poultry and infectious agents, infected and healthy birds, birds, and humans, the level of biosecurity on the farm is increased *(Ahmed et al., 2021).*

Biosecurity research on broiler farms is often based on qualitative questionnaires specifically designed for a certain disease, or focused on general biosecurity measures. To be able to objectively compare the level of biosecurity between farms or within a farm over time in a standardized way, it is necessary to quantify the level of biosecurity measures. Biosecurity measures are assessed using questionnaires (or similar tools such as checklists) in which the assessor answers several questions related to the measures applied *(Tilli et al., 2022).* Once risk factors are identified, customized health and biosecurity monitoring plans can be made for each farm *(Schreuder et al., 2023).* For this purpose, several scoring systems have been developed, and one of the most commonly used is BioCheck.UGent *(Van Limbergen et al., 2018).* Many technological recommendations have been made on biosecurity measures for large commercial farms, but these measures are not always applicable to small-size broiler farms *(Negro-Calduch et al., 2013).* For this reason, it would be very useful to analyze small-size farms as well, to establish biosecurity risks.

This study aimed to quantify the level of biosecurity measures in broiler farms of different capacities using a standardized procedure and to identify key aspects that would require improvements. In this way, in addition to large farms, the main risks would also be observed on small farms, which would contribute to the improvement of biosecurity.

Material and Methods

Study design

The research was conducted from May to September 2022. Fifteen randomly selected broiler chicken farms participated in the study. Five large-size (>30,000 chickens), five middle-size (10,000-30,000 chickens) and five small-size farms (<10,000 chickens) were analyzed. All farms are located in the province of Vojvodina and all farmers agreed to participate in the survey on biosecurity assessment. On all farms, chickens were reared on the floor. The province of Vojvodina consists of three regions (Srem, Banat, and Bačka), and poultry farming is very developed in each of them. There is a large agglomeration of broiler chicken farms. Two types of hybrids are most often present (Ross 308 and Cobb 500), and the chickens come from parent flocks raised on the territory of Serbia. Figure 1 shows a map of the province of Vojvodina with marked broiler chicken farms. All analyzed farms have previous cooperation with the Department of Epizootiology, Clinical Diagnostics, and DDD of the Scientific Veterinary Institute "Novi Sad" in the field of poultry health care.

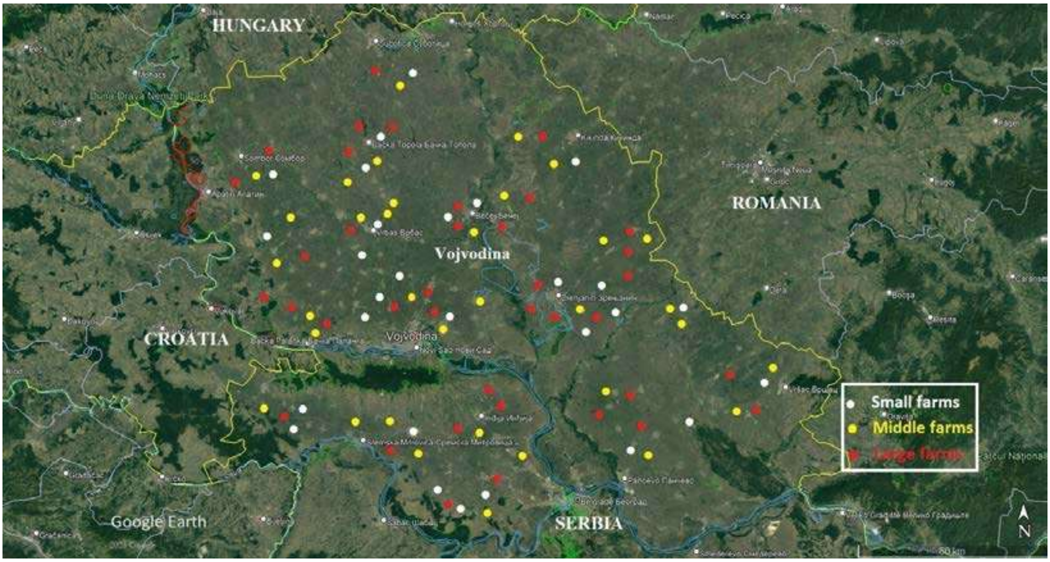


Figure 1. Map of the province of Vojvodina with marked broiler chicken farms

Biosecurity Questionnaire

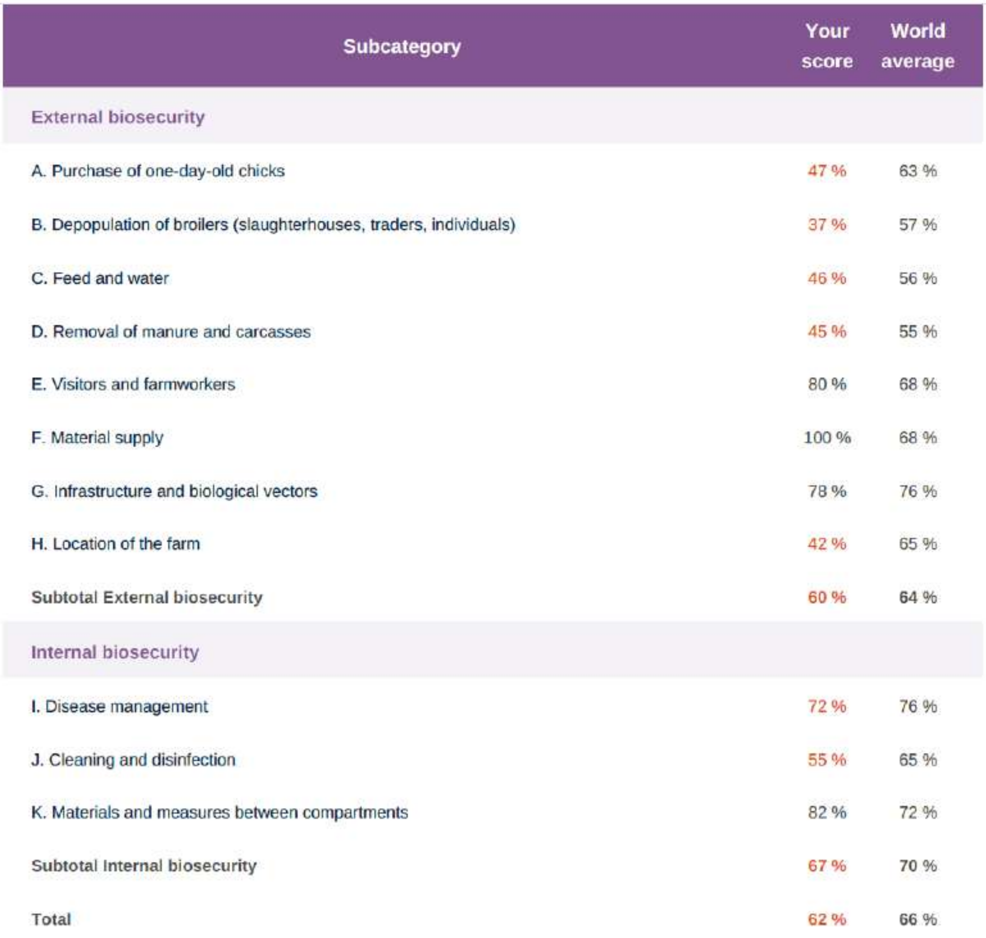
The Biocheck.UGent scoring system (https://biocheckgent.com/en) was used to quantify farm biosecurity measures according to *Tanquilut et al. (2020).* This risk-based scoring system for on-farm biosecurity quantification is credible, repeatable, and can be validated. The questionnaire aims to describe the complete status of biosecurity measures on the farm. It consists of questions about each relevant aspect of biosecurity to determine whether a preventive measure is being applied or whether a particular health problem is present or absent. The questionnaire is the result of a detailed literal study of disease transmission in poultry, from previous information obtained during the development of the BiocheckUGent tool for pigs. The literature review was performed using the existing literature on disease transmission in poultry. All possible routes of transmission are included, such as airborne, foodborne, vectorial, and environmental. Information on general biosecurity procedures was obtained from the Biocheck.UGent pig tool.

Biosecurity assessment

Assessment of the biosecurity level was carried out by filling in the BioCheck.UGent questionnaire (https://biocheckgent.com/en) on-site, after a visual assessment of the farm. The questionnaire is a risk-based scoring system that objectively estimates on-farm biosecurity, resulting in a farm-specific report that assesses external (all measures that prevent the introduction of pathogens to the farm) and internal biosecurity (all measures taken to prevent spread within the farm). It includes 97 dichotomous or trichotomous questions that are divided into several subcategories for external and internal biosecurity. Each subcategory consists of 2 to 19 questions. The overall farm biosecurity level is a weighted average of the external and internal biosecurity scores. Scores range from 0 to 100, with the last being the implementation of all biosecurity measures, indicating farmers' compliance with high biosecurity standards. To prevent interviewer bias, the questionnaire was completed during or after the farm visit.

Reporting of the Biosecurity Level

Based on the answers given in the questionnaire, the farmer receives a score between 0 and 100 for external and internal biosecurity and the corresponding subcategories. Immediately after completing the questionnaire, which takes between 20 and 30 minutes, all the results of the different subcategories are calculated online and displayed in a table together with the average results in the world (Figure 2). This allows the farmer to compare the obtained results with his colleagues. If the obtained result deviates from 100, it indicates a potential scope for improvement. The results obtained are also graphically displayed on a spider web graph (Figure 3), enabling quick visual identification of any biosecurity risks on the farm. The blue geometric figure represents the average size of external or internal biosecurity in a broiler farm (Figure 3).



Subcategory	Your score	World average
External biosecurity		
A. Purchase of one-day-old chicks	47 %	63 %
B. Depopulation of broilers (slaughterhouses, traders, individuals)	37 %	57 %
C. Feed and water	46 %	56 %
D. Removal of manure and carcasses	45 %	55 %
E. Visitors and farmworkers	80 %	68 %
F. Material supply	100 %	68 %
G. Infrastructure and biological vectors	78 %	76 %
H. Location of the farm	42 %	65 %
Subtotal External biosecurity	60 %	64 %
Internal biosecurity		
I. Disease management	72 %	76 %
J. Cleaning and disinfection	55 %	65 %
K. Materials and measures between compartments	82 %	72 %
Subtotal Internal biosecurity	67 %	70 %
Total	62 %	66 %

Figure 2. Presentation of the results of the survey on biosecurity

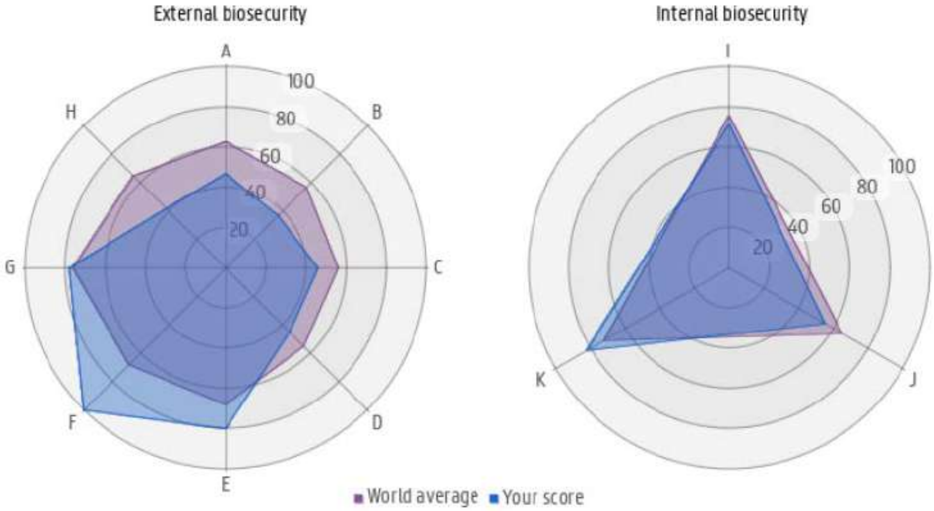


Figure 3. Graphic presentation of observed biosecurity measures on the farm

Statistical analysis

The data collected using the questionnaire were analyzed with descriptive statistics (frequency and mean value). Due to the small (limited) number of broiler farms that participated in the study and therefore the limited data set, the statistical analysis was narrowed down to descriptive statistics.

Results and Discussion

As already described in the previous section, the scoring system for biosecurity is divided into 2 categories (external and internal biosecurity) and 11 subcategories.

The results of the analysis of external and internal biosecurity measures on large-size farms are shown in Table 1. The data show that most farms implement measures at a level that is below the average in our country, and also below the world average (Table 1). The lowest overall level of biosecurity was obtained on farm III (53/100), while the best was on farm II (64/100). The overall level of biosecurity on all farms was below the world average and the average in our country (Table 1). The best level of external biosecurity was established on farm II (above the world and average of our country), and internally on farm I (still below the world and average of our country). The average values of external, internal, and total biosecurity on all farms were below the world and national level.

Table 1. Presentation of biosecurity assessment (internal and external) on large-size farms

Farms	Type	External biosecurity	Country average	World average	Internal biosecurity	Country average	World average	Total biosecurity	Country average	World average
Farm I	Large	60%	65%	64%	62%	65%	70%	62%	65%	66%
Farm II	Large	69%	65%	64%	51%	65%	70%	64%	65%	66%
Farm III	Large	58%	65%	64%	40%	65%	70%	53%	65%	66%
Farm IV	Large	62%	65%	64%	50%	65%	70%	58%	65%	66%
Farm V	Large	64%	65%	64%	40%	65%	70%	57%	65%	66%
Average		**63%**	**65%**	**64%**	**49%**	**65%**	**70%**	**59%**	**65%**	**66%**

The analysis of the biosecurity questionnaire on middle-size farms also showed a difference from the world and the average of our country (Table 2). The average level of biosecurity of all five farms is below the world and national level. The data on external biosecurity were below the world and national level, so farm X had the highest level of biosecurity (61/100), and farm VI had the lowest (26/100). Internal biosecurity was also highest on farm X (62/100) and lowest on farm VII (20/100). The average data on internal biosecurity are also below world and national levels. Overall biosecurity was highest on farm X (61/100) and lowest on farm VII (25/100).

Table 2. Presentation of biosecurity assessment (internal and external) on middle-size farms

Farms	Type	External biosecurity	Country average	World average	Internal biosecurity	Country average	World average	Total biosecurity	Country average	World average
Farm VI	Middle	26%	65%	64%	29%	65%	70%	27%	65%	66%
Farm VII	Middle	27%	65%	64%	20%	65%	70%	25%	65%	66%
Farm VIII	Middle	46%	65%	64%	53%	65%	70%	48%	65%	66%
Farm IX	Middle	39%	65%	64%	45%	65%	70%	41%	65%	66%
Farm X	Middle	61%	65%	64%	62%	65%	70%	61%	65%	66%
Average		**40%**	**65%**	**64%**	**42%**	**65%**	**70%**	**40%**	**65%**	**66%**

On small-size farms, the results of the biosecurity questionnaire showed lower level compared to the world and national average (Table 3). The highest level of biosecurity was established on farm XII (62/100), and the lowest on farm XV (38/100). The average level of total biosecurity was lower compared to the world and average of our country. Farm XII also had the highest level of internal biosecurity (72/100), which was above the world and national average. In the assessment of external biosecurity, the highest result was also observed at farm XII (57/100), however, it was below the world and average of our country.

Table 3. Presentation of biosecurity assessment (internal and external) on small-size farms

Farms	Type	External biosecurity	Country average	World average	Internal biosecurity	Country average	World average	Total biosecurity	Country average	World average
Farm XI	Small	38%	65%	64%	66%	65%	70%	46%	65%	66%
Farm XII	Small	57%	65%	64%	72%	65%	70%	62%	65%	66%
Farm XIII	Small	47%	65%	64%	53%	65%	70%	49%	65%	66%
Farm XIV	Small	39%	65%	64%	56%	65%	70%	44%	65%	66%
Farm XV	Small	36%	65%	64%	44%	65%	70%	38%	65%	66%
Average		**43%**	**65%**	**64%**	**58%**	**65%**	**70%**	**48%**	**65%**	**66%**

Although data collection using questionnaires often presents an analysis of implemented biosecurity measures in a subjective way (from the farmer's point of view), questionnaires have proven to be a useful tool for assessing biosecurity on poultry farms and a potential initiator for improving measures *(Tilli et al., 2022).* The results of our study showed a low level of implementation of internal and external biosecurity on all farms. Data on applied biosecurity measures on all three categories of farms were obtained using a quantitative tool in a standardized and reproducible way. To avoid the possibility of an unrealistic presentation of the current biosecurity, on each farm after the analysis and assessment, the examiner filled out a questionnaire. Given that in our country there is no official data on the level of applied biosecurity measures on broiler chicken farms, this study showed real differences between farms of different capacities. The Biocheck.UGent scoring system allows us to quantify biosecurity at the flock level, taking into account all relevant aspects of biosecurity *(Gelaude et al., 2014).* This scoring system can be considered a useful tool for monitoring the biosecurity level of broiler farms over time. In this way different poultry farms can be easily compared with each other and each farm can be more easily monitored when using the same scoring system. With this quantitative analysis, we study the relationship between biosecurity, health, and production characteristics, as is also carried out in pig farms *(Laanen et al., 2013).*

In large-size farms, internal biosecurity was lower than external biosecurity, which is very similar to data from pig farms *(Laanen et al., 2013).* However, the situation in the other two categories of farms was reversed. Internal biosecurity was at a higher level than external. This can be explained by the greater number of applied internal biosecurity measures, as well as the fact that these measures are influenced to the greatest degree by the grower himself *(Gelaude et al., 2014).* In our study, the level of farmers' awareness of the importance of biosecurity measures on the surveyed farms was low, mostly in the case of farms with small and middle-size. This could be explained by the fact that most of these farmers were not trained in poultry farming *(De Paul Tatfo Keutchatang et al., 2021)*. Biosecurity oversight often occurs on these farms, and they are considered high-risk. On such farms, contact with an expert (veterinarian, agricultural engineer) occurs only when a health problem appears on the farm *(De Paul Tatfo Keutchatang et al., 2021)*. Large-size farms usually have an employed veterinarian, or they engage him to monitor the health of the flock. This could be a key reason for better biosecurity measures about meddle and small-size farms *(Kouam et al., 2018).*

Biosecurity measures are not always economically acceptable, but they pay off in the long term due to disease prevention *(Dorea et al., 2010).* This includes the costs of equipment for installing biosecurity barriers as well as the time spent by farm staff during the implementation of these measures *(Sibanda et al., 2018).*

Collected data from all three categories of farms revealed deficiencies in biosecurity. Encouraging farmers and veterinarians to use biosecurity quantification tools can help identify and quantify biosecurity risks on farms. Their continued use and development can improve efficiency in dealing with biosecurity risks. For biosecurity on farms to be constantly maintained at a high level, constant communication and cooperation between farmers and experts (veterinarians, agricultural engineers) is needed, to remove all potential risks in time.

Conclusion

The findings from this study suggest that the control of implemented biosecurity measures in broiler farms of different capacities is very important. The results show that the current level of biosecurity measures is below average compared to the level of biosecurity at the global and local levels. Most of the biosecurity risks for chicken farms originate from inappropriate site selection, purchase of day-old chicks of unknown quality, and lack of procedures and training of farm employees. The effect of this research should be the development of a biosecurity plan, finding risks, and the appropriate way to educate farm owners, as well as farm employees, about the implementation of biosecurity measures.

Acknowledgment
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EXPLORING PORCINE GROWTH AND FATNESS THROUGH LIVER TRANSCRIPTOME ANALYSES IN DIFFERENT IBERIAN GENETIC BACKGROUNDS

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Invited paper

Abstract: Growth and fatness traits play a crucial role in pig production. While Quantitative Trait Loci (QTL) scans have been used to study experimental crosses between divergent breeds, differential expression analyses using RNA-Seq data from animals with contrasting traits offer additional insights into the underlying genetics and molecular mechanisms. The liver, being vital for metabolic homeostasis and involved in regulating blood sugar levels and processing dietary fats, is likely influential in the variability of these traits. Therefore, the objective of this study was to identify genes associated with pig growth and fatness traits through transcriptome analysis of the liver in two different Iberian backcrosses, using RNA-Seq technology. A total of 43 and 59 differentially expressed genes (DEGs) were found in F1 (Iberian x Pietrain) x Pietrain (BC_PI) and F1 (Iberian x Landrace) x Landrace (BC_LD), respectively, between extreme groups for growth and fatness. In BC_PI, DEGs were enriched in biological pathways mainly related to the immune system, while BC_LD showed enrichment in pathways mainly involved in cholesterol metabolism. Although the differences observed in liver were less pronounced than those found in the hypothalamus in previous studies, several promising candidate genes associated with growth and fatness traits were identified. Notably, genes such as *PPP1R13B, STK33, CXCL14, ISG20, MRC1*, and *CYP7A1* emerged as potential targets for selective breeding and genetic improvement in the livestock industry. In conclusion, this study sheds light on the genetic mechanisms underlying growth and fatness traits in pigs. However, further investigations and functional analyses are needed to fully comprehend the complex genetic pathways contributing to these traits.

Key words: pig, growth, fatness, RNA-Seq, liver

Introduction

Growth and fatness are essential traits in livestock meat production, impacting the efficiency of pig production through genetic and environmental interactions. Understanding the genetic pathways regulating these traits is crucial for optimizing animal growth and overall efficiency in the industry.

Experimental crosses between porcine breeds phenotypically extreme provide valuable insights into the genetic and molecular mechanisms underlying these traits. The use of Quantitative Trait Loci (QTL) scans has been pivotal in livestock genomic studies, unravelling the genetic architecture governing complex productive traits. For instance, the IBMAP (Iberian x Landrace) experimental cross was specifically developed to identify QTLs associated with economically important traits like growth, fatness, and carcass composition *(Perez-Enciso et al., 2000)*. Moreover, transcriptome studies that compare individuals belonging to IBMAP experimental cross with extreme phenotypes for specific traits provide complementary insights, by identifying genes that exhibit divergent expression among the groups, leading to a deeper understanding of the genetic architecture. *Perez-Montarelo et al. (2012)* conducted a differential analysis using RNA-Seq data from the hypothalamus of Iberian x Landrace backcross individuals, characterized by extreme growth and fatness traits. This analysis successfully identified strong candidate genes associated with these traits. In addition to the hypothalamus, the liver plays a crucial role in metabolic homeostasis. It regulates blood sugar levels by storing and releasing glucose as needed, and also synthesizes proteins and processes dietary fats. The liver's multifunctional role makes it a vital organ for maintaining overall metabolic balance and health in livestock species.

Therefore, the objective of the study was to identify genes associated with pig growth and fatness traits. This was achieved through transcriptome analysis of the liver in two different Iberian backcrosses, using RNA-Seq technology.

Material and Methods

In the current study, the animal material used comprised two experimental backcrosses: F1 (Iberian x Pietrain) x Pietrain (BC_PI) and F1 (Iberian x Landrace) x Landrace (BC_LD). The Iberian parental strains used in the backcrosses were selected from different Iberian strains: Guadyerbas (black hairless strain) for the Iberian x Landrace backcross, and Torbiscal (red strain) for the Iberian x Pietrain backcrosses. The BC_PI backcross was established by mating three Torbiscal boars with four Pietrain sows (F0), resulting in the production of 33 F1 animals. Subsequently, four F1 boars were bred with 33 Pietrain sows, yielding a total of 150 backcross animals. On the other hand, BC_LD was generated by mating three

Guadyerbas boars with 30 Landrace sows (F0), which produced 70 F1 animals. Following this, five F1 boars were mated with 25 Landrace sows, resulting in the production of 187 backcross animals. All pigs involved in the study were raised on an experimental farm, following standard conditions. Male pigs with extreme phenotypic values for growth and fatness were selected in each backcross through principal component analyses. The top five males for the BC_LD backcross and the top six for BC_PI with the highest values for growth and fatness indicators were selected and assigned to the High (H) groups. Conversely, the five males for the BC_LD and the six males for BC_PI with the lowest values for these traits were assigned to the Low (L) groups. For BC_PI, the mean values for their three main indicators in the L and H groups were: 77.4 - 88.4 kg of weight at 175 days, 9.6 - 13.2 mm of backfat thickness at 75 kg, and 68 - 62 % of yield of cuts from carcass. For the BC_LD, the mean value for the three main indicators were: 0.92–0.74 kg/day of average daily gain, 16.2–11.6 mm of backfat thickness, 12.6–16.7% of C18:2 in backfat, and 8.1–11.9% of C18:2 in intramuscular fat as it is described by *Pérez-Montarelo et al. (2012).*

Liver samples were collected from the 22 selected animals after slaughter. These samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis. Total RNA was extracted using RiboPure High Quality total RNA kit from Ambion (Austin, TX). The RNA samples were quantified using a NanoDrop-100 spectrophotometer from NanoDrop Technologies (Wilmington, DE) and their integrity was evaluated with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) getting RNA integrity values higher than 7.1. Fragments of 300 bp were generated, and paired-end libraries were prepared for each sample using the TruSeq RNA Sample Prep Kit v2 from Illumina (San Diego, CA). The multiplex sequencing of the libraries was performed on an Illumina Hi-Seq 2000 instrument (Fasteris, Plan-les-Ouates, Switzerland) with four and three samples per lane for BC_PI and BC_LD, respectively. This generated paired-end reads of 75 bp. The sequencing was carried out at the Centro Nacional de Análisis Genómico (CNAG).

The quality of the raw sequencing data was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). To ensure data quality, TrimGalore (http://www.bioinformatics. babraham.ac.uk/projects/ trim_galore/) was used with default settings to perform quality trimming. The filtered reads were then mapped to the pig reference genome (Sscrofa10.2) using TopHat v2.0.5 *(Trapnell et al., 2009)* with Bowtie2 v2.0.0.7. Transcripts were assembled and quantified using Cufflinks v2.0.2 *(Trapnell et al., 2012)*, with expression levels reported in FPKM (Fragments Per Kilobase of transcript per Million mapped fragments). Following the assembly phase, the Cuffdiff tool *(Trapnell et al., 2012)* from Cufflinks was employed to calculate expression values and conduct the differential expression analysis of the annotated genes and new isoforms. Genes were filtered based on the following criteria: the average mean value of FPKM per group (H and L) needed to exceed 0.5, and the fold change between the groups had to be greater than 1.5. False Discovery Rate (FDR) was considered with q-value. Finally, differentially expressed genes (DEGs) were those with a q-value < 0.05. Functional analyses of the differentially expressed genes between the H and L groups in each backcross were conducted with WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) *(Liao et al., 2019).*

Results and Discussion

A total of 738 million paired-end reads were obtained from liver transcriptome sequencing of the 12 samples of BC_PI backcross, while 1,056 million paired-end reads were obtained from the 10 samples of BC_LD backcross. The mapping efficiency across these samples ranged from 75.0% to 81.4%, indicating a significant proportion of reads successfully aligned to the pig reference genome. It is noteworthy that these percentages were higher than those reported in a previous liver study using a previous version of the genome (71.42% - 77.75%) *(Ramayo-Caldas et al., 2012)*. Using the Cufflinks tool, a total of 136,773 transcripts were found to be expressed in BC_PI, while BC_LD exhibited 133,850 expressed transcripts (Table 1). A percentage of these transcripts (6.74% and 5.94% in BC_PI and BC_LD, respectively) were found to be completely contained within a reference intron. This observation could indicate events of incorrect annotation of exons, errors and omissions in the prediction of isoforms intron retention. Furthermore, a substantial proportion of the transcripts (41.03% and 39.52%) were identified as novel isoforms, which aligns with the findings of *Muñoz et al. (2018)* who reported a similar percentage (42.46%) in the longissimus dorsi of Iberian pigs. These results indicate a lack in annotated isoforms within the porcine genome assembly.

Table 1. Classification of the transcripts identified in the liver samples from BC_PI and BC_LD backcrosses in relation to the Ensembl annotated pig genes.

Cuffcompare class	BC_PI		BC_LD	
	Transcripts, n	Transcripts %	Transcripts, n	Transcripts %
Complete match of intron	7,960	6.74	7,951	5.94
Contained in the reference	8,214	6.95	6,345	4.74
Novel isoform	48,460	41.03	52,891	39.52
Possible pre-mRNA	3,968	3.36	4,772	3.57
Exon falling into an intron	16,331	13.83	21,903	16.36
Generic overlap with a reference transcript	2,632	2.23	2,956	2.21
Polymerase fragment	2,989	2.53	2,658	1.99
Unknown	14,553	12.32	20,429	15.26
Exonic overlap opposite strand	1,000	0.85	1,500	1.12
Multiple classifications	12,014	10.17	12,444	9.30
Intron overlap opposite	1	0.00	1	0.00
Total	136,773		133,850	

In BC_PI backcross the differential expression analysis revealed 34 annotated DEGs and nine DE newly predicted isoforms. Among the total, 16 were upregulated in the H group, while the remaining 27 genes exhibited higher expression in the L group. The highest expression differences ($\log_2$FC = -3.80) were observed for the *Protein Phosphatase 1 Regulatory Subunit 13B* (*PPP1R13B*) gene which enhances the expression of proapoptotic genes and it is associated to cellular growth (Table 2). On the other hand, a total of 34 annotated DEGs and 25 DE newly predicted isoforms were observed in the BC_LD backcross. A total of 18 were upregulated in the H group and the remaining 41, were upregulated in the L group. The *Serine/Threonine-Protein Kinase 33* (*STK33*) gene showed the highest expression differences among groups ($\log_2$FC = -3.70). *STK33* is involved in mitotic DNA damage checkpoint signalling and protein autophosphorylation and associated to obesity in humans. Only two genes were in common between both backcrosses, a gene encoding for the immunoglobulin constant light chain (IGLC) and a transcript encoding for an Ig-like domain-containing protein (F1STC3) (Table 2).

Table 2. Most relevant differentially expressed genes in liver of BC_PI and BC_LD pigs

Backcross	**Gene**	**log2 (FC)**	***q-value***
BC_PI	*PPP1R13B*	-3.80	0.004
	F1STC3	-1.86	0.011
	IGLC	-1.39	0.004
	MRC1	1.32	5×10^{-4}
	ISG20	-1.26	0.001
	MZB1	-1.26	3×10^{-4}
	CXCL14	-1.22	3×10^{-4}
BC_LD	*STK33*	-3.69	1×10^{-4}
	CYP1A2	-3.69	1×10^{-4}
	CYP7A1	-2.42	1.8×10^{-3}
	CYP1A1	-1.55	0.004
	F1STC3	-1.97	0.002
	IGLC	-1.20	0.002

The expression differences observed in liver were lower compared to those observed in the hypothalamus of the same animals. In BC_PI, a total of 159 DEG and DE novel isoforms (q-value < 0.05) were identified, while in BC_LD, a total of 281 DEG and DE novel isoforms (q-value < 0.05) were detected *(Muñoz et al., 2017)*. These results suggest that the hypothalamus have a more relevant role in growth and fatness functions than liver.

A total of nine GO biological processes (GO_{BP}) and one KEGG pathway were enriched in DEGs for BC_PI (WebGestalt tool), however, none of these enrichments overcome the FDR of 5%. All of them were involved in immune system such as GO:0002376 immune system process, GO:0002702 positive regulation of production of molecular mediator of immune response or GO:0045087 innate immune response. Some of the genes involved in these processes are *CXCL14 CXC motif chemokine ligand 14* (*CXCL14*), *Interferon-stimulated gene of 20 kDa* (*ISG20*), and *mannose receptor C-type 1* (*MRC1*) all of them more expressed in the L group (Table 2). Lower expression of genes encoding proteins involved in immune response was observed in pigs with low residual feed intake (RFI) compared to those with high RFI, which are less efficient. It is worth to mention that having the immune system activated without ongoing infection is not very efficient and energetically costly *(Gondret et al., 2017)*. These results agree with those showed here since animals from L group can be considered less efficient due to lower weight at 175 days than animals from the H group (77.4 vs 88.4 kg).

In BC_LD, a total of eight GO_{BP} and two KEGG pathways were observed, and, as for BC_PI none of the overcome FDR. The most significant enriched GO_{BP} was GO:0006706: steroid catabolic process, and the most significant KEGG pathway was ssc00140: Steroid hormone biosynthesis. One of the genes involved in these pathways is *cholesterol 7α-hydroxylasel* (*CYP7A1*) gene, more expressed in the L group (Table 2) and it codifies for an enzyme involved in the synthesis of bile acids from cholesterol. Therefore, L individuals seem to activate the transformation of cholesterol to bile acids. The expression of these genes was also lower in pigs with low RFI *(Gondret et al., 2017).*

Conclusion

The differential gene expression analyses in liver between the high H and L groups of BC_PI and BC_LD pigs yielded 43 and 59 differentially expressed, respectively. While the differences were not as pronounced as those observed in the hypothalamus of the same animals, the study identified several intriguing candidate genes associated with growth and fatness traits. Notably, genes like *PPP1R13B*, *STK33*, *CXCL14*, *ISG20*, *MRC1*, and *CYP7A1* emerged as promising candidates with potential implications for pig growth and fatness.

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ADVANTAGES AND DRAWBACKS OF REARING ENTIRE MALE AND IMMUNOCASTRATED PIGS

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Invited paper

Abstract: Although surgical castration of piglets is still the predominant practice in Europe, rearing entire males (EM) or immunocastration (IC) are being increasingly used as alternatives. Present paper reviews the advantages and drawbacks of EM and IC concerning the animal welfare, productivity, pork quality, and public acceptance. Rearing of EM avoids the pain and infections associated with surgical castration while the problem of aggressiveness, injuries and stress appears during fattening. Thus from the animal welfare standpoint, IC is more favourable. Due to a better feed conversion and leanness, the rearing of EM is more profitable. However, costs occur due to slaughtering at lower weight, detection of boar taint and carcasses sorting at slaughter. The IC benefits the EM growth potential until the second vaccination, after which an accelerated growth occurs, accompanied by increased fat deposition. The main quality problem of EM meat is boar taint, however EM raw material exhibits other quality flaws related to fat tissue (low intramuscular fat, highly unsaturated fats), increased hardness of meat and lower water binding capacity. These drawbacks negatively affect the quality of meat products, though several strategies have been proposed to overcome the mentioned problems. The IC largely solves the problem of boar taint, its influence on meat quality looks positive but needs more research to further substantiate that. Stakeholder acceptance of the alternatives is country dependent and influenced by familiarity with the agricultural sector.

Key words: pigs, entire males, immunocastration, pros and cons, welfare, productivity, product quality, acceptance

Introduction

In Europe, surgical castration of male piglets is still the predominant practice in pig husbandry. The main reason for this procedure is to prevent boar taint (an unpleasant meat and fat odor due to the substances skatole and androstenone) and to manage aggressive and sexual behavior specific to male animals (*Bonneau and Weiler, 2019*). Another positive aspect of castration is ensuring high meat and fat quality and consequently high quality meat products (*Škrlep et al., 2020a*). However, the practice of castration, when performed without pain relief (i.e., anesthesia and analgesia), causes great public concern (*Prunier et al., 2006*). Due to increased public awareness of animal welfare, many stakeholders in pig production sector are seriously considering or are already implementing alternatives. Raising uncastrated (entire) males (EM) is the most likely alternative to be implemented. This practice has long been used in countries such as the United Kingdom, Ireland, Portugal, and Spain and is gradually being introduced in several Western European countries or pork production chains (*Čandek-Potokar et al., 2015*). Although rearing EM means avoiding the act of castration, it still remains problematic from an animal welfare point of view, and the economic aspect of this alternative must also be considered, in terms of better productivity compared to some other processing-related costs and poorer meat quality (*de Roest et al., 2009; Čandek-Potokar et al., 2015*).

ENTIRE MALES

Strengths

- no castration (reduced workload, improved welfare early in life, less infections)
- better productivity & economy (better feed conversion and lean meat content)
- good public acceptance (rearing)
- lower environemntal impact(less nitrogen excretion)

Weakness

- aggressive behaviour (needs adjusted management and feeding)
- presence of boar taint → on line detection of highly tainted carcasses needed
- poor quality of meat and fat (too lean carcasses, unsaturated fat) → not for high quality meat products

Opportunity

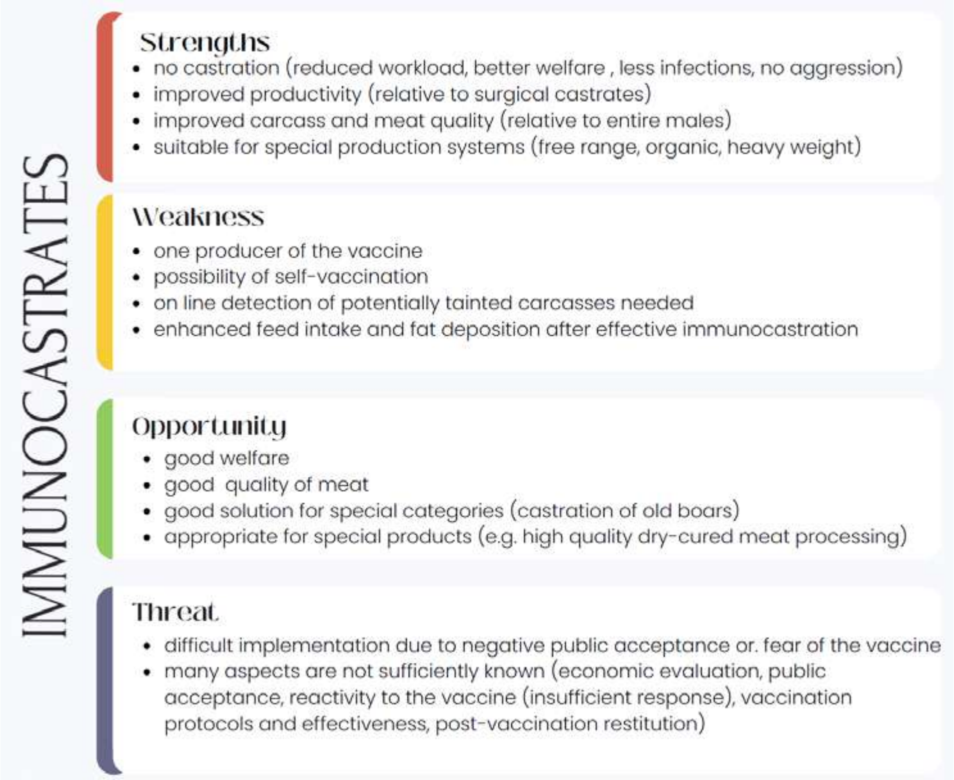
- possibility to valorise meat → marketing claiming higher animal welfare (no castration) and lower environmental impact
- possibility to valorise meat based on low and more unsaturated fat (claiming better health properties)

Threat

- potential loss of market/consumers due to boar taint or inferior product quality
- loss of interest for rearing due to aggression
- broader economic impact not sufficiently known

Figure 1. List of strengths, weaknesses, opportunities and threats of rearing entire male pigs

Regarding the rearing of immunocastrates (IC), this alternative is introduced in Europe at somewhat lower level. Despite solving several problems related to animal welfare (aggression, possibility to castrate old boars) and potentially being more suitable for some specific rearing systems (free-range, heavyweight, organic), the introduction of IC is mostly challenged by acceptance problems (*Čandek-Potokar et al., 2015; Fontanesi et al., 2017*). In immunocastration, androgen deprivation is achieved much later in the pig's life than in classical castration. To achieve a physiological response (i.e., immunize the organism against its own gondadotropin-releasing hormone (GnRH)), two consecutive administrations of an anti-GnRH vaccine are required, at least four weeks apart, with the second dose administered four to six weeks before slaughter. A rapid switch in IC metabolism occurs, increasing feed intake and promoting growth and fat deposition (*Claus et al., 2007; Batorek-Lukač et al., 2016*). Effective elimination of boar odor is also achieved rapidly, after as little as two weeks according to some literature (*Lealiifano et al., 2011*).


IMMUNOCASTRATES

Strengths
- no castration (reduced workload, better welfare , less infections, no aggression)
- improved productivity (relative to surgical castrates)
- improved carcass and meat quality (relative to entire males)
- suitable for special production systems (free range, organic, heavy weight)

Weakness
- one producer of the vaccine
- possibility of self-vaccination
- on line detection of potentially tainted carcasses needed
- enhanced feed intake and fat deposition after effective immunocastration

Opportunity
- good welfare
- good quality of meat
- good solution for special categories (castration of old boars)
- appropriate for special products (e.g. high quality dry-cured meat processing)

Threat
- difficult implementation due to negative public acceptance or. fear of the vaccine
- many aspects are not sufficiently known (economic evaluation, public acceptance, reactivity to the vaccine (insufficient response), vaccination protocols and effectiveness, post-vaccination restitution)

Figure 2. List of strengths, weaknesses, opportunities and threats of rearing immunocastrates

Welfare and management

Raising EM improves their welfare, at least early in life. They are not subjected to the pain and discomfort associated with surgical castration and postoperative complications (*Prunier et al., 2006; von Borell et al., 2009*). On the other hand, welfare may be significantly affected later in life when EM reach sexual maturity. The EM exhibit more aggressive and sexual behaviour (mounting) and are more active overall than surgically castrated males (SC) (*von Borell et al., 2009*). This manifests itself in constant confrontations between pen mates or harassment by the dominant animals. It leads to skin, ear, tail, and penis injuries, as well as lameness and leg damage (*Rydhmer et al., 2006; Weiler et al., 2016*). The problems are more commonly observed where either feeding sites or access to food is limited (*Velarde, 2007*). Increased fighting activity is also observed during regrouping (e.g., during transport or prior to slaughter) when stable rank relationships are lost (*Rydhmer et al., 2006*). Higher age at slaughter increases the risk of injury (*Weiler et al., 2016*), whereas rearing in mixed-sex groups does not seem to affect aggressiveness. The most pragmatic solution to this problem is to slaughter EM at a lower age, before reaching puberty, and/or provide an enriched environment (roughage, deep litter), adequate (feed) space, and avoid breaking up established social groups (*von Borell et al., 2020*).

In immunocastration, pain is limited only to the insertion of the needle during vaccination and is therefore considered more welfare friendly, avoiding acute pain and postoperative complications associated with surgical castration (*Prunier et al., 2006*). The literature reports either no or minor short-term reactions to the vaccine (*Einarsson, 2006*). Immunocastration reduces the aggressive and sexual behavior characteristic of EM shortly after effective immunization, as it would otherwise (considering the standard vaccination protocol) begin at 5 months of age with the onset of sexual maturity (*Zamaratskaia et al., 2008; Rydhmer et al., 2010*). This is important not only during rearing, but also in the pre-slaughter period (transport, rearing), where possible mixing of animals may trigger additional aggression related to hierarchy restoration (*Bolhuis et al., 2005*). As indicated by the incidence of carcass skin lesions and blood cortisol levels, IC showed intermediate levels of aggression compared to EM and SC. Certain rearing practices, such as restrictive feeding, can raise aggression levels back to those of EM (*Batorek et al., 2012a*). It has been suggested that the appetite of IC is greatly increased after the second vaccination (feed intake often even exceeds that of SC) and stress due to feed restriction is therefore much greater. It has also been pointed out that severe restriction (as practiced during certain periods in free-range Iberico pigs) may even significantly reduce vaccination success, thus requiring an adjustment of the feeding regime (*Hernandez-Garcia et al., 2016*). Nevertheless, immunocastration could prove beneficial in free-range systems, as it can be used for both males and females and could provide management benefits (no need to separate males and females) in addition to animal welfare (no castration of males or females) and economic benefits (higher growth, more homogeneous herd) (*Martinez-Macipe et al., 2016; Fernandez-Moya, 2011*). Energy restriction or feed dilution could be a solution to control excessive fat deposition after effective vaccination (*Batorek Lukač et al., 2021*). Another welfare-friendly practice would be immunocastration of adult boars from artificial insemination stations. Although some studies suggest that this is successful in this age class, the exact timing of boar odor elimination, vaccination protocol, interfering factors still need to be investigated (*Fontanesi et al., 2017*).

Productivity and economic aspects

Compared to castration, breeding EM may be more economically advantageous for breeders. There are no castration costs and no losses due to infection of castration wounds. In standard production systems, EM has better feed conversion (up to 15% more efficient), which is related to lower ad libitum feed intake (up to 9% less feed consumption), and exhibit higher carcass leanness (up to 20%) compared to SC. Depending on the study report, growth rate can vary significantly, with some studies indicating up to 13% higher values in EM (*Bonneau and Weiler, 2019; Lundström et al., 2009*). The reason for this lies in their metabolic status. Regarding testicular hormones (androgens, estrogens), EM exhibits a strong protein anabolic state, reducing protein degradation and promoting protein build-up and lipid catabolism (*Claus et al., 1994*). Compared with SC, the average economic benefit of raising EM in a standard production system in the Netherlands and France has been estimated at 7 to 8 EUR/pig (*ALCASDE, 2009*), including the reduction in income due to carcass sorting and a possible decrease in the price of taintedmeat (2%). In another estimate (*Béteille, 2014*), the surplus has varied between 5.2 and 10.8 EUR/pig. It should be noted here that certain additional costs are still difficult to evaluate. In some EU countries such as the UK, Ireland, Spain and Portugal, boars are slaughtered at lower weight before they reach sexual maturity. This may also imply higher herd turnover, higher demand for young piglets, lower carcass dressing and additional treatment at slaughter (removal of the reproductive tract) and consequently higher slaughter costs when calculated per kg of meat (*de Roest et al., 2009*); not to mention losses due to higher aggressiveness and potential market losses (due to negative consumer reactions to boar taint or low-quality meat products, *Fontanesi et al., 2017*). It is also worth noting that standard swine production does not cover all the specific needs of the swine sector (i.e. better quality, fatty raw material for high value meat products). In situations where high weights or age are involved (e.g., local breeds, free-range), it is not practically feasible to raise sexually mature EM (*Fontanesi et al., 2017*), so the use of other (castrated) categories of pigs remains essential.

As for IC, this category grows faster than SC or even faster than EM, considering the total fattening period (from first vaccination to slaughter) and the standard vaccination protocol. However, the IC consume more feed than EM, but less than SC. Therefore, feed conversion of IC is better than that of SC and only slightly worse than that of EM (meta-analysis by *Batorek et al., 2012b*). The reason for this is that IC is physiologically equivalent to EM until the second inoculation, allowing them to utilize their boar-like growth potential. Thereafter, rapid physiological changes (namely a drop in steroid hormone levels accompanied by relatively high IGF-1 and growth hormone levels and low leptin concentration) trigger high feed intake and accelerate growth (*Batorek et al., 2012a; Kubale et al., 2013*). It should be noted that protein deposition remains about the same, while IC mainly increase deposition (*Batorek Lukač et al., 2016*). However, the level of fat deposition in IC depends on the timing of immunisation relative to slaughter. The more time elapses between the second immunisation and slaughter, the higher the fat deposition (*Škrlep et al., 2014*), which negatively affects the economics of rearing (higher fat content leads to lower carcass leanness, which lowers the price). In general, IC pigs have greater backfat thickness compared to EM, resulting in lower lean carcass content, while a comparison of IC with SC shows the advantages of the former (lower fat carcass content, heavier hams and shoulders; *Batorek et al. (2012b*)).

The cost of immunocastration includes the price of the vaccine and the additional workload due to vaccination. On the other hand, this is compensated by higher feed conversion, faster growth, and a leaner carcass compared to SC (*Batorek et al., 2012a,b*). Although actual costs are difficult to assess due to price variations in labour, equipment, pharmaceuticals, and pig production parameters, it has been estimated (*de Roest et al., 2009*) that the direct additional cost of immunocastration ranges from 3.00 to 3.65 EUR/animal (excluding the price of slaughter line carcass examination for boar taint and additional carcass trimming). This is more than the estimated cost of surgical castration (estimated between 0.78 and 2.99 EUR, *de Roest et al., 2009; Rodriguez-Estevez et al., 2012*) or about the same if pain relief is administered (0.19 to 1.67 EUR/piglet;; *de Roest et al., 2009; Aluwe et al., 2012*). At the same time, faster growth and better feed conversion takes place compared to SC (for which reports range from 8% to 18%, depending on the breed used, *Škrlep et al., 2010; Batorek et al., 2012a*), generally resulting in an economic advantage reported in various studies to be -0.02 to + 0.12 EUR/kg (*de Roest et al., 2009; Kastelic and Košorok, 2010; Aluwé et al., 2012*). Another study by *de Roest (2015)* indicated the advantages of immunocastration over surgical castration in terms of lower infection rates (estimate: 2.75 EUR/pig). Although immunocastration does not have a large economic impact, it would also be beneficial for culled boars used for breeding or performance testing. These animals are often sold for slaughter at a very low price. Alternatively, if they are castrated by a veterinarian, this adds costs, not to mention the welfare aspects of this rather demanding procedure. However, a recent study has shown that a two-dose vaccination regimen results in progressive but variable regression of testicular function in culled boars, insufficient to elicit a complete immunocastration response in all animals (*Batorek-Lukač et al., 2022*). Therefore, a suitable vaccination regimen must be investigated for these animals.

Another important aspect is the need for classification/scanning of carcasses at both EM and IC, in order to sort out tainted carcasses (including IC, which did not respond properly to the vaccine). As described by *Font i Furnols et al. (2020)*, numerous methods have been developed so far, including those based on human assessors, chemical, physical, or spectroscopic methods. However, almost all of them have certain drawbacks related to low sample capacity, speed, price, or lack of suitability for online use; many of them still need to be sufficiently developed. Nevertheless, a recent study shows that testicular weight could be a reliable indicator of androstenone-positive carcasses (IC), while at EM the difference in testicular size between EM with androstenone levels below and above the risk threshold was less pronounced, but urogenital tract weight was reliable in these animals (*Fazarinc et al., 2023*).

Meat, fat and product quality

In general, castration in pigs leads to increased fat deposition with a concomitant increase in fat saturation (*Wood et al., 2008*). Comparing the different male sex categories, fat content and fat saturation decrease in order SC > IC > EM, although the differences vary considerably due to factors such as age, feeding, and IC vaccination timing. Interestingly, higher polyunsaturated fatty acid (PUFA) content is observed in EM, even with the same fat thickness as in SC (*Škrlep et al., 2020a*). The fat of EM may be more susceptible to oxidation (*Babol et al., 1995*), although scientific evidence for this is lacking. Compared to SC, the fat of EM contains fewer lipids, more water, and connective tissue and is therefore softer, less cohesive, and poorly attached to underlying tissues (*Wood et al., 1982*). This poses a major problem when integral meat cuts are further processed. Another problem with EM is the extreme carcass leanness along and low intramuscular fat (IMF) content (*Škrlep et al., 2020b*), which negatively affects final product characteristics such as juiciness, tenderness, flavor, and processing results (*Škrlep et al., 2020a*). At IC, the most pronounced physiological changes following effective immunization relate to increased fat synthesis (*Batorek-Lukač et al., 2016*). Indeed, carcass fat content increases proportionally to the interval between slaughter and the second vaccination (V2-S, *Lealiifano et al., 2011*), with lipid deposition being somewhat depot-dependent (leaf fat and back fat deposit faster than IMF (*Poklukar et al., 2021*). Fatty acid composition also changes very rapidly, from EM to SC within 6-9 weeks (*Tavarez et al., 2014a*), so this interval should be extended if processing into high-value meat products is planned (preference for more saturated fat).

Regarding technological meat quality traits such as colour, pH, and water holding capacity (WHC), available meta-analytical studies (*Pauly et al., 2012; Batorek et al., 2012b; Trefan et al., 2013*) suggest that there are no or only minor and practically insignificant differences. Some older studies still show higher meat pH and the presence of dark, firm, and dry meat (DFD) in EM associated with higher aggression and stress, resulting in muscle glycogen utilisation during pre-slaughter procedures (*Sather et al., 1995*). In contrast, recent research suggests that male sex categories do not respond differently to stress and meat quality is barely affected (*Holinger et al., 2018*). There is still some evidence for a stronger red meat coloration of EM, either related to the previously mentioned mechanisms of glycogen degradation, a more oxidative metabolic profile, or a lower IMF (*Škrlep et al., 2020a*). Although again of little practical significance, IC have been shown to have lighter muscle colour, most likely due to higher IMF and lower WHC (*Pauly et al., 2012; Batorek et al., 2012b; Trefan et al., 2013*). Regarding the different WHC characteristics, SC has generally been reported to have a better water binding capacity compared to EM or IC, but again this is highly study dependent. Nevertheless, some studies show that the drip loss for EM is up to 45% higher than for SC, with the most likely cause being oxidation of the unsaturated fats during heat treatment (*Škrlep et al., 2019*). The oxidation-induced lower WHC value as well as the higher collagen content in the meat of EM, were associated with the higher muscle toughness of this male sex category, while IC was still between EM and SC (*Škrlep et al., 2019; 2020b*). According to the reports of these studies, 20-25% higher shear forces were measured at EM. Nevertheless, the toughness issue remains poorly addressed, while some other processes (such as the higher proteolytic potential of EM) offer the possibility to address this drawback.

High-quality raw material is essential for the production of high-quality meat products (e.g., dry-cured products such as hams, sausages, pancetta). The more aggressive behaviour of EM often leads to a higher incidence of skin lesions on the carcass (cuts, hematomas, bruises; *Batorek et al., 2012a*), which either affect the external appearance of the product or (in case of more profound damage) trigger bacterial spoilage (*Čandek-Potokar & Škrlep, 2012*). The use of EM raw material can lead to several disadvantages related to the previously mentioned low amount of subcutaneous fat or IMF or inferior WHC, including high processing losses, excessive salt intake, and the development of an inappropriate (hard) texture and poor flavour development, as seen in either dry-cured ham (*Čandek-Potokar et al., 2020*), dry-fermented sausages (*Corral et al., 2016*), or cured belly (*Smith et al., 1983*). However, problems related to oxidation have not been reported in the literature, but this could be due to the use of antioxidants (i.e., nitrites, ascorbates) used in the majority of meat processing formulations (*Škrlep et al., 2020a*). Interestingly, EM bacon was found to be better accepted by consumers than SC bacon because it was leaner, even when boar taint was present in the samples (*Čandek-Potokar et al., 2019*). As for the use of IC, for example, the application of the standard inoculation protocol (V2-S of 4-5 weeks) resulted in dry-cured hams still more similar to EM than SC (except for the absence of boar taint) (*Čandek-Potokar et al., 2020*). Similarly, such immunocastration protocol resulted in leaner cured bellies that were also better accepted by consumers, but with a lower slicing yield (*Tavarez et al., 2014b*). Extending the V2-S period to 6-9 weeks or even vaccinating 3 times (as practised in heavy pigs) mainly eliminated the differences toward SC in dry-cured ham (*Škrlep et al., 2016; Pinna et al., 2015*) or bacon (*Tavarez et al., 2014b*).

The main drawback of processing raw material from EM is the possible presence of boar taint. To mitigate this risk, there are several strategies depending on the composition of the raw material (fat and boar taint content), the specific processing method, the masking strategy used, or the type of consumption (cold or hot) (*Škrlep et al., 2020a*). Dry curing or dry fermentation processes have not been found to sufficiently reduce or mask the concentration/perception of boar taint in dry cured ham (*Diestre et al., 1991*) or sausages (*Corral et al., 2018*). An advantage of these products is that they are usually consumed cold, which somewhat reduces the risk of negative perception, but only for products with low to moderate off-flavour (below 0.8-1.0 ppm androstenone). For sausages, a low level of masking can be achieved by microbial fermentation (addition of starter cultures), addition of spices, or chemical additives (polyphosphates, acidifiers) (*Corral et al., 2017, 2018*). Smoke, on the other hand, has been identified as a relatively efficient masking agent that can manage higher levels of boar taint in various types of sausages or other types of meat products when used intensively (*Aaslyng et al., 2018*) or in combination with dilution (mixing 25% to 50% tainted with untainted meat, *Meier-Dinkel et al., 2016*) where this process is applicable (*Škrlep et al., 2020a*). Products such as cooked ham, bacon, cooked sausages, minced meat patties, etc. are usually consumed hot/warm, which greatly increases the potential perception of boar taint especially during preparation (evaporation in the room). However, thermal treatment itself can reduce boar taint compounds in the product to some extent, but only if high temperatures are applied for a long period of time

(*Peñaranda et al., 2017*). Additional masking measures such as frying in oil (as a source of aroma compounds), applying different marinades with spices such as garlic, parsley, curry, oregano, and fennel, and embedding meat in breadcrumbs or combining tainted meat with a complex meal (e.g., with bread and cheese) have been confirmed to be effective for low to moderate levels of boar taint (*Škrlep et al., 2020a*).

Public acceptance

While acceptance for EM is quite good in countries where it is already produced, raising EM for meat consumption is new to many European countries. There, consumer acceptance of alternatives to surgical castration is generally understudied (*Fontanesi et al., 2017*). Most EU consumers are unaware of the problem of boar taint and do not even associate pork with castration (*Kallas et al., 2013*). A study conducted in 13 Eastern European countries (*Tomašević et al., 2020*) showed that awareness of pig castration is also low. Nevertheless, consumers expect meat to be safe, healthy, and tasty, and therefore may be susceptible to the problem of boar taint. As indicated in the PIGCAS project report (*PIGCAS, 2009*), pig producers and consumers were reluctant to adopt EM, preferring the traditional practice of surgical castration. According to more recent reports (*Fontanesi et al., 2017*), the attitude of some stakeholders in some production chains has partially changed in some countries, such as France, Belgium, Germany, and the Netherlands, where a larger proportion of EM is produced. Nevertheless, there are large differences between production chains and their willingness to produce EM, both within countries that have recently started producing EM and between countries. The recent trend observed in some countries to increase the production of EM applies only to standard production systems. On the other hand, raising EM in special production systems (i.e., heavy pigs, high value products) still raises concerns about management (EM aggressiveness, farmer safety), meat quality (too lean meat), and product specification (higher age and weight requirements).

Regarding existing studies on IC, acceptance within Europe is again highly country dependent. German consumers of organic pork rated EM better than IC, and the latter were rated better than SC (*Heid and Hamm, 2013*). In Sweden, consumers rated IC more positively than rearing EM or surgical castration without anaesthesia (*Lagerkvist et al., 2006*). In contrast, according to *Huber-Eicher et al. (2011)*, Swiss consumers rejected IC compared to surgical castration, but when it was performed with pain relief. Similar results were obtained by *Fredriksen et al. (2011)* in Norway. In both countries, anaesthesia is already required for surgical castration. In the Belgian study (*Tuyttens et al., 2011*), IC was also rated as very favourable, but only after thorough information about all possible alternatives, indicating the importance of consumer education. In the broader survey (*Fontanesi et al., 2017*), stakeholders in the pig production chain across Europe (including pig producers, meat processors, veterinarians, and slaughterhouses) still rated the prospects of IC as low, with the main reason being the fear of negative consumer reactions hindering its implementation. Other disadvantages cited include labour safety measures, additional workload, and lack of information on specific issues (restitution after vaccination, adaptation of vaccination protocols to specific production systems, lack of experience, and vaccination success). Consumers question the practice of IC from a food safety point of view or prefer less use of drugs, fear residues, unknown long-term effects and unnaturalness (*Mancini et al., 2017, 2018; Fredriksen et al., 2011*). On the other hand, a comprehensive international study (*Vanhonacker and Verbeke, 2011*) conducted in France, Germany, and the Netherlands, with over 4000 respondents, showed that concerns about negative reaction to IC may be overstated, as the method itself was acceptable to more than 70% of respondents. As for Norwegian and Italian consumers, one of the reasons for accepting IC was trust in their national food safety institutions (*Fredriksen et al., 2011; Mancini et al., 2018*). Animal welfare-oriented nongovernmental organisations also rated IC as acceptable, although they preferred EM. Scientific experts viewed IC as a much better alternative to SC with pain relief, as they believed it improved animal welfare and was also more economical and easier to implement (*PIGCAS, 2009*).

Conclusion

As recent trends show, the rearing EM is gradually gaining importance in the EU despite several welfare and meat quality related drawbacks. Although the immunocastration solves many of these issues (i.e. prevents boar taint, welfare problems, improves meat and fat quality compared to EM, improves productivity compared to SC), this practice has not gained much interest in the sector, at least in Europe. Fear of the public acceptance remains the main obstacle for its use. Regardless of the anticipated changes concerning the ban on castration without anaesthesia, these changes are only happening slowly, whereas the introduction of various alternatives will most probably depend on the different needs of individual niches in pig production.

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OPTIMUM CONTRIBUTION SELECTION: PRACTICAL IMPLEMENTATION IN BLACK SLAVONIAN AND BANIJA SPOTTED PIG

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Invited paper

Abstract: Breeding programmes in autochthonous pig populations are mostly oriented to conservation of genetic diversity, which includes preservation of typical exterior and avoiding inbreeding at the same time. In order to improve the ability of the breed to survive in a sustainable way, breeding programmes should include genetic improvement for the traits of interest. However, genetic improvement and conservation of genetic diversity are usually conflicting goals, and balancing between the genetic improvement for the traits of interest and preservation of genetic diversity of the population is a key problem in small populations. Various methods have been developed in order to optimize genetic gain and the level of inbreeding in the population using optimal contribution selection (OCS) framework. OCS is an optimisation of genetic contributions of selection candidates to the next generation such that expected benefit and risks are balanced. A common way to achieve this goal is to maximise genetic gain at a predefined rate of population inbreeding by restricting selection of candidates that are closely related. Also, there is a possibility to minimise inbreeding by minimising the average kinship of the population accounting also for breeding values since inbreeding arises from the mating of related parents. Both scenarios were analysed when implementing the OCS procedures on the populations of Black Slavonian pig and Banija spotted pig. While the genetic diversity parameters allowed the implementation of OCS on Black Slavonian pig, the main breeding goal in Banija spotted pig is preserving of genetic diversity.

Key words: optimal contribution selection, genetic diversity, Black Slavonian pig, Banija spotted pig

Introduction

Conservation of endangered populations of pigs has for objective the preservation of genetic diversity of the population. Moreover, the final goal is to ensure sustainable breeding, which demands development of various tools for achieving the sustainability of the breed (*Barker, 2001*). Breeding programmes for local and autochthonous breeds are mostly oriented to conservation goals, without intention for genetic improvement of the populations. However, there are examples of breeding programmes that include genetic evaluation of economically important traits, such as growth traits in Iberian pig (*Duarte et al., 2012*). Genetic gain and preservation of genetic diversity of the population are opposite goals, especially when using selection methods based on genealogical data, resulting with a selection of candidates with high breeding values, but also high kinship with other candidates. As achieving genetic improvement and maintaining genetic diversity in the population seem to be oppose breeding goals, finding optimal level of genetic improvement without substantial loss of genetic variability becomes a challenge for breeding programme creators. This is especially important in livestock breeds under conservation, which are at higher risk for loss of genetic variability and occurrence of inbreeding depression, due to the loss of different alleles and increased homozygosity. Thus, the breeding programmes for breeds with small effective population size, which usually belong to the group of endangered populations should have balanced breeding goals which include genetic improvement and maintaining genetic diversity. Optimal contribution selection (OCS) was proposed to manage this challenge (*Woolliams et al., 1999*).

Optimal contribution selection: theoretical background

Beside conservation purpose, breeding programmes in local and autochthonous breeds of pigs should have developmental role, enabling genetic improvement in the population. According to *Gandini and Oldenbroek (2007)*, strategies for moving from conservation to sustainable utilization include defining the relevant breeding goal and development of high valuable products to secure profitability and sustainability. Thus, genetic evaluation for the traits of interest might be helpful in achieving this goal. Pedigree based genetic evaluation using an animal model is still method of choice in many breeding programmes for pigs. Availability of data and lower costs compared with genomic evaluation makes best linear unbiased prediction (BLUP) the method of choice for selection in small populations. However, there is a phenomenon called Bulmer effect *(Bulmer, 1971)* that describes the reduction of genetic variability in the population caused by selection. Moreover, using BLUP as the selection method in the population increases the probability of choosing candidates from the best performing families, where individuals contribute more to the gene pool compared to those from lower performing families *(Wray and Thompson, 1990; Wooliams et al., 1999)*. Thus, an optimal solution is required that optimises genetic gain and manages the level of inbreeding in the population.

A sustainable genetic gain is obtained by creating a covariance between the Mendelian sampling terms (*a*) and the long-term genetic contributions (*r*) of the selection candidates *(Wolliams et al., 1999).* This in turn provides the framework for an effective solution for the management of genomic diversity, due to the relationship between genetic contributions and rate of inbreeding (ΔF). Various methods have been developed in order to optimize genetic gain and the level of inbreeding in the population using this framework, which is known as optimal contribution selection (OCS) *(Howard et al., 2018)*. OCS optimises contributions of selection candidates to the next generation such that expected benefit and risks are balanced *(Woolliams et al., 2015)*. The concept of genetic contributions was discussed by *Wooliams et al. (1999)* and *Bijma and Wooliams (1999)*. A common way to achieve this goal is to maximise genetic gain at a predefined rate of population inbreeding by penalising selection of individuals that are too closely related *(Wray and Goddard, 1994; Meuwissen, 1997). Meuwissen (1997)* proposed a method that maximizes the response of selection with a predefined rate of inbreeding, where the optimal solution is expressed as genetic contribution of the individuals to the next generation, which is equivalent to the number of offspring per selection candidate:

$$G_{t+1}=c_t'\,EBV_t$$

where EBV_t is the vector of BLUP estimated breeding values of the candidates for selection in generation t and c_t is the vector of genetic contributions of the selection candidates to generation t+1.

Genomic information is now used in many livestock breeding programs *(Garrick, 2011)*. The EBV’s for selection candidates can be thus based on pedigree or genomic data. Moreover, in order to set up inbreeding restriction policy, genomic or pedigree-based measures of coancestry can be used. *Clark et al. (2013)* analysed simulated and real data of dairy bulls, incorporating genomic data into OCS. The use of genomic EBV’s resulted in increased genetic gain for a given level of inbreeding when optimal contribution selection was used, especially when the population consisted of large full-sib families. Constant development of molecular methods and their reducing prices might accelerate their use in the selection of pigs and increasing the accuracy of information on relationship and inbreeding.

Caballero and Toro (2000) showed that minimisation of coancestry among individuals enables preservation of the genetic diversity, suggesting independent processes of choice of parents and creating mating schemes. However, *Fernández et al. (2001)* argued the ability of the practical implementation of optimal conservation strategies, which might be affected by the restricting factors, such as number of individuals offspring can contribute. Also, physiological restrictions can make the application of the optimal contribution strategies harder in populations. For this reason, the authors proposed single step method to optimise the management of a conservation programme when restrictions on the mating scheme exist. The single step method looks for optimal contribution of the parents and sets the optimal mating scheme simultaneously.

Optimal contribution selection in pigs

Several studies have been published using dairy cattle data *(Koenig and Simianer, 2006; Sørensen et al., 2008; Wang et al., 2017)* horse data (*Solé et al., 2013*) and sheep data (*Henden Kjetså, 2016*). The application of the optimal contribution selection on real data in pigs in the literature is scarce. *Howard et al. (2014)* analysed structural changes in the commercial pig population after applying OCS. They showed that the application of OCS in the genomic evaluation of the pigs has the potential to deliver more genetic gain for the same rate of inbreeding. *Gourdine et al. (2012)* used stochastic simulation with 25 different scenarios in local pig population and found that with optimum contribution selection, genetic progress can be achieved that is almost as great as that with truncation selection based on BLUP breeding. *Zhao et al. (2021)* analysed the long-term impact of conventional and optimal contribution conservation methods on genetic diversity and genetic gain in local pig breeds using both pedigree and genomic data. Authors concluded that the use of methods based on genomic information has better effect for combined conservation and genetic improvement. However, for an optimal outcome of applied OCS procedures, pedigree and data quality still plays an important role *(Sørensen et al., 2008; Zhao 2021).*

Practical implementation in Black Slavonian and Banija spotted pig

Implications of optimal contribution selection for litter size in the Black Slavonian pig were analysed by *Škorput et al. (2022).* The analysis has been based on litter data and pedigree from Black Slavonian herdbook. Optimal genetic contributions were determined in two different scenarios. The first scenario was to apply traditional optimum contribution selection by maximising the breeding value in the offspring for NBA (number of piglets born alive) (*Meuwissen, 1997*) while restricting the mean kinship in the offspring by defining constraints. Constraints were set on the following levels of kinship: 0.01, 0.05 and 0.1. Different levels of constraints on kinship enabled focussing on different breeding goals in the population, balancing between genetic progress and conservation (Figure 1).

The second scenario was to minimise inbreeding by minimising the average kinship of the population accounting also for breeding values since inbreeding arises from the mating of related parents. In the first scenario, different degrees of upper bound kinship between candidates caused changed different numbers of selected candidates such that the number of selected candidates increased as a constraint on kinship was stronger, with a simultaneous decrease of average EBVs.

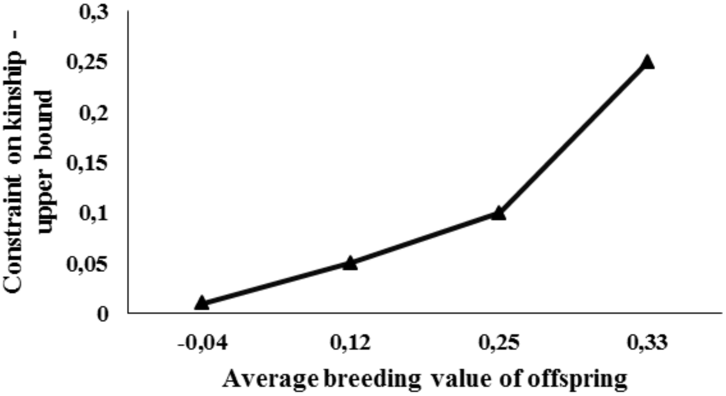

Figure 1. Relationship between the constraint on kinship and the average breeding values (expected gain) of the next generation for NBA (*Škorput et al., 2022*).

In the second scenario, when minimal targeted EBVs were set to 0.00 in order to choose only animals with positive breeding values, there was no occurrence of inbreeding in the offspring generation. With the increase of the desired genetic gain, the inbreeding in the offspring increased and the distribution of the candidates changed to select fewer female candidates, but with larger genetic contributions. The number of selected boars increased with the increase of targeted EBVs, with slightly increased maximal genetic contributions. Although the population parameters showed a reduction of genetic diversity, the results of the study showed that the use of optimal contribution selection in Black Slavonian is possible. However, additional efforts to improve pedigree and data quality are needed in order to avoid underestimation of genetic relationships between selection candidates. *Škorput et al. (2023)* estimated genetic diversity parameters for Banija spotted pig population and applied optimisation methods to control the inbreeding in the population in three different scenarios. The first scenario consisted of minimizing inbreeding by minimizing the average kinship without considering EBVs. The second scenario was to minimize inbreeding by minimizing the average kinship accounting for EBVs. The third scenario was to apply traditional optimal contribution selection by maximizing the EBV of the offspring. Due the small total and effective population size, the application of optimisation methods demonstrated that genetic improvement is possible at a high cost of loss of the genetic variability and preservation of diversity remains the first objective in the breeding programme for Banija spotted pig (Table 1).

Table 1. Number of selected breeding pigs, average inbreeding in offspring generation, minimum and maximum male, and female contribution by minimizing the average kinship of the population with account on breeding values (*Škorput et al., 2023*)

BV	No. of selected candidates (M/F)	F	Kinship	Maximal male contribution	Maximal female contribution
0.05	5/7	0.10	0.18	0.17	0.16
0.10	5/7	0.11	0.19	0.14	0.13
0.25	5/10	0.18	0.24	0.17	0.15

Practical problems that arise in field data are usually related to a low pedigree depth and consequently underestimated kinship and overestimated effective population size. This problem could be overridden by improving pedigree quality and by using molecular information that might contribute to more reliable estimates of population parameters.

Conclusion

Selection of pigs in small populations of autochthonous breeds for traits of interest could be beneficial for breed sustainability. Although maintaining genetic diversity and genetic improvement seem to be oppose breeding goals, optimum contribution selection might help breeders and organisations to achieve genetic improvement for important traits while maintaining genetic diversity in populations. In local breeds, there is no high selection pressure and optimal genetic gain and reducing inbreeding in future generations might be achieved using reliable pedigree or genomic data.

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IMPROVING THE REPRODUCTIVE CHARACTERISTICS OF THE PUREBRED IBERIAN PIG: A CHALLENGING ENDEAVOR

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Abstract: The reproductive performance of the Iberian pig breed is generally lower compared to most commercial breeds, which poses a challenge for selection programs aimed at improvement. In addition, the relatively small size of the herds and the strong influence of environmental effects are additional factors that need to be addressed in these programs. This study focuses on a review of several factors affecting the productivity of Iberian sows. These factors include: boar genotype, farrowing season, parity order, inbreeding depression and sow age at first farrowing. Finally, variance component analyses were performed for the reproductive traits under consideration. The results emphasize the importance of incorporating these factors into the analysis models. In addition, these findings supported estimates of genetic and environmental parameters obtained in previous studies.

Key words: litter size, maternal aptitude, environmental factors, genetic parameters

Introduction

The Iberian pig, like other European local breeds, is known to have worse maternal characteristics compared to most commercial world breeds *(Rodríguez et al., 1994)*. Breeding programs aim to address this deficiency through appropriate genetic evaluations and selection indexes. However, in medium-small size herds, which are highly influenced by environmental conditions and not integrated into global breeding schemes allowing for high selection pressures, the potential for improvement remains limited.

The present work examines several aspects and strategies that can be considered to improve the prolificacy and maternal aptitude of the Iberian breed. The research was carried out on the herd owned by the company Sánchez Romero Carvajal Jabugo (SRC), which is known for producing high-quality products derived from Iberian pigs fattened in a traditional open-air system known as *Montanera*. Since 2011, SRC has been implementing a breeding program comprising two lines, the sire and the dam line. The sire line focuses on traits related to growth, carcass composition and meat quality, while the dam line's main objective is to improve reproduction traits, specifically prolificity and maternal aptitude *(Muñoz et al., 2018)*. The company manages several farms of medium-size (between 750 and 200 sows) in the southwest of Spain.

Data

We have data from an extensive study comprising 23,525 litters from 5,990 sows with an average of 3.93 litters per sow. The data was gathered over a period spanning from September 2011 to April 2023 in four farms with different environmental and facility conditions: Monte Castilla (MC), Tejarejo (TJ), Coto Gomeles (GM), and Dehesón del Encinar (DH) farms, consisting of 12,771, 4,479, 5,664, and 611 litters, respectively. Notably, MC and TJ farms share the same environment. The study addresses several important traits routinely measured in the farms, including the total number of piglets born (TNB), number of piglets born alive (NBA), number of weaned piglets (NWP), litter weight at birth (LW0, including stillborn piglets), litter weight at 21 days (LW21), interval between consecutive births (IBB), and weaning-mating interval (IWM). The base population comprises 1,056 sows without a previous pedigree. The breeding program incorporates boars from both the Iberian and Duroc breeds. Artificial insemination was used in MC and TJ farms, while GM and DH farms utilized natural service for breeding. The litters crossed with Duroc are born between the end of October and March, and this production is intended for sale to industries unrelated to SRC. From April to October, pure Iberian boars are used to produce pigs to be fattened in the open-air *Montanera* system. Subsequently, these pigs are slaughtered and marketed by SRC at its facilities in Jabugo (province of Huelva). The number of litters born from Duroc and Iberian boars is 4,784 and 18,741, respectively.

Effect of boar genotype and farrowing season

The influence of farrowing season on reproductive performance in Iberian pigs has been extensively studied and documented *(Dobao et al., 1983; Vázquez et al., 1994)*. However, in our specific population, there is a seasonal variation in the use of boar genotypes, either Iberian or Duroc, which makes it challenging to distinguish between the two effects. Moreover, since no crossbred litters are born in spring or summer, this seasonal pattern could potentially interfere with the interpretation of our results. To address this issue, the reproduction traits were carefully analyzed using GLM (General Linear Model) procedure with SAS (SAS Institute Inc., Cary, NC, USA, 2002-2012). In our GLM model, we included the interaction between the boar genotype and the herd, as well as between the boar genotype and the farrowing season. Additionally, we considered parity order as fixed effect and incorporated the covariates TNB and NWP for LW0 and LW21, respectively, in the model.

The sow's seasonal anestrus *(Rodríguez-Estévez, 2010)* is even more significant in poorly selected breeds, with prolificacy or fertility being highly dependent on the sow. For this reason, comparative analyses based on the boar genotype used in mating does not provide much information in this population, especially because the use of Iberian or Duroc boars is linked to a specific season. Therefore, no inferences could be made about the effect of boar type on TNB, NBA, or the interval traits.

Regarding litter weight, a trait influenced by the sire, litters crossed with Duroc showed systematically higher weights than those of Iberian, according with a breed with higher growth capacity together with the effect of the heterosis between breeds. The least square means (LSM) estimates for crossbred litters with Duroc range between 10.72 and 10.89 kg for LW0 and between 36.14 and 37.18 kg for LW21. Those values for purebred litter were between 8.76 and 10.03 kg for LW0, and between 31.13 and 32.72 kg for LW21.

The seasonal effect of farrowing date has been analyzed only in pure Iberian litters to avoid any potential interaction with the boar genotype. Concerning TNB and NBA, it is evident that summer is the least prolific season (-0.16 and -0.20 piglets, respectively), while no significant differences were observed among the other three seasons. In the intervals traits, considered the farrowing date at the end of the interval, spring is the season with the longest time elapses between parities or between weaning and mating. On the other hand, autumn and summer were the shorter intervals, followed by winter.

The litter weights traits showed very different patterns depending on whether they were recorded at birth or at 21 days. For LW0, there was a slight increase in litter weight in spring respect winter and summer (0.07 kg) and it was noticeably lower in autumn (-0.30 kg). On the other hand, for LW21, the clear increase in litter weight was observed in winter respect spring and autumn (0.43 kg), and a strong reduction in weight in summer (-1.16 kg).

These findings clearly highlight the significant impact of seasonal effects on the production of Iberian pigs.

Effect of parity order

Some authors have advised considering the first parities of the sows as distinct traits from subsequent parities, suggesting a different genetic control of reproductive characteristics depending on the parity order. Specifically in Iberian pigs, *Fernández et al. (2008)* recommended treating NBA differently, considering the first parity as one trait and the second and subsequent parities as another trait. Similarly, for LW21, the first and second parity would be treated as one trait, while from the third parity onwards it would be considered as another trait. This approach would involve conducting multivariate analyses for reproductive traits, considering the distinct treatment of parities. However, before implementing such an analysis in our population, it would be essential to justify this decision by estimating the variance components for the different parities.

To verify the suggestions proposed by *Fernández et al. (2008*), a multivariate analysis was conducted for the two traits currently included in the selection index: NBA and LW21. To perform this analysis, the VCE program *(Groeneveld et al., 2008)* was used fitting an animal model that incorporated several systematic fixed effects such as farm, boar genotype and farrowing season. Furthermore, random effects were taken into account, encompassing the birth batch and the direct genetic effect including the kinship matrix. In addition, NWP was included as covariate for LW21 analysis.

The heritability estimates for NBA showed variation, ranged from 0.13 in the first parity to 0.21 in the fourth parity, with a slight decrease to 0.08 in the sixth or subsequent parities. However, despite these slight differences in heritability values for successive parities, the genetic correlations were consistently high, always above 0.80, except for correlations between the sixth and subsequent parities with the first (0.67) and the third (0.77) parities. Similarly, for LW21, the heritability estimates ranged from 0.30 to 0.41 across different parities, indicating a moderate to high heritability for this trait. The genetic correlations for LW21 were also consistently high, above 0.80, except for the first parity with the sixth and subsequent parities (0.70).

Considering the slight variations in heritability values of successive births and the high genetic correlations observed between successive parities for both

NBA and LW21, the results do not currently advise the implementation of a multivariate analysis for prolificacy and maternal aptitude in the breeding program.

Effect of inbreeding

To assess the potential impact of accumulated inbreeding due to the selection program, inbreeding depression for these traits has been estimated. For this analyses, we managed a database consisting only of litters from sows with an inbreeding coefficient (F) greater than zero. The inbreeding coefficient was calculated using pedigree data with R Studio software *(Wellman, 2019)*. To estimate inbreeding depression a linear model was fitted in proc GLM of SAS including F of the sow as a covariate. Additionally, another model was tested, where the F was adjusted as a fixed effect with levels defined based on specific ranges. The number of litters included was 4,100 from 1,576 sows.

The results obtained from both models were consistent and showed the same direction of effect. In terms of interval traits, no significant effect of inbreeding was observed. However, for TNB and NBA, there was a significant regression value of -0.131 and -0.132 piglets, respectively, for each 1% increase in F. Regarding litter weight traits, the regression was not significant in LW0, although the estimate was negative (-0.043 kg). On the other hand, for LW21 the regression was significant, showing a decrease of -0.267 kg per 1% increase in F.

In the analysis of F as a fixed effect with predefined intervals, two intervals with the highest levels of inbreeding (defined as greater than 13% and less than 25%, and equal to or greater than 25%) consistently showed lower estimate of least square means compared to the other intervals for TNB, NBA and LW21. For LW0, the interval effect of F was not found to be statistically significant. However, it is worth noting that the interval equal to or greater than 25% displayed a lower LSM value compared to the other intervals, suggesting a possible trend of inbreeding depression in this category as well.

Since 2021, the increase in inbreeding has been considered in the farms when selecting future breeders through controlled mating, which optimizes genetic progress without raising inbreeding levels. Additionally, inbreeding limits of 12.5% for future dams and 6.25% for future boars have been established.

Therefore, inbreeding depression must be taken into account in the breeding program in the future and be incorporated in the genetic evaluations.

Impact of Sow Age at First Farrowing

The impact of sow age at first farrowing (AFF) on subsequent production has been addressed by several authors *(Babot et al., 2003; Engblom et al., 2008)*. A trial carried out in 2022 in a reduced group of gilts from this population, showed that the age of the sow at farrowing (between 345 and 458 days) had a significant effect, producing an increase in the weight of the piglets of 0.290 kg per born piglet *(Gutiérrez et al., 2022)*. In our herd, AFF is relatively high compared to more prolific commercial pig breeds. Many sows have their first farrowing at more than one and a half years of age, with a mean AFF of 17.7 months. To analyze the effect of age at first farrowing on reproductive traits, we used data from sows with at least two parities and employed two approaches within the GLM procedure in SAS. On one hand, AFF was included as a covariate in separate analysis for each parity order, aiming to assess how it influences reproductive traits as the sow's productive life progresses. On the other hand, in an analysis of all the data, AFF was included in the model as a fixed effect. The levels for AFF were defined, similar to the approach used for inbreeding, based on the set of AFF values present in the database. The total number of litters analyzed was 16,632 coming from 2,914 sows.

The results of the individual analysis for each parity revealed that AFF significantly affects prolificacy and maternal aptitude traits in the first parity. The regression values of AFF on TNB and NBA were 0.070 and 0.063 piglets, respectively, for each additional month of AFF. Similarly, for LW0 and LW21, the regression values were 0.032 and 0.169 kg, respectively, per each additional month of AFF. The effect of AFF was still present in the second parity for TNB, with a regression value of 0.039 piglets, and for LW21 persisted with a regression value of 0.127 kg. However, the effect was attenuated for NBA with a regression value of 0.022, although it did not reach statistical significance (p-value=0.113), and disappeared for LW0. In successive parities, AFF had minimal influence on the traits, except for LW21, where the regression remained positive and significant until the fifth parity.

The analysis including all parities with AFF as a fixed effect revealed that the influence of AFF was significant for TNB and LW21, with p-values of 0.0013 and <0.0001, respectively. Younger ages at first farrowing had some detrimental effects on both traits, with lower LSM estimates observed at the 13, 14, and 15-month intervals of AFF for TNB. Additionally, lower LSM values were evident for LW21 at the 13-month interval. For NBA and LW0, the 13-month interval showed lower LSM values, but without statistical significance.

In summary, the analysis reveals a detrimental effect of young sow age at the time of first farrowing, especially on the weight of the litter at 21 days, although this effect is not as evident in terms of birth weight. However, since this effect is negligible on NBA, the benefits of a longer reproductive life of the sows that enter the first mating at a young age outweigh the slight decrease in litter weight during the early stage of production, from weaning to 50-60 kg. Table 1 show, for several age intervals, the percentage of sows with seventh or more parities respect to the number of sows at first parity. This percentage is clearly lower in the intervals of greater first farrowing age.

Table 1. Number of sows at first parity, number of sows raising a seventh or subsequent parities, and percentage between them in certain sow age intervals at first farrowing

Age intervals (months)	Number of sows at first parity	Number of sows raising a seventh or subsequent parities	Percentage
<13	19	11	58
13	69	31	45
14	231	91	39
15	407	175	43
…			
21	175	57	33
22	138	47	34
>22	187	58	31

Estimation of variance components

One of the main steps in our breeding program involved estimating variance components for the reproduction traits with known low heritability. In this study, we presented the analysis performed using the VCE program *(Groeneveld et al., 2008)* by applying a bivariate animal model to the following pairs of traits: TNB and NBA, NBA and LW0, NBA and LW21, NBA and IBB, LW0 and LW21, and LW21 and IBB. The model included the following systematic effects: farm, boar genotype, farrowing season, and parity order (ranging from 1 to 7 or greater than 7, with a value of 0 assigned to founder sows whose parity order was unknown). Additionally, the random effects included the birth batch, permanent environmental effect of the sow, and the direct genetic effect, which included the kinship matrix. TNB and NWP were used as covariates with LW0 and LW21.

The estimated genetic parameters confirmed previous findings in this breed reported by *Rodríguez et al. (1994), Fernández et al. (2008)* and *García-Casco et al. (2012)*. Low heritability values were observed for TNB with a value of 0.083, NBA ranging from 0.067 to 0.080, and IBB with values of 0.059 or 0.039. On the other hand, for maternal aptitude traits, the heritability values were moderately higher: LW0 with a value of 0.101 and LW21 ranging from 0.156 to 0.164. In terms of genetic correlations, NBA showed a high correlation with TNB (0.96), moderately correlated with LW0 (0.46), somewhat lower with LW21 (0.19), and moderate but negative with IBB (-0.36). A medium-high correlation (0.60) was identified between the two litter weights, while a very low correlation (0.033) was observed between LW21 and IBB.

In the current breeding program, a genotypic aggregate is utilized, where weights were assigned to NBA and LW21 based on their economic values. Notably, the economic weight for NBA is significantly higher than that for LW21. Additionally, an empirical aggregate is calculated, with LW21 contributing 20% to the overall index, in order to enhance maternal aptitude. The values presented here reveal the challenges faced in achieving significant responses to selection in this type of herd, particularly concerning litter size.

Near future

Population size and low heritability are challenging obstacles to overcome in this breeding program. As a potential solution, marker-assisted selection could serve as a complement to conventional BLUP genetic evaluations for these traits. To achieve this, the design of a SNP panel including SNPs mapped in key genes such as *ESR1*, *GHR*, *PRLR*, and *LHR*, among others, is currently undergoing, and association analyses between genotypes and phenotypes using the reproductive data of 500 sows are being conducted. These analyses hold great promise and are likely to provide valuable insights into the genetic basis of the reproductive traits in our herd. If successful, this approach could mark a significant step forward in our selection efforts and help improve the overall performance and productivity of the herd.

In future evaluations, there are plans to include IBB in the breeding goal. However, this inclusion will be limited to two farms, MC and TJ, which have a continuous farrowing management system. In contrast, the other two farms, GM and DH, follow a discontinuous farrowing management system, and thus, IBB cannot be included in the breeding goal at this time. To address the weighting of IBB in the genotypic aggregate, economic weight calculation or adopt a more empirical approach to weighting need to be considered. Both methods have their merits, and the final decision will depend on various factors, including the specific goals of the breeding program, the economic importance of IBB, and the available data and resources.

The problem of the negative impact of global high temperature in production is expected to worsen in the future due to the ongoing climate emergency. To address this issue, we are starting to include the temperature and humidity index (THI) in our animal model, following the approach described by *Muñoz et al. (2022)*. THI will be calculated at the time of mating or a few weeks after it for TNB, NBA and LW0, and at birth for LW21. The inclusion of THI offers a more appropriate alternative to the simple seasonal effect so far included in the analysis models. By considering THI, we can better adjust for the impact on the reproductive characteristics of temperatures above normal in October, September, March, or April, similar to those experienced during summer. The primary objective is to select sows with improved production data specifically during periods of high temperatures.

The inclusion of the inbreeding effect of the sow in the models faces a challenge due to a high number of sows with F equal to zero based on pedigree information. To overcome this issue, we can address it by estimating molecular inbreeding. An ongoing study involving massive genotyping of 500 slaughtered pigs from several *Montanera* seasons will provide valuable molecular genetic information. By using this data, molecular inbreeding can be predicted, allowing for a more comprehensive analysis of inbreeding effects on reproductive traits, and enable us to make more informed decisions in managing inbreeding and maintaining the genetic diversity of the herd for sustainable and improved performance over time.

Finally, it is crucial to continue studying and addressing the challenges encountered in the breeding program, with a focus on implementing strategies to improve litter weight and prolificacy while simultaneously preserving meat quality. To achieve this, we plan to conduct an analysis of variance component estimates using a multivariate model including NBA, LW21 and the percentage of intramuscular fat of the loin. However, combining the sow database containing reproductive records with the slaughter pig database containing growth and carcass data is a complex task. To effectively address this, we will need to employ appropriate genetic-statistical models that can accurately estimate the genetic and environmental parameters

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STREPTOCOCCUS SUIS, TWO-FACED GAME CHANGER

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Invited paper

Abstract: *Streptococcus suis* infection is one of the major health problems in the swine industry worldwide. During the last decade, the number of reported human cases due to *S. suis* has dramatically increased, and while most sporadic human cases of infection appear to be due to close occupational contact with pigs/pork products. *S. suis* infection is considered to be multifactorial, with transition from subclinical to clinical that depends on many factors. These factors can be divided in two groups, host-based and external factors. Pathogenesis of *S. suis* infection can be divided into 4 phases: adherence to and colonisation of mucosal and epithelial surfaces, invasion into deeper tissues and entering the bloodstream, crossing blood-brain barrier and inflammation. *S. suis* virulence-associated factors are divided into the following 4 groups: surface/secreted elements, enzymes (such as including proteases), transcription factors and regulatory systems and other factors (such as transporting and secreting systems). Therefore significant research support is needed to obtain a vaccine as a valuable and universal protection against disease caused by *S. suis* strains and thus national and international support will be crucial for the aim many researchers hope for.

Key words: *Streptococcus suis*, pigs, commensal, pathogen, virulence factors

Introduction

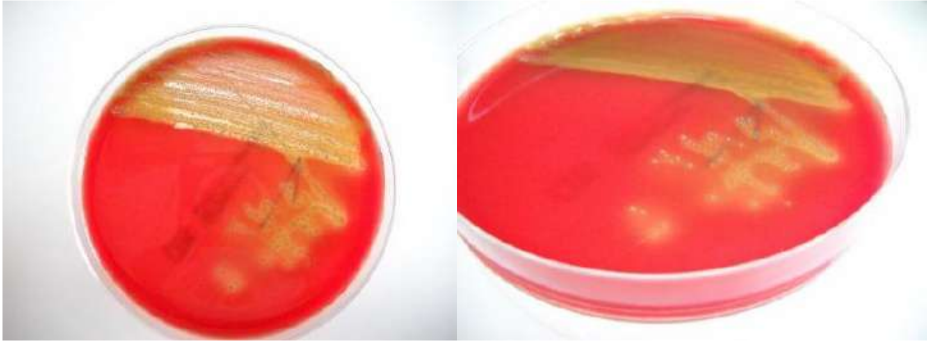
Streptococcus (*S. suis*) is a commensal of the swine respiratory system, in particular of the tonsils and nasal cavities, but it can also cause serious infections with high mortality rate.

Streptococcus suis infection is one of the major health problems in the swine industry worldwide. This pathogen is the most prominent cause of meningitis and septicemia in the porcine industry, however, other pathological conditions have also been described, such as arthritis, endocarditis, pneumonia, and septicemia with sudden death.

S. suis is primarily considered a major swine pathogen, but it has been increasingly isolated from a wide range of mammalian species, birds and even fish species. These findings suggest the existence of complex epidemiological patterns of the infection, since other animal species might also be a source of swine infection (*Gottschalk et al., 2010b).* Human *S. suis* infections have usually been considered sporadic (*Arends and Zanen, 1988*). During the last decade, the number of reported human cases due to *S. suis* has dramatically increased, and while most sporadic human cases of infection appear to be due to close occupational contact with pigs/pork products, particularly in Western countries (farmers, veterinarians, butchers, food processing workers, etc.), two epidemics were recorded in China in 1998 and 2005 (*Goyette-Desjardins et al., 2014*). However, the important outbreak in China that occurred in 2005 and that affected more than 200 people with a mortality rate of nearly 20% changed the perspective on the threat of *S. suis* to human health.

Microorganism characteristics, number and distribution of serotypes

Streptococcus suis is a facultative anaerobic, Gram-positive coccoid bacterium that has the ability of capsule synthesis and secretes heamolysin. The cell wall antigenic components of *S. suis* are similar to those displayed by group D streptococci (*Stanojkovic et al., 2012*). However, *S. suis* is not genetically associated with group D streptococci *(Kilpper-Balz and Schleifer, 1987).* The organism grows well on media usually used for isolation of streptococci, most frequently sheep blood agar, and forms glistening, round, slightly grey alpha haemolytic colonies (Picture 1). *S. suis* has vary variable biochemical properties (*Stanojković et al., 2014*) and thus must be confirmed by serotyping.



Picture 1. Alpha haemolytic colonies of *Streptococcus suis* on CNA 5 % sheep blood agar (*Stanojković et al., 2012*)

Previously, *S. suis* had been classified into 35 serotypes (serotype 1/2, and 1–34) (*Higgins and Gottschalk, 1995*) and then reduced to 33 serotypes because serotypes 32 and 34 were determined to be *Streptococcus orisratti*, streptococci that can be often isolated from rats (*Hill et al., 2005*). More recently, it was proposed to remove serotypes 20, 22, 26 and 33 from the *Streptococcus suis* taxon (*Tien et al., 2013*). Hence, it is currently considered that there are 29 true *Streptococcus suis* serotypes.

During the last 12 years, more than 4500 serologically confirmed strains recovered from diseased pigs have been reported. Globally, the most dominant serotypes isolates from clinical cases in pigs are serotypes 2, 9, 3, 1/2 and 7, while 15.5% were so called non-typable strains. However, there is clear geographical distribution of serotypes.

Goyette-Desjardins et al. (2014) summarize strain prevalence in Europe and America. In Canada the most prevalent serotype is serotype 2, while in United States serotype 3 is the most prevalent. In these countries there is only a slight difference in percentages of prevalent strains, demonstrating similar distribution of serotypes when data from Canada and the USA are combined. Both, serotypes 2 and 3 are the most prevalent from diseased pigs with 24.3% and 21.0% prevalence respectively, followed by serotypes 1/2, 8 and 7 (*Goyette-Desjardins et al., 2014*). This can be explained by easy and free movement of animals from United states to Canada and vice versa. In South America, all results came from Brasil, stating that serotype 2 is the most prevalent with 57.6% reported cases followed by serotypes 1/2, 14, 7 and 9. In Asia, the majority of results regarding serotype affiliation came from China and South Korea. In China the most prevalent serotypes detected in infected pigs are, in decreasing order of prevalence, serotypes 2, 3 4, 7, and 8. On the contrary, in South Korea serotype 2 had a prevalence of only 8.3%, the same as serotypes 8 and 33 while the most dominant were serotypes 3 and 4 with 29.2|% and 20.8% respectively, while serotypes 16 and 22 had distribution of 4.1%. Other Asian countries reported many human cases of disease but strains isolated from pigs only refer to slaughterhouses and healthy pigs. Similarly, in Japan there have been 10 human *S. suis* cases reported but studies on the distribution of isolates from ill pigs have not been published ately and all of the research dates before 1987. In Cambodia, Philippines, Laos and Singapore, human cases were diagnosed recently but there are no data avilable on the epidemiology of *S. suis* infections in pigs.

In Europe, the largest number of *S. suis* serotypes isolated from clinically ill pigs belongs to serotypes 1 to 8 *(Reams et al., 1996; Higins and Gottschalk, 2005)*. Most of the *S. suis* serotype distribution reports date before year 2000. *S. suis* serotype 2 was the most common in clinical cases in Italy, France and Spain, whreas serotype 9 was more frequent in the Netherlands, Germany and Belgium. Recent conducted reaserch on serotype distribution in Spain suggest that serotype 2 is no longer the most prevalent serotype, and that serotype 9 is the one most frequently isolated from diseased pigs. Behind serotype 9 is serotype 2, followed by serotypes 7, 8 and 3 (*Luque et al., 2010*). In Netherlands, serotype 9 was the most prevalent in data collected between 2002-2007 followed by serotypes 2, 7, 1 and 4. Contrary to the fact that serotype 9 becomes most prevalent in some countries, there were no human cases reported that were associated with this serotype. In Belgium and United Kingdom, serotype 1 was the predominant in ill pigs while in Denmark serotype 7 was the most frequent one. In Southern Europe, serotype distribution was done in Serbia where serotype 2 was the only serotype found in piglets that had clinical symptoms of meningitis (*Stanojkovic et al., 2015*). Beside that various *S. suis* serotypes were found in healthy animals (*Stanojković, 2012*).

S. suis infection in pigs

The natural habitat of *S. suis* is the upper respiratory tract of pigs, more particularly the tonsils and nasal cavities, but also the genital and digestive tracts (*Goyette-Desjardins et al., 2014*). Almost 100% of pig farms worldwide have carrier animals, and that puts *S. suis* as one of the most important bacterial pig pathogens with quite established infection patterns.

Transmission of *S. suis* among animals is considered to be mainly through the respiratory route. Indeed, investigation of presence of alpha haemolytic streptococci, enterococci and streptococci-like bacteria in tonsil and nose swabs of clinically healthy pigs in one research (*Stanojković et al., 2012*) showed that most species belonged to *S. suis (*64%*).*

The sow is also a source of infection. Gilts and sows may harbor *S. suis* in the uterus or vagina, but no male reproductive organs have been shown to be infected. Piglets born to sows with uterine or vaginal *S. suis* infections become infected at birth, before birth, or soon after birth (*Robertson and Blackmore, 1989*), but mostly when passing through the birth canal.

Transmission of virulent strains between herds usually occurs by the movement of healthy carrier animals. The introduction of carrier pigs harboring virulent strains (breeding gilts, boars, weaners) into a noninfected recipient herd may result in the subsequent onset of disease in weaners and/or growing pigs (*Higgins and Gottschalk, 2005*). Horizontal transmission is important especially during outbreaks when diseased animals shed higher numbers of bacteria, increasing transmission by direct contact or aerosol (*Cloutier et al., 2003*).

S. suis type is an important contaminant of feces, dust and water. In water, the organism survives for 10 min at 60°C and for 2 h at 50°C. At 48°C, *S. suis* can survive in carcasses for 6 weeks (*Clifton-Hadley et al., 1986).* At 0°C, the organism can survive for 1 month in dust and for over 3 months in feces, whereas at 25°C, it can survive for 24 h in dust and for 8 days in feces. *Dee and Corey (1993)* have also been shown that to transmission of *S. suis* strains can be through fomites, such as manure-covered work boots and needles. *S. suis* can be inactivated using many disinfectants, such as diluted bleach. Organic matter reduces effectiveness of chemical disinfectants and should be completely removed with thorough washing prior to application. Even though *S.suis* survives in water up to 2 hours at 50°C but only 10 minutes at 60°C, use of heated pressure washers compared with non-heated is of limited value since water cools rapidly on surfaces negating potential benefit (*Clifton-Hadley and Enright 1984*).

Vectors of *S. suis* can play a role in disease transmission. Houseflies can carry *S. suis* strains for 2 for 5 days, and have been shown to easily transmit the disease migrating between farms (*Enright et al., 1987*). Mice can be experimentally infected orally or intranasally with *S. suis* type 2, and the transfer of organisms from orally infected mice to uninoculated mice has been established (*Williams et al., 1988; Robertson and Blackmore, 1990*). Transmission of disease between mice and pigs is believed to occur (*Williams et al., 1988*).

All categories of pigs can be affected by the disease caused by *S. suis*, including suckling piglets, older piglets and fatteners. *S. suis* carriage rates may vary between herds and can range from 0% to up to 80-100% *(Amass et al., 1997)*. More than one serotype of *S. suis* often colonizes individual pigs. In one study, 31% of pigs had only one serotype of *S.suis* in their nasal cavities, 38% had two or three serotypes, and 6% had more than four serotypes *(Monter Flores et al.,* *1993).*According to *Silvonen et al. (1988)* even if all the pigs in the herd are infected with some strains of *S. suis* clinically apparent disease varies and is usually below 5%. The prevalence of and the morbidity and mortality from *S. suis* vary among herds.

Even when the pig carrier rate is near 100%, the incidence of the disease varies from period to period and is usually less than 5% *(Clifton-Hadley et al., 1986)*. Clinical signs can vary between herds, depending on the pathogenesis of the disease. Pigs with per acute *S. suis* infection may be found dead with no previously noticed signs of disease or die within hours of the onset of clinical signs. In the acute form of the disease, clinical signs may include fever (up to 42°C), depression, anorexia and lassitude, followed by one or more of the following: ataxia, incoordination, tremors, opisthotonus, blindness, loss of hearing, paddling, paralysis, dyspnea, convulsions, nystagmus, arthritis, lameness, erythema, and/or abortion *(Staats et al., 1997)*. So, we can conclude that meningitis is the major feature of *S. suis* infection in pigs but other organs (joints, heart, lungs, reproductive organs etc.) can also be affected.

S. suis infection in humans

Different from pigs infection, the main route of entry of *S. suis* in humans is thought to be through contact of cutaneous lesions, most usually on the hands and arms, with contaminated animals, carcasses or meat, although in some cases, no wound was detected; bacteria may colonize the nasopharynx, as observed in swine; and the gastrointestinal tract, as suggested by diarrhea as a prodromal symptom *(Fongcom et al., 2001; Wertheim et al., 2009)*. The outbreak in China in 2005 caused by *S. suis* affected more than 200 people, with almost 20% mortality rate. This epidemic has completely changed the perception of the danger which this pathogen presents to human health *(Stanojkovic et al., 2014)*. Period of incubation ranges from just a few hours to few days *(Fongcom et al., 2001)*. Just like in pigs *S. suis* produces meningitis as the main feature of disease but cases of endocarditis, pneumonia, peritonitis, arthritis and other less common clinical signs can be seen as the part of generalized septicemia *(Arends and Zanen, 1988)*. Also, there have been described per acute infections related to this pathogen which were usually in the form of streptococcal toxic shock-like syndrome (STSLS) with almost 20% death cases. In Western countries, *S. suis* disease has been considered a rare event in humans. Most cases of human infection are related to close contact with meat or live animals: pig farmers, abattoir workers, persons transporting pork, meat inspectors, butchers, and veterinarian practitioners *(Tang et al., 2006)*.

According to *Hoa et al. (2011)* slaughterhouse pigs are a major reservoir of *Streptococcus suis* serotype 2 capable of causing human infection. *Cheung et al.*

(2008) examined 78 samples of raw pork lean meat from retail markets and wet markets and determined that *S. suis* can be found in every sample although in different levels (MPN/g). Similar meat prevalence in Serbia *(Stanojković et al., 2016)* showed serotype 2 was the most isolated serotype from fresh pork with 46,1 % of isolated *S. suis* serotypes followed by serotype 9, 7, 3, 1 and 4. Slaughtered pigs had similar prevalence of *S. suis* strains just like those data reported for clinically ill pigs.

Stanojkovic et al. (2016) found that there was a significant difference in the presence of *S. suis* strains on the basis of sample collected. In above mentioned authors research hog head was highly contaminated with *S. suis* serotype 2 strains (prevalence of 25%). This result is maybe expected since *S. suis* is normal inhabitant of respiratory system such as tonsils, and also slaughtered pigs are held in that kind of position that allows water to spread bacteria from hind part of the body to the head. Same authors found that presence of *Streptococcus suis* serotype 2 in liver, kidneys, shoulder, ham, loin and belly was 20%, 12%, 5%, 5%, 5%, and 10% respectively. Have found overall prevalence of *S. suis* serotype 2 in pork of 12.8%. Same authors mentioned that prevalence of *S. suis* serotype 2 in fresh meat was 10.8% but it was not clear referring to the part of the body that fresh meat was taken from. These authors detected 15.4% prevalence of *S. suis* serotype 2 in liver and other offal and demonstrated that *S. suis* accumulates in the kidney during *S. suis* infection.

It can be concluded that processing and consuming of uncooked or partially cooked pork meat in Asian countries is major risk factor for infection.

Different from Asian countries infected persons in Western countries are usually adult males and this can be readily explained, since many acquire the disease following occupational exposure to pigs or pork products. Affected humans had usually close contact with pigs or meat and very often small cuts on their hands *(Stanojkovic, 2012). Stanojkovic et al. (2012)* found that *S. suis* can readily isolated from butchers knives. Also, there are reports that confirm carrier state in humans, especially abattoir workers *(Sala et al., 1989; Rohas et al., 2001). Strangmann et al. (2002)* determined nasopharyngeal carriage rate of *S. suis* serotype 2 in the high-risk group (butchers, abattoir workers, and meat processing employees) was 5.3%, while those without contact with pigs or pork consistently tested negative. This kind of nasopharyngeal carriage rate has also been shown in pigs *(Higgins and Gottschalk, 2005)*.

In humans, *S. suis* usually produces a purulent meningitis but also endocarditis, cellulitis, peritonitis, rhabdomyolysis, arthritis, spondylodiscitis, pneumonia, uveitis, and endopthalmitis have also been reported *(Gottschalk et al., 2010b; Wertheim et al., 2009)*. Also, there have been described per acute infections related to this pathogen which were usually in the form of streptococcal toxic shock-like syndrome (STSLS) that has been associated with most of the death cases in China 2005 epidemics *(Lun et al., 2007)*. The most important often mentioned sequela of *S. suis* infection are vestibular dysfunction or unilateral or bilateral hearing loss.

S. suis as commensal

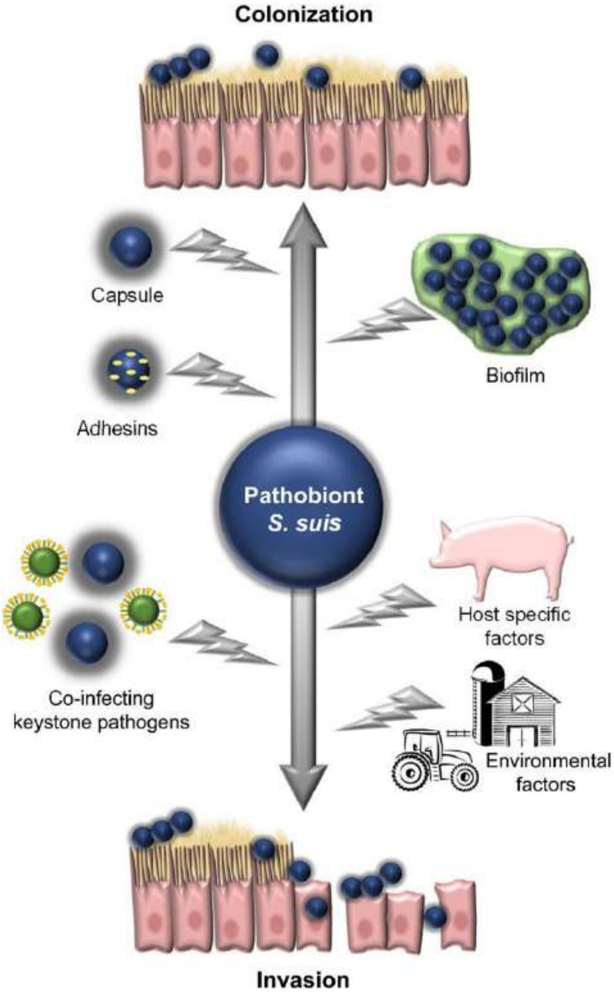
S. suis is a commensal bacterium with a natural habitat being usually the tonsils and nose cavities of healthy pigs. *S. suis* two million (mega) base pairs (2 Mbp) genome contains sequences that encode variety of factors such as adhesins and enzymes which enable it to colonize pigs tonsils in cohabitation with other bacteria Bacterial adherence is the first step and maybe crucial for development of a carrier state.

In recent years there have been many researches regarding mechanisms of adherence and tissue tropism of *S. suis*, but at the moment exact adhesin responsible for infection can not be precisely explained. Study by *Chuzeville et al. (2017)* reports that *S. suis* serotype 2 and 9 strains express genes coding for multimodal adhesion proteins known as antigen I/II (AgI/II) which in the presence of salivary glycoproteins AgI/II leads to the aggregation of *S. suis*, adherence, and colonization of the upper respiratory tract of pigs. In serotype 9, the AgI/II is responsible for aggregation and biofilm formation and these aggregated group of bacteria are partially resistant to and protected from high acid content (low pH) in the stomach which leads to colonization of the intestine (*Chuzeville et al., 2017*).

Adhesin FhB (factor H-binding protein) of *S. suis* is responsible for binding of the blood complement H factor which results in enhanced adherence of the bacteria to epithelial and endothelial cells and protecting bacteria from complement mediated phagocytosis. (*Roy et al., 2016*).

Polysaccharide capsule is the major factor involved in the pathogenesis of *S. suis* infection. Capsule is down regulated by *S. suis* genes and its thickness depends on the environment in which the bacterial cell resides. According to *Gottschalk and Segura (2000)* capsule thickness increases during epithelial invasion and adhesion and is involved in enabling *S. suis* to escape phagocytosis. Capsule synthesis is regulated is regulated by transcriptional regulator catabolite control protein A (CcpA) (*Willenborg et al., 2011*). Non-encapsulated phenotype strains lack the ccpA gene.

Biofilm is one of the components that enable the bacteria to colonize tissues, resist host defense and antibacterials, but is also involved in pathogen-comensal relationship. Biofilm formation is regulated by luxS gene (coding for the enzyme S-ribosylhomocysteinase, LuxS). It has been reported that virulent strains of *S. suis* have a higher ability to produce biofilms than avirulent strains (*Wang et* *al., 2011a*). Biofilm formation results in lower expression of virulence-associated genes with less damage to the host tissue, thus can explain the colonization of host with virulent strains while being harmless with no signs of disease.



Picture 2. *S. suis* infection (*Vötsch et al., 2018*)

Commensal to pathogen transition

S. suis infection is considered to be multifactorial, with transition from subclinical to clinical that depends on many factors. These factors can be divided in two groups, host- based and external factors. Genetics, age, pre-existing diseases and infections, immunosuppresssion are one of the most prominent host-based factors. Climatic changes in the breeding objects, poor hygiene, bad ventilation, weaning, overcrowding, and other external stressors are usually the key starters of clinical infection (*Vötsch et al., 2018*). *S. suis* strain virulence and the presence of virulence factors is also an important feature for pathogenesis. Human infection usually depends on the route of infection and immunocompetence of the human host.

Pathogenesis of *S. suis* infection can be divided into 4 phases: adherence to and colonisation of mucosal and epithelial surfaces, invasion into deeper tissues and entering the bloodstream, crossing the blood-brain barrier and inflammation. All of these phases are mediated by specific virulence factors and none of the phases will be explained here regarding their complicity and in limited knowledge for the some parts of pathogenesis.

Feng et al. (2014) classified *S. suis* virulence-associated factors into the following 4 groups: surface/secreted elements, enzymes (such as including proteases), transcription factors and regulatory systems and other factors (such as transporting and secreting systems). First group of the surface/secreted elements enzymes includes:

- capsular polysaccharides (*cps*);
- extracellular protein factor *(epf)*;
- fibronectin binding adhesin (*fbps*);
- muramidase released protein (*mrp*);
- protein of 38 kDa localized on bacterial surface (*38 kDa*);
- secreted thio-activated heamolysin (suilysin)
- surface-associated subtilisin-like serine protease (*SspA*)
- histidine triade immunogenic cell surface protein (*htpS*);
- Sat surface protein (*sat*);
- serum opacity factor (*ofs*);
- surface antigen protein (*sao*);
- sortase A (*SrtA*), catalyzing cell wall sorting reaction;
- pili

The second enzyme group of virulence factors is represented by more than 20 bacterial enzymes such as:

- GlnA, glutamine synthetase (*glnA*);
- Gdh, glutamate dehydrogenase (*gdh*);
- enolase (*eno*) catalyzing dehydration of 2-phosphoglycerate to phospho-enolpyruvate enzyme catalyzing lipoteichoic acid (LTA)-d-alanylation (*dltA*);
- peptidoglycan N-acetylglucosamine deacetylase (*pgdA*);
- inosine 5-monophosphate dehydrogebase [Impdh] (*impdh*);
- N-acetylneuraminic acid (sialic acid) synthetase (*neuB*);
- UDP *N*-Acetylglucosamine 2-Epimerase (*neuC*)
- glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)
- DNase (112 kDa)
- IgA1 (*IgA1*) protease cleaving immunoglobulin A;
- superoxide dismutase (*sod*)
- adenosine synthase (*Ssads*)
- LuxS or S-ribosyl homocysteinase

Transcription factors include more than 15 elements which include:

- AdcR (*adcR*)
- catabolite control protein A (*ccpA*)
- ArgR (*argR*)
- Rgg (*rgg*)
- Fur (*fur*)
- PerR (*perR*)

The fourth group of transporters/secretion systems comprises following factors:

- VirA (*virA*)
- Trigger factor (*Tig*)
- FeoBA *(feoBA)*
- Type IV like-secretion system (T4SS-like system)

Conclusion

Streptococcus suis is a swine pathogen that causes important economic losses in the swine industry worldwide. This bacterium has great ability to adapt to the present host, being commensal, but also to be the cause of wide range of pathological findings, including meningitis, septicemia and endocarditis, but also the cause of sudden death. The task of battling the disease is not easy because of the great diversity among virulent *S. suis* strains with many virulence-associated factors. It is very difficult to implement effective preventive measures in pigs but also for the persons that come into close occupational contact with pigs and pig products, especially employees of the meat industry. The goal of providing a functional vaccine against *S. suis* infection is being undertaken with promising results.

Therefore significant research support is needed to obtain vaccine as a valuable and universal protection against disease caused by *S. suis* strains and thus national and international support will be crucial for the aim many researchers hope for.

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RELEVANT BIOSECURITY MEASURES TO PREVENT THE SPREAD OF AFRICAN SWINE FEVER IN THE DOMESTIC PIG PRODUCTION SECTOR IN SERBIA

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Invited paper

Abstract: African swine fever (ASF) is currently the most important viral infectious disease affecting domestic pig production worldwide. The first case of ASF in Serbia was confirmed in 2019 in a backyards domestic pig population. Since then, numerous outbreaks in domestic pigs and wild boar have been reported throughout the country despite the efforts of the veterinary authorities to control the disease. The lack of an effective vaccine is one of the main constraints, and the only currently available option to prevent ASF infections is the application of biosecurity measures. However, in the currently prevailing extensive smallholder and backyards farming systems, farm biosecurity is largely non-existent. The aim of this review was to identify specific relevant biosecurity measures as the way to minimise ASF-risks factors and prevent disease spreading in the current pig production sector in Serbia. Moreover, the main risk factors for ASF spreading and transmission at the domestic/wild boar interface, biosecurity practices in different production systems, and possible future control measures are discussed. The identified relevant biosecurity measures as well as risk factors need to be strictly addressed in order to prevent further ASF spread in Serbian pig production sector.

Key words: African swine fever, biosecurity, domestic pig, Serbia

Introduction

African swine fever (ASF) is a contagious viral disease in hosts belonging to the *Suide* family, caused by a double-stranded DNA virus, a member of the family *Asfarviridae*, genus *Asfivirus* (*Bellini et al., 2016; Penrith et al., 2023*). Since the ASF virus (ASFV) was first introduced in Georgia (2007), it has progressively spread on the European continent (*de la Torre et al., 2022*). Today the geographical expansion of ASF continues despite the implementation of disease control measures (*Penrith et al., 2021; Scollo et al., 2023*). The severity of the epidemiological situation is also indicated by the fact that, since ASF first case in Europe, disease elimination has only been successful in two countries (*Bellini et al., 2021*). The most of ASF outbreaks were recorded in wild boars, suggesting that the wild boar population is currently the predominant host of ASF (*Omelchenko et al., 2022; Viltrop et al., 2022*). Indeed, when ASF was introduced to the Baltic States in 2014, the epidemiological role of wild boars became evident and a new infection cycle was described in European continent: the wild boar–habitat cycle (*Chenais et al., 2019; Cwynar et al., 2019*). Today, more than ten years after detection of the first ASF case, the disease became endemic in wild boar population in many countries in old continent (*Viltrop et al., 2022*). However, the ASF-infected wild boar population pose a serious threat to the domestic pig industry (*Chenais et al., 2019*). Moreover, recent outbreaks in domestic pigs frequently overlapped with ASF in wild boars, suggesting that regional prevalence in wild boars is an important risk factor for domestic pig populations (*Plut et al., 2023*). More importantly, the occurrence of ASF in an affected country results in severe constraints for swine producers. Therefore, biosecurity measures, as well as surveillance ASF activities in domestic and wild population are of utmost importance, not only for disease prevention and control but also to detect new cases as soon as possible (*Alarcón et al., 2021; Bellini et al., 2021*).

The impact of ASF vary in different areas of the world and in the different pig farming systems (*Bellini et al., 2016; Scollo et al., 2023*). Since there is still neither an effective treatment nor an effective vaccine, strict biosecurity measures have to be applied in order to prevent ASF in domestic pig production (*Glišić et al., 2023*). Moreover, given the reservoir in the wild boar population, national and international restrictions based on strict biosecurity measures today are the only prevention against the ASF spread in domestic pig sector (*Bellini et al., 2021; Plut et al., 2023*). In the Western Balkan countries smallholder pig farming is common and ASF is present within the domestic pig value chain (*EFSA, 2019; Penrith et al., 2023*). Indeed, in this part of Europe, ASF is severely affecting small-scale domestic pig holders and backyards (*Polaček et al., 2021; Plut et al., 2023*). The observed epidemiological ASF pattern is associated with the characteristics of the domestic pig sector with large percentage of backyards where pig keeping represents an important meat source and a traditional heritage (*Prodanov-Radulović et al., 2022; Prodanov-Radulović et al., 2023*). Indeed, the most important difference between the Western Balkan countries and EU member countries is the structure of pig production sector (*Plut et al., 2023*).

Pig farming is an important sector in Serbia, with many different types of farms producing pork for both the domestic market and for own consumption.

Intensive commercial systems exist mainly in the north of the country, where pigs are kept in indoor facilities and biosecurity measures are most stringent (*Prodanov-Radulović et al., 2020a; Prodanov-Radulović et al., 2020b*). However, in the vicinity of intensive production units, in the surrounding villages, there are a considerable number of smallholdings and backyards, which are more traditional, semi-extensive or extensive pig units. Consequently, today human-related activities are recognized as the cause of ASF transmission from extensive and introduction into intensive pig production sector (*Nešković et al., 2021; Polaček et al., 2021*).

The aim of this review is assessment of relevant biosecurity measures implemented in the practice, in order to identify the main risk factors for ASF spread in the domestic pig sector in Serbia. Through an available epidemiological ASF data analysis, we evaluated the most significant factors in the disease spread, considering all the peculiarities related to domestic pig production in Serbia and interface with specific human-related activities.

Overview of the biosecurity in the pig production sector in Serbia

In Serbia the existence of highly variable pig farming system was recently reported (*EFSA, 2019; Prodanov-Radulović et al., 2020a*). Considering the pig production, the main and highly important difference as compared to the EU member countries is related to the structure and organisation of domestic pig sector (*Prodanov-Radulović et al., 2020b; Prodanov-Radulović et al., 2021*). Serbia has the highest pig density of all countries in the Balkan region, with a total population of 2.7 million pigs (*EFSA, 2019*). The estimated number of pigs has fluctuated over the years, and nowadays it shows constant decreasing (*Polaček et al., 2021*). However, in terms of percentage, over 50% of the domestic pig population is located in production units with very low or no biosecurity measures (*Prodanov-Radulović et al., 2020a; Prodanov-Radulović et al., 2022*). In the EU member countries, pig farms are classified into three categories: non-commercial farms (pigs kept only for own consumption); commercial farms and outdoor farms (*Bellini et al., 2021*). This classification considers the commercial attitude of the holdings and it controls two important facts: the risk of ASF spreading by trading pigs and the risk for the farm of being exposed to source of infection (*EFSA, 2019*). However, the pig farming in Serbia include five different pig production holdings: commercial industrial pig production (intensive production) with the highest level of biosecurity; family farm of type A (farm with more than 10 animals and biosecurity measures which are less stringently implemented); family farm of type B (farm with more than 10 animals and low level of biosecurity); backyards (few animals/less than 10 pigs kept mainly for self-consumption, with often inefficient or total absence of biosecurity)(*Prodanov- Radulović et al., 2021; Prodanov-Radulović et al., 2022*). Finally, extensive semi-enclosed and free-range production includes animals kept in semi-fenced or unenclosed areas without the use of biosecurity measures, where domestics often share the habitat with wild boars (*Cwynar et al., 2019; Omelchenko et al., 2022; Prodanov-Radulović et al., 2023*).

It should be emphasized that, in Serbia, biosecurity measures for pig production are not officially required by law and are given only in the form of general recommendations (*Polaček et al., 2021*). The government instruction on ASF only requires that commercial (intensive) and type A family farms have an official written and implemented biosecurity plan specific to the farm conditions. However, it does not provide any details on what the biosecurity plan must contain (*Plut et al., 2023*). It is well known that preventing ASF transmission on backyard systems is key to sustainable ASF control (*Milićević et al., 2019; Glišić et al., 2023*). Although the risk factors for ASF transmission are known and frequently discussed, the situation regarding these factors in different livestock production environments in Serbia is largely undetermined.

The biosecurity measures in extensive pig production systems in Serbia

The designation backyard holding stands for a quite heterogeneous family small-scale pig farming system with low biosecurity. Backyard farms are considered prone to ASF introduction and thus are of particular interest in disease prevention and control (*Mutua and Dione, 2021; Penrith et al., 2021*). In the Serbian villages, smallholdings and backyards are common, often with different livestock species (pigs, sheep, cattle, chicken) (*Prodanov-Radulović et al., 2022*). The majority of households have also breeding categories together with the piglets and fatteners. The primary purpose for keeping pigs is family meat supply but also an extra income (*Polaček et al., 2021*). The existence of breeding animals in the backyards is recognised as highly-risk and relevant biosecurity factor in the extensive production. Indeed, breeding boars in the small-scale holdings are frequently moved around for breeding purposes (*Plut et al., 2023*). On the contrary, according to the EU regulations, sows or boars cannot be held on non-commercial farms for mating purposes (*Bellini et al., 2021*). The animal loan practices, i.e., sharing of boars for natural mating in several villages (*Mutua and Dione, 2021*) represent the critical risk factor, due to ASF transmission through direct pig-to-pig contact (*Olesen et al., 2020*). In extensive systems pig feeding strategies depend on feed resources availability. However, domestic pigs frequently have access to swill feeding (*Chenais et al., 2019; Mutua and Dione, 2021*). According to Serbian law, swill feeding is banned but it is difficult to control in distant rural areas. In general, swill feeding is considered to be the most likely source of ASFV in many countries (Bulgaria, Romania) (*de la Torre et al., 2022*). Swill feeding represents an important risk for indirect ASF transmission because of the long-term survival of the virus in pig meat (*Alacron et al., 2021; Bellini et al., 2021*). Another important biosecurity risk is related to home-slaughtering practice. Backyard pigs are mostly slaughtered at home, whenever new meat supplies for family are needed (*Polaček et al., 2021*). Home-slaughtering is considered as a feature of non-professional pig production, which is a known constraint to ASF control (*Olesen et al., 2020; Bellini et al., 2021*). However, domestic pigs home slaughtering is allowed in Serbia. Further, low biosecurity practices and the human factor that creates link to wild boars around the villages are deemed to be the most dangerous combinations for the spread ASFV in domestic pig sector (*Nešković et al., 2021*). Indirect contact through visiting the yard by the neighbours or via shared mechanical equipment cannot be excluded. Thus, direct or indirect contact to contaminated fomites, which entered the stable via human activities, is regarded as source of infection (*Bellini et al., 2016*). Consequently, in the case of ASF outbreak in one backyard, the whole village needs to be regarded as one epidemiological unit (*Milićević et al., 2019*). Moreover, outdoor keeping, semi-free range or free-range pigs is common in some regions in Serbia (*Prodanov-Radulović et al., 2022*). This type of pig keeping represents one of the weakest links in the biosecurity chain and the biggest risk factor for ASF introduction. The free-ranging pigs can act as a bridge in transmitting ASFV between wild boars and domestic pigs (*Plut et al., 2023; Prodanov-Radulović et al., 2023*). In the EU, concerning current ASF situation, the EU Commission has banned outdoor keeping of pigs as the main strategy to avoid ASF spread (*Bellini et al., 2021*).

The biosecurity measures in commercial (intensive) pig farms in Serbia

Despite the fact that all mentioned pig production units are highly extensive, according to the Serbian Law, they are commercial holdings. Actually, if the farmer carries out the official pig labelling, it can obtain the veterinary health certificate and sell/trade the pigs in the country (*Prodanov-Radulović et al., 2022*). However, the intensive commercial pig farms include the classical pig farm structure according to the technological pig production process: farrow-to-finish and/or farrow-to-piglets or fattening only (*Prodanov-Radulović et al., 2020a*). However regarding relevant biosecurity measures, there is a difference between old types of pig farms (built in the period 1970-1980s) and farms built after 2000 (*Prodanov-Radulović et al., 2020b*). In the past, pig holdings were owned by the state. In the 1990s, farms were privatized but the new owners have not been obligated to invest in the modern concept of biosecurity. Indeed, today we have examples of farms, with capacity up to 1200 sows that is still managed by the old type of farrow-to-finish production system. Some of the biosecurity measures that we know today, which are essential for sustainable pig production, are simply not possible to be implement without major investments (*Prodanov-Radulović et al., 2022*). Indeed, the external biosecurity measures cannot always be applied (*Polaček et al., 2021*). The situation is similar for most of the farms and the problem of entrance of transport vehicles to the farm perimeter was identified (feed transport, rendering services, slurry transport and transport to the slaughterhouse) (*Prodanov-Radulović et al., 2021*). Nowadays**,** the major problem from the external biosecurity aspect is also the lack of workers. It is very difficult to find workers who do not have backyards or have contact with domestics and/or wild boar after working time (*Nešković et al., 2021*). In the EU member countries, formal biosecurity plans are generally focused on commercial holdings that exceed a specific size defined by the national legislation (*EFSA, 2019; Bellini et al., 2021*). Regardless of the type of production, high levels of farm biosecurity are considered the most important tools for disease prevention (*Alacron et al., 2021*). Biosecurity measures on farms at the farm entrance (cleaning and disinfection) have an important role in any type of pig production (*De Lorenzi et al, 2020*). *Nešković et al.* (*2021*) recently described an ASF outbreak at a large commercial pig farm in Serbia, where the weakest points identified were the entrances, surrounding farm environment and the possibility that employees were involved in activities linked to backyards and/or wild boars. This research indicate that biosecurity measures applied in intensive pig farms do not always work in practice and are highly influenced by ASF surrounding area contamination and the habits of people involved in pig production (*Glišić et al., 2023; Plut et al., 2023*). The tenacity and resistance to inactivation of ASFV are important aspects that make external biosecurity control hard to manage (*De Lorenzi et al, 2020*).

Conclusion

In order to prevent the ASF spread from wild boar to domestic pig populations, relevant biosecurity measures must be implemented and controlled in the complete pig production sector. The results of the epidemiological research indicate that human-related activities are frequently recognized as the nature-independent factor that contributes to ASF spread. This risk factor that could be avoided through a targeted awareness campaign and education of farmers, pig owners, as well as possibly through investment funded by the relevant authorities.

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SIGNIFICANCE OF *LISTERIA* ISOLATED IN ABORTED MATERIALS FROM COWS

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Abstract: *Listeria* is a microorganism widely distributed in nature and can be found in different types of ecological surroundings, water, wastewater, soil, different types of food, organs, and excreta of animals and humans. The effect of pathogens on health status in animals and humans is very similar. A perinatal and adult form of the disease is present. The course of the perinatal form is most often asymptomatic in females, with mild flu-like symptoms 2 weeks before the abortion. The clinical findings in adults are manifested by the appearance of meningitis with accompanying nervous symptoms. For livestock production, especially in cattle, infection with listeria can lead to abortion, which on the one hand leads to significant economic losses, while on the other hand, since it is a zoonosis, it threatens the health safety of farm employees. Considering that Listeria represents ubiquitous microorganisms, as well as the fact that, in addition to listeria, cow abortions can be caused by various other microorganisms (viruses, bacteria, or protozoan parasites), it is not easy to precisely diagnose the biotic or abiotic causes of abortion. In our study, we monitored the presence of *Listeria monocytogenes* in aborted fetuses of cows. Our goal is to determine the prevalence of these microorganisms in the analyzed samples as well as their importance for the occurrence of abortions and animal health. In 3.5 years, we analyzed a total of 65 aborted samples, of which 7 were positive for *Listeria monocytogenes*. It can be concluded that a relatively small number of samples were examined in the observed period, which indicates a low interest of livestock keepers in monitoring abortion samples. On the other hand, concerning the number of examined materials, the percentage of *Listeria* isolates (>10%) is relatively high.

Key words: abortion, cows, *Listeria monocytogenes*

Introduction

Listeria is microorganisms that, as saprophytes, are widely distributed in nature, especially in places with plant material in the process of decomposition. For these reasons, ruminants, through oro-fecal infection, represent one of the important links of maintenance of this bacterium in nature (*Fenlon, 1999; McCarthy, 1990*). From the genus *Listeria*, only two species are potentially pathogenic: *Listeria monocytogenes* and *Listeria ivanovii* (*Vazquez-Boland et al. 2001*). One of the most important routes of listeria infection in ruminants is the consumption of silage contaminated with this bacterium (*Fenlon, 1999, Wesley, 1999*).Listeria tolerates relatively low pH values, as well as increased salt concentration, and can multiply at refrigerator temperatures (*Lou and Yousef, 1999*). There are two forms of listeriosis, listeriosis associated with abortions in cows and listeriosis in adults. Listeriosis that leads to miscarriage occurs as a result of the transfer of listeria to the fetus through the placenta and the development of chorioamnionitis (*Vazquez-Boland et al., 2001*). Listeriosis in adults is more common (up to 70% of total listeria infections) and is characterized by meningoencephylitis with an altered state of consciousness (behavior), significant movement disorders, and in some cases paralysis (*Vazquez-Boland et al., 2001*).

Cattle abortions are one of the significant problems in livestock production. On the one hand, they lead to health problems of infected animals and can affect the health of farm workers, and on the other hand, they affect the costs of raising and exploiting animals (*Norman et al. 2012; Anderson, 2007; Holler, 2012).* Abortions can be caused by biotic and abiotic factors. Of all the possible factors that contribute to abortion, it is not easy to determine, which are the direct causes that led to the disruption of the reproductive process and whether it is a multi-causal or a single effect. Some research indicates that a clear etiological factor can be determined in cattle abortion ranging from 23,3-45,5% (*Anderson, 2007; Campero et al., 2017*).The influence of production category, race, climate, vaccination regime, feeding method, and zootechnical management, as non-infectious factors, are often not clearly defined and therefore difficult to diagnose. Unlike them, infectious factors are practically dominant and can be confirmed much more easily and precisely (*Anderson, 2007, Bon Durant, 2007*). Laboratory methods of isolation are more demanding, time-consuming, and sometimes less sensitive, but still represent the gold standard in the diagnosis of abortion (*Seleem et al., 2010*).

In our study, we microbiologically analyzed parts or whole aborted fetuses of cows of different ages. Our goal is to determine whether *Listeria monocytogenes* is present in the examined samples and in what percentage, as well as to point out the importance of this type of bacteria in the occurrence of cow abortions.

Material and Methods

In Serbia, by legal act, the Program of Measures, prescribed the obligation for producers to send every aborted fetus of cattle for laboratory testing. The analysis of the aborted fetus includes virological, bacteriological, and parasitological tests, which are carried out using different methods. Our investigation was conducted for more than three years and covered the territory of the northern part of Serbia, the Autonomous Province of Vojvodina.

In the period from 2020 to the first half of 2023, a total of 65 aborted bovine fetuses were examined. Parenchymatous organs (liver, kidney, spleen), and parts of the gastric and intestines were analyzed. The brought materials were placed in a stomacher bag and placed into the liquid medium for pre-enrichment, semi-Fraser (Promedia). It is taken 25 g of the aborted fetus and poured with 225 ml of the specified substrate (*ISO 11290*). The inoculated material was incubated at 30^0C. After incubation for a period of 24h ± 2, a solid medium of Compass Listeria agar (Biokar) is inoculated from semi-Fraser, and 0.1 ml is transferred to 10 ml of Fraser broth and incubated at 37^0C for a period of 24h ± 2. All colonies that created a halo effect on Compass Listeria agar, regardless of whether it was from semi-Fraser or Frazer broth, they were tested by biochemical chararacteristic and CAMP test with *Staphylococcus aureus* and *Rhodococcus equi* (*Yadav et al. 2010*). Along with the tested isolates, the reference *Listeria monocytogenes* was also tested.

The isolates, which, like the reference strain, gave a slight increase in beta hemolysis towards *Staphylococcus aures* and no changes towards *Rhodococcus egui*, were tested by molecular method (PCR) to confirm the diagnosis. The isolated strains of *Listeria monocytogenes* (molecular size 234bp) were verified by conventional molecular method, confirming the presence of *hlyA* gen (*Chena Jin-Qiang et al. 2017*).

Confirmation of *Listeria monocytogenes* strains was done from an overnight culture on tyrptosa broth (Biocar, Alone, France) wich used for DNA extraction. All bacteria were first boiled for 15 minutes, then exposed to ice for 10 minutes and centrifuged at 13500 rpm for 5 minutes (Hermle, Wehingen, Germany). The *hlyA* gene, gene specific for *Listeria monocytogenes,* was detected using the polymerase chain reaction (PCR) method. The PCR method was performed with a commercial kit (Qiagen, Hilden, Germany) with primers (Invitrogen, Glasgow, United Kingdom), forward *hlyA*-F (CGG AGG TTC CGC AAA AGA TG) and reverse *hlyA*-R (CCT CCA TAT AGT GAT CGA TGT T) according to (*Aznar and Alarcon, 2003*). PCR conditions in thermocycling apparatus (Bibby Scientific LTD, United Kingdom) were: an initial cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, with the annealing temperature of 55°C for 45 s, followed by 72°C for 45 s, and finally 72°C for 5 min.

Electrophoresis was performed in a 2% gel, to which ethidium bromide (Serva, Heidelberg, Germany) was added, on a Labnet apparatus (Horisontal gel System, Enduro, USA), and visualization of PCR fragments of size 234 bp on a UV transilluminator (UVP, Upland, USA).

Results and Discussion

Abortions in cattle represent a significant production problem. What is particularly problematic for producers and veterinarians is the fact, that, it is often difficult to determine what is the real cause of an abortion. Miscarriage is often caused by multicausal factors, although even then it is not easy to determine what started the process, i.e., what is the primary factor, and what exactly are associated causes. According to literature data (*Hovingh, 2009*), it can be seen that abortions in cattle can be caused by infectious factors (bacteria, viruses, protozoa, and fungi), toxic agents, various types of stressogenic factors, and genetic predispositions. The occurrence of abortion can be influenced by pregnancy with twins or the presence of mastitis (*Santos et al., 2003*). Investigations (*Norman et al., 2012*) showed that the largest number of abortions in California (USA) was caused by infectious agents 37.1%, that non-infectious agents were responsible in 5.5% of cases and that even 57.3% were undetermined. Such data indicate the complexity of the issue of abortion in cows and the difficulties in attempts at prevention and/or therapy.

Table 1. contains data on analysed samples of aborted fetuses of cows from 2020 to April 2023. It should be pointed out that the samples that arrived at the laboratory for analysis were part of the monitoring prescribed by the state, by the legal act, the Program of Measures. This means that the costs of laboratory testing are paid by the state.

Table 1. Results of examination of the cows aborted fetuses

	2020	2021	2022	2023	Total	%
Examined	4	31	24	6	65	100
Positive for infectious agents	4	25	20	6	55	84.61*
Listeria positive findings	-	3	3	1	7	10.76**

* percentage of positive materials for infectious agents in relation to the total number examined
**percentage of positive *L. monocytogenes* findings in relation to the total number examined of material

During our investigation, a little shorter than three and a half years, a total of 65 samples of aborted cows were examined. Table 1. shows the total number of positive materials for infectious agents. In Serbians Rulebook, aborted fetuses of cows are examined for the possible presence of several pathogens: brucellosis, leptospirosis, listeriosis, bovine viral diarrhea (BVD), infectious bovine rhinotracheitis/infectious bovine pustular vulvovaginitis (IBR/IPV), Q fever, toxoplasmosis and infection caused by neospora *(Rulebook on Establishing the Program of Animal Health Protection Measures of the Republic of Serbia for 2023)*. For this reason, Table 1. provides data on how many tested materials were positive for some of the infectious cow abortion samples. It can be seen that slightly less than 85% of the tested samples were positive for some of the pathogens (viruses, bacteria, protozoa) and that about 15% were free of biological agents. What is important to emphasize, is that none of the positive materials carried only one of the causative agents of cow abortion, i.e. all samples were burdened with several bacteria, viruses, and/or parasite species. In comparison with the literature (*Morrell et al., 2019*), it can be seen that our percentage of positive materials for the presence of infectious agents is significantly higher (84.61%) in comparison with the 52% that were confirmed in Argentina. In contrast to these findings, the presence of pathogens that cause abortions in cows in Brazil (*Sarangi et al., 2021*) was very high: BVD 98.4%, *Brucella* 98.3%, BHV 87.5 and *Leptospira* 54.69%. Similar to the findings of the authors from Argentina, a study from Uruguay (*Macías-Rioseco et al., 2020*) indicates that out of 102 analyzed samples of fetuses and fetuses with placentas, only in 54 cases (53%) was determined the cause of the abortion. The presence of an infectious agent was proven in 51 samples. Investigations showed that in as many as 47% of the samples, it was not possible to determine what caused the abortions.

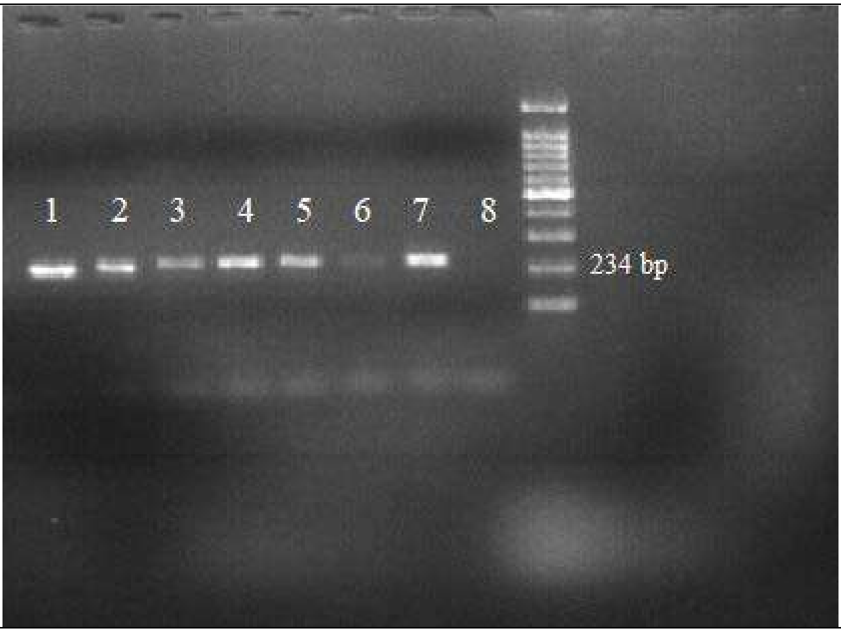
Table 1. shows that of the total number of analyzed materials (65), *Listeria monocytogenes* was isolated in 7 cases, i.e. 10.76%. By reviewing the research of other authors, it can be seen that the presence of *L.monocytogenes* in our samples is higher compared to theirs. Out of 150 examined abortions in Argentina, *L. monocytogenes* was isolated in only one case, which represented only 0.68% (*Morrell et al., 2019*). The standards of the laboratory method of bacterial isolation were applied, as in our work. In the analysis of causes of abortion in the USA (*Jamaluddin et al., 1996*) out of 595 samples, only one case (0.17%) was found to contain *L. monocytogenes* and two cases (0.34%) *L. ivanovii*. Generally, compared to our findings, the listeria finding was many times smaller. Testing by molecular methods (PCR), in Brazil, researchers found that out of 64 analyzed materials, 4 or 6.25% of the samples were positive for the presence of *Listeria monocytogenes*.

Marked halo effect on Compass Listeria agar | Marked CAMP effect of *Listeria*

Figure 1. Confirmation of the phenotypic characteristics of the isolated strains

In Figure 1, the halo effect (opaque precipitate) is shown on the left, i.e. the presence of an opaque precipitate produced by listeria on Compass Listeria agar. This reaction proves the presence of the phospholipase C enzyme possessed by *L.monocytogenes*. Decomposition of the phosphatidyl inositol, which is presented in the substrate, this reaction becomes visible. Similarly, by hydrolysis of X-β glycosides and oxidative dimerization of the created compounds, a blue-green color is created in the center of the grown colony. The occurrence of the described phenomena proves the presence of very important properties of listeria in the process of phenotypic determination. On the right side, in Figure 1, you can see the CAMP effect that *Listeria monocytogenes* creates with *Staphylococcus aureus*. The presence of *L. monocytogenes* enhances the complete (beta hemolysis) produced by S. aureus which is characteristic of listeria. The obtained effects are marked with lines.

In our work, we decided to confirm the isolated strains of *L. monocytogenes* by molecular methods. Figure 2 shows the results of molecular confirmation (PCR) of isolated strains of *Listeria monocytogenes*. As you can see in the picture, the size of the tested *hlyA* gene is 234bp, which corresponds to the obtained results.



1-7: molecular confirmation of *Listeria monocytogenes*; 8: negative control

Figure 2. Image of *Listeria monocytogenes* gel, molecular confirmation

In our investigation, we did not use molecular methods for diagnostic purposes, respectively, to prove the presence of *Listeria monocytogenes* in the received samples. The Rulebook of animal health protection measures for the year 2023 foresees, proving the causative agent in the pathological materials of aborted fetuses, aerobic cultivation, i.e. isolation of *Listeria monocytogenes*. For these reasons, molecular methods were used only to confirm isolated *Listeria* strains.

Conclusion

The examination determined the presence of *Listeria monocytogenes* in 7 brought samples. Concerning the total number of tested samples, 10.76% of the material was positive for this bacterium. In comparison with the results of similar tests, it can be concluded that the number of positive samples in our analysis is many times higher even when compared with molecular tests. The question is what are the reasons for this difference, but it certainly represents a topic for further research.

Following the total number of materials that were analyzed during the observed period of 3.5 years, it can be concluded that even though the laboratory tests are not the cost of animal owners, a relatively small number of samples arrived for analysis. This could mean that there is no interest in determining etiological factors, especially infectious ones, that caused an abortion. Why the owners do not consider abortions not only a health but also an economic problem remains to be determined?!

From the obtained results, it can be seen that 84.61% of the brought samples were positive for some of the biological factors (bacteria, viruses, parasites), which means that slightly more than 15% of abortions could be caused by abiotic factors, that is, some of the zootechnopathies. Based on this, it could be concluded that better health control, respectively, laboratory practice can reduce the number of abortions. Due to the complexity of the causes of abortion, it is not easy to make such an assessment.

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COENUROSIS OF SHEEP IN SERBIA - CASE REPORT

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Abstract: Coenurosisa is a zoonotic disease caused by the larval forms of *Taenia multiceps* which are great host's animals from the family of canids. The disease is global distribution but is most present in the Mediterranean, Middle East, Central Africa and Australia. Larvae exhibit tropism toward brain tissue, and cysts are usually located in the left hemisphere of the brain of small ruminants. Involvement of the brain can cause increased intracranial pressure, seizures, loss of consciousness, and focal neurologic deficits. During 2022 we examinee one flocks of 78 milking sheep reared on hilly pastures located below the Zmajevac hill (397 m), between the branches of the Osimćki mountains and the river Zapadna Morava, at an altitude of 178 m. Symptoms of ataxia, such as unsteady gait and stumbling, and depression were observed in 12 animals. In 5 sheep, continuous aimless or circular movement, wandering, was also manifested. In addition to these symptoms, in one sheep that died, visual disturbances were observed depression and unilateral blindness. All affected sheep were slaughtered and a pathoanatomical examination was performed. In the brain of all animals we revealed the presence of infestation with one to four coenuri 4.1-5.5 cm in size. The sites of predilection were the left hemisphere (48%), followed by the right hemisphere (40%) and the cerebellum (12%). The coenurus has a thin wall surrounding a single cavity that contains a clear fluid. When the cysts were opened, numerous scoleces were found inside, from 2 to 3 mm in diameter attached to the cyst wall. Each scolex has four circular suckers and two rows of hooks on a rostellum. The hook lengths are 147 to 165 µm and 87 to 125 µm.The results show that *C. cerebralis* was a major cause of the nervous manifestations of cenurosis in clinically affected sheep.

Key words: *Coenuris cerebralis, Taenia multiceps,* sheep

Introducton

The grazing way of feeding enables sheep to have constant contact with transitional hosts, eggs and larval forms of parasites, so that there is no sheep that is not infected with at least one parasite species *(Ash and Truong, 2003; Pavlović et al., 2012; Pavlović et al., 2013; Pavlovic, 2009).*

Permanent pastures pose the greatest health risk for sheep, especially if they have been used unplanned for many years. According to M.Hall's famous quote, "permanent pastures perpetuate parasites", which has been proven many times *(Vlassoff et al., 2001; Pavlović et al., 2012)*. Uncultivated pastures with poor floristic composition cause nutritional imbalances, but cultivated pastures that are improperly used are also a place of constant infections, especially by parasitic agents *(Pavlović et al., 1991; 1995; 2018; Ash and Truong, 2003).* The cumulative effect of accumulated agents is reflected through reinfections and superinfections and conditions their permanent circulation *(Truong and Baker 1998; Truong et al., 2000)*. The presence of parasites leads to numerous health problems and significant economic losses due to the reduction of production results *(Pavlović et al., 2003a,b).*

In addition to the parasites that are hosted by domestic and wild ruminants, a large number of parasite eggs are found on pastures, for which these animals are transitional hosts. Namely, animals from the genus canida (dogs, foxes, wolves and jackals) and felids (cats, wild cats) are the true hosts of a large number of cestodes for which small ruminants are transitional hosts *(Pavlović, 1994; 2023; Pavlovic, 2023; Pavlovic et al., 2008; Ivanović and Pavlović, 2017, Petrovic et al., 2021; Pavlovic and Ivanovic, 2022).*

Through the eggs thrown out in the feces, the transitional hosts become infected and develop larval forms of the parasite that are incised on their organs *(Jovanović et al., 2012).* After slaughter or death, these organs, if not removed, become a source of infection of the real hosts. Echinococcosis/hydatidosis, cenurosis, cysticercosis, sarcocystosis, etc. are the most important diseases caused by this method *(Pavlović and Ivanović, 2005; 2006; 2022; Jovanović et al., 2012; Ivanović and Pavlović, 2015; Petrović et al., 2021).*

One of these diseases is coenurosis - a disease caused by larval forms of the tapeworm *Tenia* (syn. *Multiceps*) *multiceps* (Soulsby, 1977). This tapeworm lives in the small intestine of dogs and other canids *(Pavlović, 1994; Pavlović et al., 2008)*. The transitional hosts of this tapeworm are ruminants, equids, and humans can also be infected. *Coenurus cerebralis*, the larval stage or metacestode of *T. multiceps*, develops in the central nervous system of sheep, goats, cattle, buffaloes, yaks, horses and pigs, as well as other domestic and wild ruminants *(Nourani and Kheirabadi, 2009*). These are transparent bubbles 3-5 cm in size.

Usually 2-3 blisters are found in which there is a different number of scolex and there can be 10-500 of them *(Pavlović and Anđelić-Buzadžić, 2011).*

Materials and Methods

During 2022 we examination flock consisting of 78 milking sheep reared on hilly pastures located below the Zmajevac hill (397 m), between the branches of the Osimćki mountains and the river Zapadna Morava, at an altitude of 178 m. Symptoms of ataxia, such as unsteady gait and stumbling, and depression were observed in 12 animals. In 5 sheep, continuous aimless or circular movement, wandering, was also manifested. In addition to these symptoms, in one sheep that died, visual disturbances were observed depression and unilateral blindness. All affected sheep were slaughtered and performed a pathoanatomical examination.

Results And Discusion

At autopsy in the brain of all animals we revealed the presence of infestation with one to four cysts 4.1-5.5 cm in size. (Picture 1). The sites of predilection were the left hemisphere (48%), followed by the right hemisphere (40%) and the cerebellum (12%). The cystss has a thin wall surrounding a single cavity that contains a clear fluid. When we opened the cysts they were found in them numerous scoleces from 2 to 3 mm in diameter attach to the cyst wall. Each scolex has four circular suckers and two rows of hooks on a rostellum. The hook lengths are 147 to 165 μm and 87 to 125 μm.

Picture 1 and 2. Brain with *Coenuris cerebralis* cysts

Based on the morphological characteristics of the scolex, we found that it is the *Coenurus cerebralis*, larval form of *Taenia multiceps.* Because of the characteristic clinical symptoms in transitional hosts (uncoordinated movement), this disease is colloquially known as gid, sturdy, or staggers *(Bhalla and Negi,* *1962; Akkaya and Vurusaner, 1998; Achenef et al., 1999).* The disease is of global distribution and occurs most often in Mediterranean countries, North Africa, Central Asia and Australia *(Bhalla and Negi, 1962; Edwards and Herbert, 1982; Akkaya and Vurusaner, 1998; Achenef et al., 1999; Desouky et al., 2011; Giadinis et al., 2012).*

What is the epizootiological state of coenuruses in Serbia is not known, considering that the last researches were done more than 50 years ago and only sporadically. Given that the disease is evidently present in herds of small ruminants in Serbia, and that adults of this tapeworm have been found in dogs, foxes and wild canids in Serbia, it is necessary to carry out extensive research on the prevalence of this parasitic disease in transitional hosts - mainly small ruminants *(Pavlović et al., 2017).*

Taenia (syn. *Multiceps*) *multiceps* is a tapeworm that lives in the small intestine of dogs and other carnivores. It is widespread throughout the world and in a large number of wild canids and dogs *(Edwards and Herbert, 1982; Soulsby, 1977; Pavlović and Anđelić-Buzadžić, 2011).* The parasite is typically prevalent in rural areas, where the dog-sheep route appears to be the most important transmission pathway. The disease may also potentially be spread by wild animals, such as foxes and wild ungulates. Farmers can also contribute significantly to environmental contamination with this parasite by opening the skulls of diseased sheep out of curiosity or to establish their own personal diagnosis, enabling stray dogs to freely access *Coenurus* cysts or directly contaminating dogs by feeding them with infected tissue. In Serbia, it was found in foxes, wolves, jackals and dogs *(Pavlović, 1994; Pavlović et al., 2008; Cirović et al., 2015 a,b).* It is 0.4-1 m long. The scolex is pear-shaped with 4 round leeches and a rostrum armed with two crowns of hooks. The front legs are wider than longer, in the middle they are of the same length and width and have a serrated appearance, and the rear legs are longer than wider *(Soulsby, 1977).* In mature articles there are eggs that are excreted into the environment. Coenurosis affects sheep during their first year, and mainly lambs aged 3–4 months. There are rare reports of clinical signs in sheep older than 3 years, in animals with a general immunodeficient condition where they fail to produce antibodies, or in intensively bred sheep that have never been exposed to the parasite before.

In the small intestine of an intermediate host, the oncosphere hatches from an egg and travels through the intestinal wall, primarily reaching the CNS via the bloodstream, where it encysts and matures over several months into an infective coenurus *(Gauci et al., 2008).*

There are several strains of this tapeworm that are morphologically similar but differ genetically, which leads to a partial change in larval tropism *(Scala and Varcasia, 2006; Oge et al., 2012; Varcasia et al., 2014).* The metacestode may also develop and mature in subcutaneous, intramuscular tissues and peritoneal areas as well as in organs such as the heart and lungs of sheep and goats *(Schuster et al., 2010; Oryan et al., 2014; Christodoulopoulos et al., 2016; Noamn, 2019)*. Through genetic analysis, it was determined that these are genetic variations, which was also confirmed by mitochondrial DNA research, proving that strains Tm1-Tm3 differ in their tropism and that they definitely belong to a different genotype of M.multiceps *(Varcasia et al., 2014)*. However, the largest number of these tapeworms belongs to common strains that show neurotropism and incyst in the brain or spinal cord *(Welchman and Bekr-Ochir, 2006; Oryan et al., 2014)*. After migrating through the blood and arriving at the predilection site, the larvae are fixed. The larval development of the parasite takes place on the meninges, and less often on the spinal cord of sheep, goats, cattle, less often horses, pigs and humans.

The oncosphere develops in the following stages: on day 8–10 post-infection (PI), it reaches the CNS and then migrates actively in the CNS from day 10 to 33 to reach its final destination; on day 40, it turns into a pyriform vesicle with just visible scoleces; after 2 months, it is the size of a cherry. Three months after infection, the cyst matures with well-formed protoscoleces, and finally, after 7–8 months, it reaches its final size of 5–6 cm in diameter. The life cycle is complete when the definitive host ingests the coenurus containing the mature protoscoleces.

True hosts become infected when they eat the brains with cysts of diseased transient hosts. In the right host, this taeniasis manifests itself in the form of digestive disorders with alternating diarrhea and constipation, weakness and weight loss *(Giadinis et al., 2005; Polizopoulou et al., 2016)*.

Pathological changes begin during larval migration. A hexacanth embryo on its migration path through nervous tissue leaves meandering fields of bleeding and necrosis until it stops. Having reached the meninges, they stay there and form cysts known as *Coenuris cerebralis (Nourani and Kheirabadi, 2009; Oge et al., 2012; Pavlović et al., 2017)*. After 2-5 weeks of penetration into the nervous system, the cyst reaches a size of about 3 mm and after six weeks about 1 cm. Two months after reaching the central nervous system, it is about 2 cm in size and the beginning of protoscolex formation can be observed in it. A mature cenurus blister completes development and becomes infectious 6-8 months after infection. It is a transparent bubble of size 3-5 cm and usually 2-3 bubbles are found, rarely more *(Kheirandish et al., 2012; Varcasia et al., 2022)*. They contain a clear liquid and each one has a different number of scolex (depending on the size of the bladder, there can be 10-500 of them).

Due to the characteristic clinical symptoms in transitional hosts (uncoordinated movement), this disease is also called fidget spinner. Ataxia manifests itself as unsteady gait and stumbling. Characteristic is the endless movement in a circle, to the side opposite to the localization of the cenrus bubble. Continuous aimless or circular movement, wandering is also manifested. Vision disorders are manifested in the form of hypermetropia, drops in the field of vision, or blindness. As the bubble grows, compressive atrophy of the brain tissue occurs, and if it is present on the surface of the brain, the pressure can be so strong that atrophy and bulging of the corresponding part of the head bone occurs *(Nourani and Kheirabadi, 2009).*

Pathological changes and symptoms depend on the size and location of the cyst *(Welchman and Bekr-Ochir, 2006)*. The acute form of the disease usually occurs 17-23 days after infection and is a consequence of the migration of oncospheres through the brain tissue. Symptoms of meningoencephalitis appear, rapid and strong pulse and excitement, which is replaced by depression and tremors, paresis and paralysis *(Giadinis et al., 2006; Polizopoulou et al., 2016).* Deaths are possible.

In the chronic form, which occurs 3-5 months after infection, disorders of locomotion and vision are manifested, animals move in circles, loss of vision is possible. If the cenurus is located in the spinal cord, paralysis and paresis occur. Animals lose weight and die from cachexia *(Nourani and Kheirabadi, 2009).*

Human infection occurs by accidental consumption of parasite eggs. In them, larval development takes place as in other hosts with similar clinical symptoms *(Ing et al., 1996; Benifla et al., 2007).*

Conclusion

Cenurosis is a zoonotic disease caused by the larval forms of the tapeworm Taenia (syn. Multiceps) multiceps, whose real hosts are animals from the canid family. The disease is of global distribution, but it is most present in the Mediterranean area, the Middle East, central Africa and Australia. Larvae show a tropism towards brain tissue and cysts are most often located in the left hemisphere of the brain of small ruminants. Diagnosis is based on clinical symptoms *(Scott, 2012).* Suspicion is confirmed by the finding of papillae nervi optici edema (increased intracranial pressure) with spot bleeding. Papilledema occurs 1-2 months before clinical symptoms appear. Therapy in the acute phase of the disease can be performed with praziquantel and benzimidazoles (50-100 mg/kg) *(Ghazaei, 2005).* An operative procedure is also possible, which is routinely performed in some countries (e.g. Greece) *(Komnenou et al., 2000; 2005).*

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INVESTIGATION OF THE USE OF PROPOLIS IN BROILER FEEDS AND ITS EFFECTS ON HEALTH AND PERFORMANCE PARAMETERS

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Abstract: Numerous scientific studies have shown that propolis, with its various bioactive compounds, has enormous potential as a natural additive in poultry nutrition. Research findings have shown that propolis added to poultry diets supports immune function, improves gut health, increases antioxidant activity, and provides positive effects on overall performance. In this study, 180 equivalent chicks from 27.000 head Ross 308 broiler chicks were divided into groups with equivalent body weight averages and 60 chicks were randomly assigned to each group, and control and experimental groups were formed. In the study, 0-13 days chick starter feed, 14-24 days chick feed, 25-37 days chicken rearing feed and 38-42 days pre-slaughter finisher feed and water were provided ad libitum. Propolis used as an additive was supplied with drinking water as 1 cc/L and 2 cc/L. In the study, the data of the 2nd week, the data of the control group I. and II. Compared to the animals in the experimental groups (1 cc/L water and 2 cc/L water), the body weight averages were found to be higher. However, it was determined that the live weight averages of the animals in the experimental group given the 4th and 5th data 1 cc /L propolis were higher than the other groups (P<0,05 P=0,042). At the end of the study, ALT values were included in the reference values for all groups in the analysis of blood parameters. Total serum cholesterol was found to be lower in the control group, different from the reference value. As a result, the findings; It indicates that 1 cc/L propolis improves body weight, organ characteristics and blood parameters.

Key words: propolis, broiler, performance, blood parameters

Introduction

As a result of long-term use of antibiotics used as growth stimulators in animal nutrition, they can gain resistance against pathogenic microorganisms. The World Health Organization stated that antibiotics are no longer very effective in protecting human health, as they gain immunity against microorganisms as a result of incorrect and long use. The use of antibiotics in compound feeds is limited or prohibited by EU countries. As a result of this situation, the use of some natural and synthetic feed additives has come to the fore. Today's poultry nutrition industry has introduced probiotics, prebiotics, organic acids, plant extracts and essential oils as alternative feed additives to growth factors. The livestock sector, which is constantly in search to eliminate the losses, is investigating the use of propolis in mixed feeds in bee products, considering its effectiveness in health protection, especially in poultry nutrition. Propolis is a resinous substance that honey bees (*Apis mellifera L.*) collects from the branches, sprouts and buds of plants, bark and carries them to the hive with pollen baskets on their hind legs *(Seven et al., 2007; Özcan et al., 2003).* Bees undergo a biochemical change with the enzymes they secrete with the secretory glands in their heads, and by mixing some beeswax, they turn it into a sticky organic substance that does not dissolve in water *(Yıldız and Ünal., 2022; Seven et al., 2007)*. Known as bee glue, propolis is used by bees; It is used as a barrier to protect against insects and microorganisms, to disinfect the hive, to repair cracks or openings in the hive, to protect the hive against adverse weather conditions such as rain and wind *(Çoşkun and İnci., 2020).* Variations in the complex chemical composition, color and aroma of propolis may vary depending on its geographical origin, plant variety and time of collection. Depending on these variables, the structure of raw propolis consists of 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% other organic compounds *(Pasupuleti et al., 2017; Gómez-Caravacave et al., 2006)*.It becomes hard and brittle when cold (below 15 ^{0}C), meltable when hot (80-105 ^{0}C). With the increase in studies, more than 300 chemical components of propolis have been identified. Apart from resins, the main chemical compound groups are waxes, polyphenols (phenolic acids, flavonoids) and terpenoids *(Przybylek and Karpinski, 2019).* Terpenoids and polyphenols are considered the most active compounds *(Pimenta et al., 2015)*.12 different derivatives of flavonoid group including pinocembrin, acacetin, chrysin, rutin, luteolin, kaempferol, apigenin, myricetin, catechin, naringenin, galangin and quercetin have been identified.Another critical group of propolis compounds is aromatic acids. Among them, ferulic is found in cinnamic salicylic, benzoic, caffeic and p-coumaric acids *(Przybylek and Karpinski, 2019; Bankova et al., 2000)*. Propolis contains beneficial minerals such as vitamins B1, B2, B6, C and E, magnesium (Mg), calcium (Ca), potassium (K), sodium (Na), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe), as well as several enzymes such as adenosine triphosphatase, acid phosphatase, succinic dehydrogenase, 2006. *Seven et al. (2007)* stated in a review they published that propolis is used for different purposes in folk medicine, apitherapy, biocosmetic and pharmaceutical industry due to its biological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, cytotoxic, immunomodulatory, antiulcer, local anesthetic, antitumor, immunostimulator *(Çelik, 2019)*. By harnessing the power of plant extracts, the poultry industry can optimize the health and well-being of their flock while meeting the demands of environmentally conscious consumers. In this context, one of the natural substances that contributes to this and is emphasized is propolis. Numerous scientific studies have shown that propolis, with its various bioactive compounds, has enormous potential as a natural additive in poultry nutrition. Research findings have shown that poultry producers can support immune function, improve gut health, increase antioxidant activity, and improve overall performance by including propolis in their diets. Nutritional additives such as propolis seek to improve intestinal health as an alternative to the global ban on in-feed antibiotics used as growth promoters (AGP). The objective of this study was to evaluate the effect of propolis supplementation in diet of broilers.

Materials and Methods

Animal, Feed and Propolis Materials

In the commercial broiler house where the experiment was carried out, 180 chicks were randomly allocated for research among 27.000 head Ross 308 broiler chickens from a private sector integrated poultry company. The feeds used in the research were obtained from the company that belongs to the private sector and also provides the animal supply. During the experiment (42 days), all groups were given chick starter feed, chick starter feed, broiler development feed and pre-slaughter finisher feed.

Table 1. Nutrient content of complete feed used to feed 0-13 days old broiler chicks

Nutrients	%
Crude protein	21.44
Crude fiber	3.24
Raw ash	4.23
Crude oil	5.35
Lysine	0.36
Methionine	0.33
Threonine	0.13
Calcium	0.54
Phosphorus	0.44
Sodium	0.18

Vitamins	(for 1 kg of feed) IU/Kg
Vitamin A (IU/kg)	9,900
Vitamin D (IU/kg)	4,050

Trace Elements (for 1 kg of feed)	mg/kg
Iron (Ferrous sulfate)	20
Copper (Copper sulfate)	16
Zinc (Zinc sulfate)	120
Iodine (Calcium iodate)	1.25
Manganese (Manganese oxide)	120
Selenium (Sodium selenite)	0.3

The raw materials that make up the content of the feeds used in the study. Corn, wheat, soybean meal, soybean extruded, sunflower seed meal, meat-bone meal, vegetable oil, dicalcium phosphate, calcium carbonate, sodium chloride, sodium sulfate, vitamin-mineral premix.

Table 2. Nutrient content of the whole feed used to feed 25-37 days old broilers

Nutrients	**%**
Crude protein	19.29
Crude fiber	3.19
Raw ash	3.58
Crude oil	6.41
Lysine	0.42
Methionine	0.27
Threonine	0.11
Calcium	0.45
Phosphorus	0.38
Sodium	0.18

Vitamins for 1 kg of feed) IU/Kg	
Vitamin A (IU/kg)	7,500
Vitamin D (IU/kg)	3,000
Trace Elements (for 1 kg of feed) mg/kg	
Iron (Ferrous sulfate)	20
Copper (Copper sulfate)	16
Zinc (Zinc sulfate)	120
Iodine (Calcium iodate)	1.25
Manganese (Manganese oxide)	120
Selenium (Sodium selenite)	0.3

The raw materials that make up the content of the feeds used in the study. Corn, wheat, soybean meal, soybean extruded, sunflower seed meal, meat-bone meal, vegetable oil, calcium carbonate, sodium chloride, sodium sulfate, vitamin-mineral premix.

Table 3. Nutrient content of the whole feed used for pre-slaughter feeding of broiler chickens aged 38-45 days

Nutrients	**%**
Crude protein	19.28
Crude fiber	3.41
Raw ash	3.45
Crude oil	6.13
Lysine	0.43
Methionine	0.22
Threonine	0.1
Calcium	0.41
Phosphorus	0.36
Sodium	0.18

Vitamins (for 1 kg of feed) IU/Kg	
Vitamin A (IU/kg)	7,500
Vitamin D (IU/kg)	3,000
Trace Elements (for 1 kg of feed) mg/kg	
Iron (Ferrous sulfate)	20
Copper (Copper sulfate)	16
Zinc (Zinc sulfate)	120
Iodine (Calcium iodate)	1.25
Manganese (Manganese oxide)	120
Selenium (Sodium selenite)	0.3

The raw materials that make up the content of the feeds used in the study. Corn, wheat, soybean meal, soybean extruded, sunflower seed meal, meat-bone meal, vegetable oil, calcium carbonate, sodium chloride, sodium sulfate, vitamin-mineral premix

Propolis, Research Environment, Drinkers and Feeders

Propolis was obtained from *Apis mellifera* beehives located in the Çanakkale region and in Kaz Mountains at an altitude of 1000-1300 meters above sea level. Propolis with a concentration of 25% was prepared as ethanolic propolis. The propolis used in the diet supplementation of the broilers was characterized by having brown tones. Regarding plant origin, 14 plants were identified as the primary sources of resins, among which the following stand out: black poplar (*Populus nigra*), black willow (*Salix nigra*), pine (*Pinus nigra*), Turkish oak (*Quercus cerris)*, chestnut (*Castanea sativa*), strawberry tree, (*Arbutus unedo*) and heath (*Erica cornea*) specie. Each propolis sample was shaken twice daily for 25 days and kept in the dark to prevent the change of some photosensitive metabolites. After 25 days, ultrasonic shaking was performed for 24 hours and each of the samples was filtered. After obtaining the ethanolic extract, it was kept in amber glass bottles until further processing. The research was conducted in a commercial broiler house owned by the private sector. For the animals to be used in the experiment, 3 compartments were created with a 2x2 meter length and 50 cm high stainless cage wire, with an area of 4m2. Rice husk was used as litter material in the compartments. Automatic water nipples are not included in the created partitions. In the first 13 days of the study, drinking water was provided by hand-filled plastic chick drinkers with a volume of 5 liters, 2 for each of 60 animals. The feed additive ethanolic propolis used in the experiment was added to the drinking water of the animals daily and in a controlled manner at the doses specified in the method section.

Method

This study was carried out in Ihlamur broiler farm in Tuzak village of Hendek district of Sakarya. The chicks, which were brought to the hen house at the age of one day, were randomly distributed in the pens that were prepared beforehand for the trial. Since Poultry is a company with Good Agricultural Practices certificate, precautions and practices are carried out meticulously in terms of food and animal safety. Heating of the henhouse was provided by automatic fans using solid fuel boilers. The ambient temperature was started with 33,5 C on the first day. It was lowered by 0,3 C in a controlled manner every day. Humidity and temperature balance in the henhouse is kept under control by automatic systems. Illumination of the poultry house is provided by LED bulbs.

Research Plan

In the experiment, 180 day-old chicks were randomly divided into three groups, one in the control group and two in the experimental group, into the compartments prepared as 60 chicks each. Chicks remained in the same compartments from one day old to slaughter (42 days). The animals were treated with four different feed combinations of the experiment: 0-13 days chick starter feed, 14-24 days chick starter feed, 25-37 days chicken grower feed, and 38-42 days pre-slaughter finish. During the experiment, feed and water were given ad libitum. Propolis, which is the subject of the experiment, was given to the animals via drinking water. While no additives were used in the control group, ethanol propolis extract was added to the drinking water of the other groups at doses of 1 cc/L and 2 cc/L, respectively.

Calculation of Body Weight Gain and Determination of Feed Consumption

At the beginning of the experiment, the chicks were weighed and their average weight was determined. Care was taken to ensure that the groups were equal in average animal weight. Based on the day the experiment started, weekly live weights were determined and recorded by weighing regularly every week. The body weight gain of each group was determined by subtracting the body weight of the previous week from the body weight of the week of weighing. Weighings were measured on scales sensitive to 5 g. Since the additive, which is the subject of the experiment, was given with drinking water, each group consumed the same feeds in the same periods. Feed was not measured weekly as it was delivered by automatic feeders connected to the feed silos of the house. Based on the amount of feed used from the arrival of the chicks to the slaughter, the feed efficiency ratio was determined by the pre-slaughter live weights of the animals used in the experiment.

Slaughter Process of Broilers and Blood Analysis

At the end of the experiment (day 42), 12 animals representing each group were slaughtered. The average time between taking the feeders from the front of the animals and slaughtering is 10-12 hours. The delivery to the special chicken slaughterhouse was provided by a special vehicle. Pre-slaughter weighings were carried out. The groups were separated from each other and hung on the cutting line, and the cutting process was carried out by professional workers. During slaughter, 5 cc blood was taken from each animal into yellow capped blood tubes. The blood samples were sent for analysis in the presence of cold chain. The blood obtained during the slaughtering process was taken to a private laboratory for analysis. In these analyzes; Alanine aminotransferase (ALT), Total Serum Cholesterol was evaluated comparatively by determining its levels.

Weighing the Internal Organs and Examination of the Liver at the Macroscopic Level

After cutting, plucking, head-neck separation, and foot separation processes were carried out in automatic machines. The internal organs were removed by a professional team and a skinned carcass was obtained. The liver and gizzards were placed in bags and taken to a special laboratory for weighing and macroscopic examination of the liver. Weighing of the removed internal organs was made with precision scales and recorded. Ante-mortem macroscopic examination and examination of the livers were performed and a report was written.

Statistical Analysis

Analysis of variance was applied to the data obtained in the randomized plot design. Pairwise comparisons for the features with significant differences were made with the Tukey test.

Results and Discussion

First of all, the body weight averages obtained at the level of all groups in the first 4 weeks of the experiment were not statistically significant (P>0.05). Due to its anti-inflammatory, antibacterial and antioxidant properties that help maintain intestinal integrity, propolis has been suggested as one of the natural remedies that support intestinal health *(Simone-Finstrom M. et al., 2017).* The fact that the experimental group animals given 1cc/L propolis showed higher body weight gain than the 2cc/L and control group animals in the measurements made at the third and fourth weeks, which is consistent with the literatures. Especially in the group given 1 cc/L propolis, the relatively better growth performance may be related to the regulation of the intestinal microbiota of propolis, nutrient savings due to the decrease in the number of competing microorganisms, reduction or elimination of microorganisms that cause subclinical infections, and reduction of toxins or metabolites that inhibit growth. Especially in the group given 1 cc/L propolis, the relatively better growth performance may be related to the regulation of the intestinal microbiota of propolis, nutrient savings due to the decrease in the number of competing microorganisms, reduction or elimination of microorganisms that cause subclinical infections, and reduction of toxins or metabolites that inhibit growth. When the liver weight was examined, it was found to be higher than the control group, the group given 1 cc/L propolis and the group given 2cc/L propolis, and it was statistically significant ($P<0.05$). One of the main reasons for this is that the intense smell of propolis (2cc/L) has reduced feed consumption in animals. In a similar study, *Behboodi H.R. et al. (2022)*, it has been reported that the addition of propolis to the diets of broiler chickens may cause structural and functional changes due to the modification of gene expression in enterocytes in terms of intestinal health, and it helps to protect intestinal health. In the analysis of post-slaughter blood parameters, the average of the total serum cholesterol values in the control group was below the reference values, and the difference between the groups was statistically significant. ($P<0.05$). ALT values are in the I. experimental group and II. The difference between the experimental group was statistically significant ($P<0.05$). However, these values are among the reference values. There are differences in liver and gizzard weight averages in propolis groups. In the comparison between the groups according to their liver weights, it was noted that the liver weight of the animals in the control group was higher than the groups given propolis and it was statistically significant. ($P<0.05$).This is due to the anti-oxidant properties of propolis, which are considered to improve lipid metabolism, liver morphological structures and biological functions. These results are in agreement with the findings of *Klaric et al. (2018)*. Similarly, *Çelik et al., (2019)* reported that propolis supports immunity by reducing fatty liver due to its very strong immunomodulatory effect.

Live Weights and Body Weight Gain

When we consider the weekly body weight averages and compare the groups, the first 4 weeks data were not found to be statistically significant ($P>0.05$). In the last weighing before slaughter, the live weight averages of the animals in the group given 1cc/L propolis were higher than the other groups, but the average live weight in the group given 2cc/L propolis was measured at the lowest level among all groups and is statistically significant. ($P<0.05$ $P=0.042$).

Table 4. Average of weekly live weights of experimental groups (g)

Weeks	Control Group		Experimental Group 1 (1cc/L)		Experimental Group II (2cc/L)		P
	Mean	SE	Mean	SE	Mean	SE	
1	238.24	1.91	225.92	5.38	234.72	3.49	0.201308
2	697.47	14.35	635.08	25.89	639.63	18.38	0.156073
3	1199.0	7.72	1198.9	4.4	1150.88	34.89	0.300157
4	1669.55	23.2	1749.68	24.24	1689.77	48.88	0.267605
5	2367.99^{AB}	18.38	2396.66^{A}	25.7	2300.32^{B}	23.45	0.042875

SE: Standard Error of Mean

When we look at the weekly body weight gains, the average body weight gains between the groups vary every week and are statistically insignificant (P>0.05).

Table 5. Averages of weekly live weight gains of experimental groups (g)

Weeks	Control Group		Experimental Group 1 (1cc/L)		Experimental Group II (2cc/L)		P
	Mean	SE	Mean	SE	Mean	SE	
1	198.24	1.91	185.92	5.38	194.72	6.97	0.201
2	459.23	13.3	409.17	20.64	404.91	18.59	0.166
3	501.53	7.41	563.81	26.95	511.26	18.74	0.194
4	470.55	20.4	550.79	27.35	538.89	21.27	0.073
5	698.45	8.32	646.97	5.1	610.55	38.08	0.12

Organ Weights and Consumable Portion Weights

The mean gizzard weights of the animals in the 1 cc/L propolis given group were higher than the control group, and this difference was statistically significant (P<0.05). When the liver weight was examined, it was found to be higher than the control group, the group given 1 cc/L propolis and the group given 2cc/L propolis, and it was statistically significant (P<0.05).

Table 6. Average weights of organs at the level of groups (g)

	Control Group		Experimental Group 1 (1cc/L)		Experimental Group II (2cc/L		P
	Mean	SE	Mean	SE	Mean	SE	
Liver	52.93^{A}	5.52	48.27^{B}	5.2	47.87^{B}	4.61	0.017
Gizzard	31.73^{B}	5.84	39.07^{A}	5.32	34.8^{AB}	5.52	0.003

When we look at the consumable parts and carcass weights after slaughter, the differences between the groups are statistically insignificant (P>0.05).

Table 7. Consumable Portion Weights of Groups

	Control Group		Experimental Group 1 (1cc/L)		Experimental Group II (2cc/L		P
	Mean	SE	Mean	SE	Mean	SE	
Thigh	583.2	33.82	576.1	40.9	566.8	37.58	0.493
Wing	207.13	11.34	213.33	19.71	212.13	15.01	0.524
Chest	627.0	58.3	652.6	37.53	626.8	47.2	0.256
Carcass	2049.1	141.8	2064.9	83.1	2015.4	133.0	0.531

Blood Parameters (ALT and Total Serum Cholesterol)

In the analysis of post-slaughter blood parameters, the average of the total serum cholesterol values in the control group was below the reference values, and the difference between the groups was statistically significant. (P<0.05). ALT values are in the I. experimental group and II. The difference between the experimental group was statistically significant (P<0.05). However, these values are among the reference values.

Table 8. Some Blood Parameters of Groups

	Control Group		**Experimental Group 1 (1cc/L)**		**Experimental Group II (2cc/L**		**P**
	Ort.	SH	Ort.	SH	Ort.	SH	
Total Serum Cholesterol)	111.92^{B}	16.21	139.5^{A}	24.24	149.08^{A}	25.74	0.001
Alanine transaminase (ALT),	12.57^{AB}	4.2	15.75^{A}	7.42	9.615^{B}	3.404	0.023

*(Reference value ranges information was obtained from İzmir Bornova Veterinary Control Institute) for Alanine transaminase ALT (u/L) is 4-20 and for Total Serum Cholesterol (mg/dL) is 125-200.

When the body weight averages were examined, the weight averages of the group given 1cc/L propolis were found to be higher than the other groups, while the lowest body weight averages were found in the 2cc/L group (P<0.05). The quality of water is of great importance in order to achieve healthy production and efficient results in poultry. Because regardless of the direction of water cultivation, it is the most consumed food and vehicle for poultry, it has a vital importance. We can say that the animals in the 2 CC /L propolis added group were affected by the sharp smell of propolis, consumed water and dense feed relatively less, and their live weights were affected. Although there were differences in weekly body weight gains, the difference was not statistically significant for any week (P>0.05). There are differences in liver and gizzard weight averages in propolis groups. In the comparison between the groups according to their liver weights, it was noted that the liver weight of the animals in the control group was higher than the groups given propolis and it was statistically significant. (P<0.05).This is due to the anti-oxidant properties of propolis, which are considered to improve lipid metabolism, liver morphological structures and biological functions. When the effects on blood parameters were examined, it was found that propolis had a lowering effect on high blood glucose level, triglyceride, LDL cholesterol, urea, creatine levels, and increased HDL cholesterol. In liver function tests such as AST, ALT, ALP, which increase with fatty liver, the use of propolis was effective in reducing the values, and it reduced liver fat in the trial groups given propolis.

Conclusions

Findings from this study did not affect the performance criteria of 1 and 2 cc/L of ethanolic propolis; however, dietary practices show that broilers receiving 1 cc/L propolis tend to increase their body weight and daily feed consumption when compared to the control group (p>0.05). It was observed that the chickens fed with

1 cc/L propolis added feed had better feed values compared to the other groups at the start, rearing and the whole trial period. Particularly, the observation of relatively better growth performance in the group given 1 cc/L propolis may be related to the regulation of intestinal microflora by relatively low levels of propolis, the reduction of nutrients due to the reduction of the number of competing microorganisms, and the reduction or destruction of microorganisms that cause subclinical pathogenicity. The fact that the experimental group animals given 1cc/L propolis showed higher body weight gain than the 2cc/L and control group animals in the measurements made at the third and fourth weeks, which is consistent with the literatures. Especially in the group given 1 cc/L propolis, the relatively better growth performance may be related to the regulation of the intestinal microbiota of propolis, nutrient savings due to the decrease in the number of competing microorganisms, reduction or elimination of microorganisms that cause subclinical infections, and reduction of toxins or metabolites that inhibit growth. Observation of relatively better growth performance, especially in the group given 1 cc/L propolis, regulation of intestinal microflora with relatively low levels of propolis, reduction of nutrients due to reduced number of competing microorganisms, and reduction or destruction of microorganisms that cause subclinical pathogenicity. Although there was no improvement in carcass and cut yields, there was a drop in abdominal fat percentage ($p<0.05$). Previous research has shown that a variety of parameters, including propolis type and dosage, amounts of active ingredients, duration of feeding, physiological characteristics (such as age, weight, and/or breed), and animal species, can have an impact on propolis' effects on the performance, health, and welfare of poultry. Propolis' effectiveness may vary depending on the trial conditions (stressed or unstressed, and the types of stressors), according to the literature that is currently accessible and its inconsistent results. Propolis's reported biological effects as an antioxidant, antibacterial, immune-stimulant, and growth promoter are supported by the favorable results on the performance and health of chicken, and they suggest that its usage as a natural feed additive is promising. Findings from this study did not affect the performance criteria of 1 and 2 cc/L of ethanolic propolis; however, dietary practices show that broilers receiving 1 cc/L propolis tend to increase their body weight and daily feed consumption when compared to the control group ($p>0.05$). It was observed that the chickens fed with 1 cc/L propolis added feed had better feed values compared to the other groups at the start, rearing and the whole trial period. Wheras chickens have an underdeveloped sense of taste, but a relatively keen sense of smell. Propolis has an aromatic odor and a stimulating effect on the salivary and gastric glands, and the addition of propolis (2 cc/L) to the feeds above tolerable levels reduced the daily feed consumption of the chickens. We think that propolis supplementation significantly increased cellular-mediated immunity compared to

the control group, since no signs of mortality and morbidity were observed in the propolis-treated group. As a result, we can state that there are important signs that the addition of propolis improves many assessment indicators in chickens and may be as effective as commonly used antibiotics. There still needs to be more investigation on the most effective methods for using it on chicken farms.

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CURRENT APPROACHES TO THE RELATIONSHIP OF ZEARALENONE AND FERTILITY IN LIVESTOCK

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Abstract: There has been an increase in the rates of infertility, embryonic death, and stillbirth in ruminants with increasing productivity levels in recent years. The basis of these problems in livestock depends on nutrition, however, these problems are not always related to unbalanced rations, but also to the consequences of global climate changes in the world. The source of these threats should be identified and a solution to the main problem should be sought. Zearelenone (ZEN), which causes infertility with its estrogenic effect in ruminants, is among the issues that animal nutritionists should pay attention to. Because this product can contaminate many crops such as corn, wheat, and soybean that make up the diet and harm farm animals. ZEN is a resorcyclic acid lactone, a mycotoxin with non-steroidal estrogenic action. Due to its structural similarity to estradiol, ZEN can chronically affect the reproductive performance of female animals. This mycotoxin, which can be metabolized in the rumen, causes the formation of five metabolites. The effects of these mycotoxins and their metabolites can be reduced by the use of enzymes, probiotics, antioxidants, clay minerals, and activated carbon, which can bind toxins or aid degradation. In this review, it is aimed to present the effects of zearalenone, which has caused significant fertility problems in ruminants in recent years, and current approaches that can be used to eliminate the negative effects of zearalenone.

Keywords: livestock, mycotoxins, zearalenone, fertility

Introduction

Increases in global warming and field-based contamination cause an increasing in the amount of mycotoxins in feeds, as well as the level of contamination with more than one mycotoxin. For example, stress, precipitation, increases in humidity levels, and insects that occur during the growth and harvest time of plants can cause damage to plants. These problems in fodder crops can occur during harvest time, storage and can lead to dangers that can cause toxicity to animals. The most important mycotoxin that has a negative effect on fertility in ruminants is ZEN, which has an estrogenic effect.

Even in very low amounts, the presence of fungal secondary metabolites in the organism is called mycotoxin intoxication. *Aspergillus, Penicillium, Fusarium, Penicillium, Alternaria,* and *Claviceps* fungi produce the most abundant mycotoxins in live (*Steyn, 1998; Döll and Danicke, 2011*). Among these mycotoxins, *Fusarium* spp., which has saprophytic and phytopathogenic characters, can be found widely in the fields with temperate and hot climates and can contaminate forage crops. (*EFSA, 2013*). In addition, spontaneous outbreaks of *Fusarium* mycotoxicosis have been reported in Europe, Asia, Africa, New Zealand, and South America (*Cortinovis et al., 2013). Fusariums* have produced mycotoxins trichothecene, fuzarin, fumonisin, beauvericin, enniatins and moniliformin, including zearalenone. It is known that Fusarium toxins also have toxicological effects on farm animals (*Fink-Gremmels and Malekinejad, 2007*). ZEN, synthesized by the fungi *F. graminearum, F. culmorum, F. grainis, F. equiseti, F. crookwellense,* and *F. semitectum,* is a mycotoxin that needs attention (*D'Mello et al., 1997*). ZEN is biosynthesized by the polyketide route as contaminant of cereal crops worldwide (*Bennett and Klich, 2003*). ZEN produced by *Fusarium* contaminates maize most and colonizes to a lesser extent barley, oats, wheat, sorghum, millet, and rice. In addition, the presence of toxins has been detected in grain by-products such as flour, malt, soybean, and beer (*Zinedine et al., 2007*).

ZEN affects the development of the ovaries and fertility in animals (*Dai et al., 2016*), even at the level of 1.04 mg/kg ZEN can reduce the level of follicles through massive follicular activation in the pig ovary. However, *Yang et al. (2018)* has also reported that ZEN promotes porcine ovarian development through estrogen receptors. Ingestion of metabolites of ZEN with estrogenic activity in farm animals at a level of 1-5 mg/kg in pigs may cause vulvovaginitis, vagina, and rectum prolapses (*Pitt, 2006*). It has been reported to reduce FSH and LH concentration in sheep and cows (*Nakamura et al., 2015; Riesen et al., 1977*) and testicular weight in rams (*Riesen et al., 1977*). In addition, it can impair oocyte maturation and embryo development in vitro, as well as inhibit granulosa cell proliferation and impair sperm function (*Chiminelli et al., 2022*). Young pigs are more susceptible to this toxin than cattle and sheep.

As a result, ZEN produced by *Fusarium* can cause economic losses by affecting the yield parameters in farm animal production. However, since the studies on the subject are not sufficient, new studies are carried out to see the effects and deactivation of this metabolite. In this review, it is aimed to learn the current effects and deactivation of ZEN, and will also provide an approach to new mycotoxication problems that may occur with global warming.

Structure and properties of Zearalenone

ZEN is a resorcyclic acid lactone, expressed chemically with the formula 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-*β*-resorcylic acid lactone and molecularly with the formula $C_{18}H_{22}O_5$ (*Seeling and Dänicke, 2005*). This toxin is white color, crystalline, and has a melting point of 164-165 °C, insoluble in water, but soluble in aqueous alkali and various organic solvents. Moisture level and oxygen availability are critical factors in ZEN growth. Fusarium grows in a laboratory environment in the presence of humidity above 20% and at a temperature between 20°C and 25°C within 3 weeks (*Gupta et al., 2018*). Even exposure of this fungus to a temperature of 8-15 °C for several weeks may be sufficient for toxin production. The ZEN breeding process in the field is similar to the environment that can stimulate zearalenone production in the laboratory, providing alternating weather conditions at medium and low temperatures. It is possible for ZEN to reproduce quite rapidly while in the field during wet weather in the summer or in the early fall following damage to corn by hail (*Paterson and Lima, 2010*). However, some species (*F. roseum*) can have two different temperature alternatives for ZEN production, high (24-27°C) or low (12-14°C). In maize, the effect of this toxin (*F. graminearum*) is called red ear rot and may be more associated with a cool, wet growing season and the presence of insect pests. In wheat, *Fusarium* is associated with the presence of excess moisture during flowering and early grain formation. ZEN contamination is often observed in maize, but wheat, barley, sorghum, and rye are also potential hosts. At the country level overall, the main sources of ZEN are wheat, rye, and oats in Europe; In Canada and the United States, it is in corn, corn products, and wheat products. *Gruber-Dorninger et al. (2019)* in their study where they collected worldwide feed material samples from 2008 to 2017, reported that there were 44% positive samples for ZEN in maize and 75% positive samples for DDGS in maize. In both soybean grains and soybean meal, ZEN was also reported to be the most common mycotoxin, with detection in 36% and 61% of samples, respectively. They found ZEN at the level of 33%, 20%, and 34% in wheat, barley, and rice, respectively. ZEN typically has low contamination of cereal grains in the field, but this rate may increase further when humidity rises above 30-40% under storage conditions (*Gupta et al., 2018*).

ZEN consumed by animals undergoes rapid biotransformation and is excreted from the body. The consumption of meat and meat products and their uptake into the human body is therefore uncertain (*Creppy, 2002*). ZEN and its metabolites can pass into the milk of dairy animals fed with feeds containing high levels of ZEN. *Prelusky et al. (1990)* in their study, 6.1 µg/L ZEN, 4 µg/L α-ZEN, and 6.6 µg/L in the milk of cows fed 6000 mg ZEN (12 mg/kg body weight (v.a.) equivalent) orally. They detected β-ZEN, but ZEN and its derivatives were not detected in the milk of 3 cows supplemented with 50 to 165 mg of ZEN (equivalent to 0.1 to 0.33 mg/kg bw) for 21 days. It has been suggested that ZEN leads to the synthesis of at least five metabolites, including ZAN, α-ZEN, β-ZEN, α-ZAL, and β-ZAL in the metabolism of ovine animals (*Miles et al., 1996*). There are six metabolites of ZEN that can cause toxicity with itself (Figure 1).

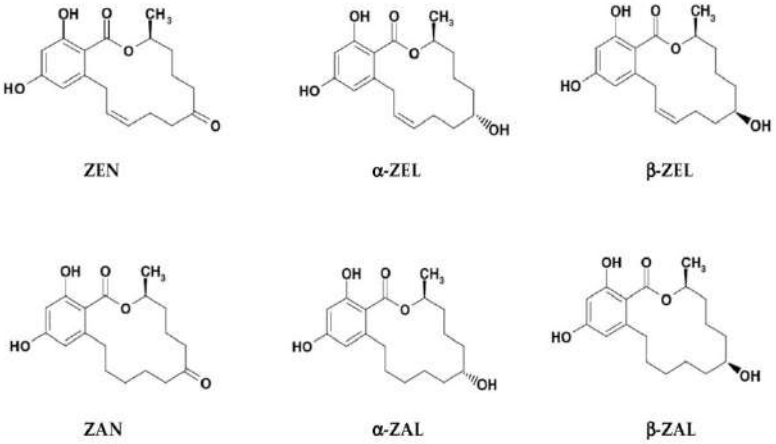

Figure 1. Chemical structures of zearalenone (ZEN/ZEA) and its modified forms α-zearalenol (α-ZEL/α-ZEN), β-zearalenol (β-ZEL/β-ZEN), zearalanone (ZAN), α-zearalanol (α-ZAL), and β-zearalanol (β-ZAL) (modified based upon *Urraca et al., 2004*).

Effects of zearalenone on biotransformation and reproduction

ZEN is rapidly absorbed after oral ingestion and can have various effects on animals. Although there are significant differences in the metabolic profile and process of ZEN between animal species, after consumption of ZEN in pigs it is rapidly absorbed and metabolized in intestinal cells (*Zinedine et al., 2007*). In ruminants, it is largely converted to the hydroxy-metabolite α-zearalenol by metabolism, predominantly by rumen protozoa, as well as in the liver (*Fink-Gremmels, 2008*). It is also metabolized in the rumen by reduction to β-zearalenol (*Kiessling et al., 1984*). However, sometimes there are cases where it cannot be completely destroyed, which may change due to changes in diets or metabolic diseases such as rumen acidosis and the filling of the detoxifying capacity of the rumen microflora. Alpha-zearalenol is 4 times more estrogenic than zearalenone, while the effect of β-zearalenol is equal to zearalenone (*Peng et al., 2018; Fink-Gremmels, 1999*). In rumen metabolism, the increase or decrease of zearalenone toxicity depends on its absorption by the gastrointestinal tract, its ability to be metabolized in the liver by hydroxysteroid dehydrogenase, and competition at cytosolic receptor sites in animal species (*Gupta et al., 2018*).

Due to its structural similarity to estradiol, ZEN can chronically affect the reproductive performance of female animals. ZEN is a non-steroidal, estrogenic mycotoxin (*Wood, 1992; Author and Omurtag, 2008*). The mycotoxins produced by *Fusarium* spp. are generally of two types: 1) non-estrogenic types, including deoxynivalenol and T-2; 2) Mycoestrogens, including ZEN and zearalenol (*Diekman and Green, 1992*). Zeranol may delay implantation and puberty in heifers, increasing the incidence of non-ovulatory oestrus, and thus delaying the development of the reproductive system (*Moran et al., 1990*). Because the lactone ring in its structure resembles many of the structural features of the aromatic rings of estradiol and apparently conforms to the binding portion of the mammalian estrogen receptor, ZEN and its metabolites exhibit distinct estrogenic and anabolic properties that affect the reproductive systems of many animal species (*Kleinova et al. 2002; Fink-Gremmels and Malekinejad 2007*; *Minervini and Dell'Aquila, 2008*). ZEN and its derivatives also cause estrogenic effects through competitive binding to the cytosolic estrogen receptor. The receptor-toxin complex is rapidly transferred to the nucleus, where it binds to specific nuclear receptors and produces estrogenic responses through gene activation, resulting in the production of mRNAs encoding proteins normally expressed by receptor-estrogen complex binding (*Riley, 1998*). However, it has been reported that dietary ZEN intake does not affect embryo production in superovulated cows (*Takagi et al., 2013*). Its structure is flexible enough to allow ZEN to bind to the mammalian estrogen receptor alpha, although it has a lower affinity than the natural estrogens 17b-estradiol, estriol, and estrone (*Pillay et al., 2002*). This may cause permanent pathological changes in the reproductive system, which may lead to infertility (*Liu and Applegate, 2020*). Based on the "uterotropic activity" evaluated in rodents, the estrogenic activity and modified forms of ZEN were classified in the following order: *α-ZEL > α-ZAL > ZEN ≈ ZAN ≈ β-ZAL > β-ZEL* (*EFSA, 2017*).

Contamination of ZEN at even extremely low concentrations can cause fetal death during pregnancy or neonatal weight loss in mammals (*Wang et al., 2013*). This mycotoxin has been associated with infertility, decreased milk production and hyperestrogenism in cows (*D'Mello et al., 1999*). *Fitzpatrick et al.* (*1989*) reported significant differences in the affinity of the metabolite α-Zol to the estrogen receptor between different animal species. Due to its estrogen-like effect, ZEN can inhibit ovarian activity and subsequently have adverse effects on the reproductive system of female animals (*Zinedine et al., 2007; Hu et al., 2020*). According to *Liu et al.* (*2023),* ZEN reported that it can inhibit goat granulosa cell proliferation, and while significant increases in apoptosis rate and oxidative stress levels are observed in these cells, it can reduce their estrogen synthesizing ability. *Etienne and Dourmad (1994)* also reported that ZEN can also affect the uterus by reducing LH and progesterone secretion and changing the morphology of uterine tissues. In male pigs, ZEN can suppress serum testosterone, testicular weight, and spermatogenesis, while inducing feminization and suppressing libido. Heifers fed a diet contaminated with 300 ppb ZEN has been reported to have significantly reduced viable oocytes compared to the control group (*Silva et al., 2021*). However, *Weaver et al. (1986*) studied the fertility level in Holstein heifers given 250 mg of 99% purified zearalenone in a gelatin capsule daily. In the study, they stated that there was no effect on serum progesterone concentration and no lesion attributable to zearalenone was observed in the heifer. *Elweza et al. (2022*) reported that in vitro, sperm-uterine encounter, pre-exposure of bovine endometrial epithelial cells to ZEN may reduce sperm motility parameters and decrease their survival in the bovine uterus, thereby reducing fertility. *Song et al. (2022*) reported that the use of DON and ZEN at 10 µM and 30 µM, respectively, in donkeys significantly impaired gene expression related to androgen and estrogen secretion. *Pizzo et al. (2015*) conducted a study to evaluate the effect of zearalenone metabolite α-zearalenol on cell proliferation and steroidogenesis of bovine large follicle granulosa cells. They reported that estradiol inhibited cell growth, indicating that α-Zol did not act as an estrogen agonist. *Zhu et al. (2012)* investigated the effect of high concentrations of zearalenone on the proliferation and apoptosis of porcine granulosa cells and found that zearalenone dose-dependently reduced the proliferation of porcine granulosa cells, and that it was dose-dependently mediated by caspase-3- and caspase-9-dependent mitochondrial pathways. They reported that it may be related to its ability to induce apoptosis and necrosis of porcine granulosa cells. However, β-zearalenol can act on granulosa cells and provide a stimulating function for hormone release (*Pizzo et al., 2016*). *Fushimi et al. (2015*) investigated the effects of low zearalenone levels in vivo on anti-Müllerian hormone endocrine levels and reproductive performance of cattle, and feed with zearalenone contamination below 1 ppm was given. Serum anti-

Müllerian hormone concentrations were observed as 438.9 ± 48.6 pg/ml and 618.9 ± 80.0 pg/ml in the 1st and 2nd flocks, respectively. In the study, they stated that although there is no difference in antral follicle populations between herds, this level of zearalenone may affect ovarian antral follicle populations even though it does not affect fertility in cows. *Takagi et al. (2008*) aimed to simultaneously detect ZEN and its metabolites in bovine follicular fluids by mass spectrometry and to examine the in vitro effects of ZEN on bovine oocytes. In the study, they reported that ZEN and its metabolites can be found in bovine follicular fluid and may affect meiotic competence, but not fertilization and development rates.

Zearalenone deactivation and protection strategies

It can be categorized into 4 groups aimed at reducing the presence of mycotoxin. These can be classified as physical methods, thermal methods, chemical methods and feed additives that provide mycotoxin control. However, while previous three methods are aimed at reducing the presence of mycotoxins during the processing of feed materials, the last method is aimed at reducing the effects of mycotoxin-contaminated rations on the animal body (*Peng et al., 2018*). When the feed materials are evaluated to determine the presence of mycotoxins in the herd, it should be checked whether the color changes are observed on the green roughage, mold, and fungus formations on the roughage and whether the silages are harvested at the appropriate time. It should be ensured that the plants are not stressed during growth and harvest. If it is necessary to evaluate the presence of mycotoxins with animal controls, milk yield should be evaluated, decreases in pregnancy rates and increases in abortion rates should be evaluated. When evaluating feed consumption, checks should be made whether the imbalances in totally mixed ration consumption or whether the residual feed in the feed mixer creates a suitable environment for mold and fungus growth.

Ruminants may be exposed to different mycotoxins due to their complex ration content resulting from different feed ingredients including roughage and grains. Unlike monogastric animals, rumen microbiota can reduce some mycotoxins to less toxic or non-toxic metabolites through enzymes released by microorganisms (*Doğan and Dal, 2022*).

Li et al. (2023a) investigated the effects of isorhamnetin, an antioxidant flavonoid, on oocyte damage caused by zearalenone in pigs. They reported that Isorhamnetin enhanced mitochondrial function and protected oocytes from ZEN-induced damage by inhibiting early apoptosis, oxidative stress, and endoplasmic reticulum stress in porcine oocytes. In addition, *Li et al. (2022*) planned a study to investigate the effects of isorhamnetin on ZEN-induced damage in porcine ovarian granulosa cells and to elucidate its molecular mechanism. They reported that

Isorhamnetin can protect ovarian granulosa cells from damage caused by ZEN by promoting proliferation, attenuating apoptosis, and oxidative stress.

Li et al. (2023b) planned a study to see the effects of using cyanidin-3-O-glucoside (C3G), a metabolite found in or produced by Saccharomyces cerevisiae, against the negative effects of zearalenone on porcine ovarian granulosa cells (pGCs). The results reported that C3G could effectively rescue ZEN-induced apoptosis in pGCs, especially significantly increasing cell viability and proliferation.

Gruber-Dorninger et al. (2021) planned a study to see the effects of using enzymes (ZENzyme®, Biomin) that can reduce estrogenic effects in cattle by hydrolyzing the ester bond of the lactone ring of zearalenone. With this study, they reported that these enzyme derivatives could be a promising strategy to counter the estrogenic effects of ZEN in cattle.

Niderkorn et al. (2006) evaluated the ability of different strains of lactic (LAB) and propionic acid bacteria (PAB) to degrade *deoxynivalenol* (DON) and fumonisins in their study on mycotoxin degradation in silage in vitro. The strains selected in the study were capable of removing up to 88% of zearalenone and that fermentative bacteria could bind the main Fusarium mycotoxins.

Chlebicz and Śliżewska (2020) carried out a study in which they looked at the ability of *Lactobacillus* spp. (12 strains) and *Sacchromyces cerevisiae* (6 strains) to destroy toxins, including zearalenone. They observed detoxification rates of aflatoxin B1, T-2 toxin, and zearalenone by Lactobacillus at 60%, 61%, and 57%, respectively, and by yeast for 65%, 69%, and 52%, respectively. They reported that these probiotics could potentially be used to reduce toxin concentrations in animal feeds. In another study, *Bertuzzi et al. (2022)* reported that *Bacillus subtilis* QST 713 was able to inhibit the growth of zearalenone mycotoxins in their study to control mycotoxigenic fungus formation in agricultural crops.

Fruhauf et al. (2019) evaluated the estrogenic activity of hydrolyzed zearalenone and decarboxylated hydrolyzed zearalenone metabolites formed by the hydrolysis of zearalenone using zearalenone-degrading enzymes (zearalenone-lactonase Zhd101p) in pigs. In the study, they stated that both metabolites exhibited significantly reduced estrogenicity in vitro and in vivo.

Among the mycotoxin binders, inorganic clays and activated carbon (AC) act as a mycotoxin binder: 1) chemical interactions involving different types of cation exchange capacity such as ion-dipole, Van der Walls forces or hydrogen bonds; and 2) MTB can reduce mycotoxin load in the gastrointestinal tract in animals based on its physical properties such as pore size or mycotoxin structure and shape (*Kihal et al., 2022). Kihal et al. (2022)* studied the effect of mycotoxin binders [AC, bentonite, clinoptilolite, hydrated sodium calcium aluminosilicate (HSCAS), montmorillonite (MMT), sepiolite, yeast cell wall (YCW), and zeolite] against 6 mycotoxins, including zearalenone. They stated that mycotoxin adsorption capacity was 83% for AC, 76% for MMT, 62% for bentonite, 55% for HSCAS, 52% for sepiolite, 52% for clinoptilolite and 44% for YCW. They stated that the adsorption for ZEN was 48%.

Conclusion

Changing climate conditions on a global scale and their side effects require revision and regulation of facts. Among these, it has become important to comply with the hygiene rules and biosecurity measures in feed, which is the main component of animal husbandry. Mycotoxins in feeds have important effects on animal health and production, but Zearalenone derivatives produced by *Fusariums* cause a decrease in reproductive performance in animals. In addition to various factors that can cause reproductive disorders in high-yielding farm animals, the effects of Zearalenone are also mentioned. Zearalenone can lead to a decrease in reproductive activities due to hormonal and functional changes in farm animals. In general, to prevent this mycotoxin problem, forage crops should be planted and harvested in accordance with the region. However, more research is needed to see the ZEN contamination levels in feeds and the effects of ZEN on reproduction.

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THE USE OF STARCH IN THE MEAT PROCESSING INDUSTRY

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Invited paper

Abstract: Starch is extensively used as an additive in the modern food industry. Various starch sources are applied in the meat industry with multiple functions, such as water binding, emulsifiers, fat substitutes, stabilisers and texturisers. However, in many processing conditions, using native starch does not always give the desired result, given functionality. Modification of native starch is therefore carried out to give the starch the properties desired in these cases. This article overviews different starch properties, their modifications and their application in the meat industry.

Key words: native starch, starch modifications, fat mimicry, meat products

Introduction

Starch is a form of plant energy reserve and, second to cellulose, it is the most widespread biological material on earth. It is usually closely packed as small granules in the plants' tubers, seeds, leaves or roots. Starch usage is known from ancient times; however, only a couple of centuries ago has starch become industrially available for usage in food. *Bergsma (2000)* describes that the first “industrial processes” for manufacturing wheat or potato starches date back to 1772. Nowadays, in the industrial production of starch, only a few plants are used: maize (corn), potato, tapioca (Cassava), pea, rice and wheat. The predominant crop of each continent primarily determines these preferences for starch sources. For example, in North America, the most commonly used starch in food applications is corn-based, while potato starch is the most predominant in Europe. Rice and tapioca starches are commonly used in the food industry in Asia.

Each starch source has unique characteristics important to its selection for use in a meat formulation. When stained by potassium iodine solution, the starch source can be identified microscopically by the shape, diameter range, and colouration of its granules. The microscope is the tool most commonly used to identify starch when its source is unknown and to check its cook status and functionality in a food product *(Tarté, 2009)*.

In general, potato and corn (maize) starches are preferable for the meat processing industry. Compared to other starches, potato starch has the largest granules and, therefore, the highest water-binding capacity. One significant advantage of potato starch is that it will start to gelatinize at the same time as the meat proteins are losing the most water. Another essential characteristic of potato starch is its rapid viscosity development due to naturally occurring phosphate groups, making it the preferable choice for pasteurized meat products, where the final internal temperatures are not exceeding 72-75˚C. Potato starch is often considered ideal for meat products application *(Zhang and Barbut, 2005)*.

Due to higher amylose content (approx. 25%), native corn starch in meat systems will form firmer gels after cooling (that can contribute to better slicing performance). However, it will have lower water binding capacity and higher syneresis than potato. Because of the higher gelatinization temperature of native corn starch, its usage is more suitable in sterilized meat products.

With a small amount of amylose (approximately 18%), tapioca starch gives a soft gel when pasted. It is used mainly in meat systems with softer textures (for example, in spreads).

Amylopectin or “waxy” starches have no linear amylose molecules, so they will not gel and give a much softer texture in meat products. Their water binding capacity (peak viscosity) is much higher than native starches. Another advantage is that they will not retrograde since there is no amylose, and they are best suited to achieve long-term water holding capacity: higher yield, less cook loss and purge, but softer texture. There are two primary sources of waxy starches on the market: waxy corn (maize) and waxy potato. The starch source, in this case, has the same effects as the native form of this starch: waxy potato has higher peak viscosity (water binding) and lower gelatinization temperature than corn.

The amylose content related to the starch variety influences the gelatinization temperature and the final texture. The gelatinization temperature and the maximum starch functionality must be achieved during the heat treatment. Choosing the right starch source is critical depending on desired product characteristics and process conditions. Otherwise, the starch will not be fully functionalized, and its total water-binding capacity and stability will not be reached. This review aimed to summarize the functionality of native and modified starches in meat systems and better explain starch's role in meat products.

Starch composition

Starch is a biopolymer, and regardless of which plant is produced, it consists of two large glucose polymers, linear and branched. The former is referred to as amylose, and the latter as amylopectin. Both molecules are constructed of glucose, and their glucose chain linkages differentiate them: amylose is a linear glucose polymer chain with α-(1,4)-glucosidic linkages, while amylopectin is a more branched glucose polymer with α-(1,4) and α-(1,6) linkages *(Eliasson, 2004)*. Starches generally contain 17 to 24% amylose and 76 to 83% amylopectin. Different starch raw materials give different functional properties, mainly due to a different composition of amylose and amylopectin, but also to different granular sizes and other minor components like lipids, proteins and endogenous phosphate *(Biliaderis, 1998)*.

Native starch granules are insoluble in cold water due to the strong hydrogen bonds holding the starch polymers together. However, the granules swell when the aqueous starch suspension is heated in water. A classic diagram of a starch cooking curve, the viscosity development, is represented in Fig1. Every native form of starch during the cooking process (heating and cooling) goes thru several stages: first, it reaches its pasting/gelatinization temperature, starts to swell, reaches its viscosity peak, and lose its viscosity as the heating continuous (breakdown phase), and in the end, it increases in viscosity in a cooling phase (phenomena known as setting or retrogradation) *(Kaur et al., 2012)*.

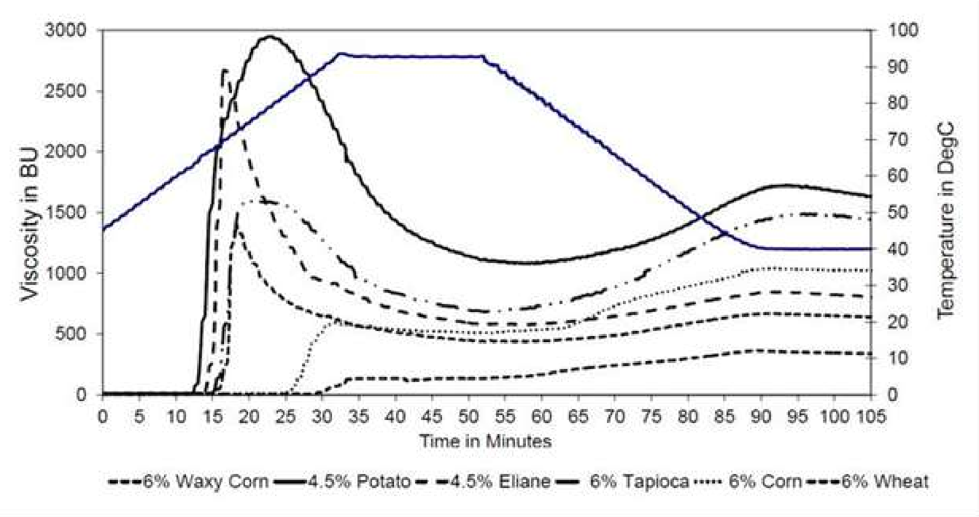

Figure 1. Gelatinization profiles of different starches

Fig. 1 also shows the difference between the cooking curves of different native starch sources. Compared to others, potato starch has the highest peak viscosity and starts to gelatinize earlier, followed by tapioca and waxy maize (maize starch composed of almost 100% amylopectin). Maize starch has the highest "setback" or increase in viscosity during the cooling phase, primarily due to the highest amylose content (amylose is responsible for gelling and retrogradation of cooked starch during cooling).

Starch modifications

In general, native starches have limitations in terms of heat, shear, acid and freeze-thaw resistance, and by modifying the native starch, we can improve their performance. When modifying starch, the alteration occurs at the molecular level, with a very low change in the superficial appearance of the granule. Some modifications also include adding some functional attributes to the starch, such as emulsification properties. The most important chemical, biochemical and physical modifications of starch include *(BeMiller, 1997; Eliasson, 2004; Kaur et al., 2012)*:

- Cross-linking – an essential chemical modification that influences the starch to have shorter texture, inhibited swelling and enhanced stability at low pH, heat, sheer and freeze-thaw stability compared to its native form. The most common cross-linking whey is the replacement of hydrogen bonds between starch chains with stronger covalent bonds.
- Stabilization – by addition of blocking substituents to the hydroxyl group of the starch chain. Stabilized starches have lower retrogradation and gelatinisation temperature than their native form.
- Acid hydrolysis – the other terms for this type of modified starches are: acid thinned, thin boiling and fluidity starch. The effects of this modification are: lower hot viscosity and strong gel after cooling.
- Oxidation – occurs when starch has been simultaneously degraded (into smaller chains) and stabilized. These starches have lower hot viscosity, lower gel strength (compared to acid-thinned starches) and lower retrogradation.
- Enzyme hydrolysis – is a biochemical modification where a range of enzymes are used to produce starch hydrolysates (α-amylase, β-amylase, iso-amylase, etc.).
- Maltodextrins – smaller chain fragments obtained by acid or enzymatic hydrolysis of starches.

- Lipophilic substitution – is a unique form of modification where the octenyl succinate groups are linked to starch, which makes starch capable of stabilizing oil-in-water emulsion. Emulsifying starches are generally not the best water binders, as their primary function is to emulsify. Their common name is OSA starches.
- Pregelatinization – is a physical modification that can be applied to native or modified cook-up starches. The starch is pregelatinized by simultaneously cooking and drying. In this process, a cold-water swelling starch is produced.

With some exceptions, in Europe, all chemically and biochemically modified starches have an E-number, as they are classified as food additives and regulated by Directive 95/2/EC on “food additives other than colours and sweeteners” *(European Parliament and Council, 2006)*:

- E 1404: oxidized starch
- E 1410: mono-starch phosphate
- E 1412: di-starch phosphate
- E 1413: phosphorylated di-starch phosphate
- E 1414: acetylated di-starch phosphate
- E 1420: starch acetate
- E 1422: acetylated di-starch adipate
- E 1440: hydroxypropyl starch
- E 1442: hydroxypropyl di-starch phosphate
- E 1450: starch sodium octenyl succinate
- E 1451: acetylated oxidized starch

The starches that are not considered as food additives in EU and therefore have no E-number are:

- Native starches (including “waxy” starches – amylopectin starches)
- Pregelatinized native starches
- Enzymatically treated starches
- Acid hydrolyzed starches
- Dextrinized starches
- Other physically modified starches, such as: thermal inhibited starches, annealed and heat-moisture treated starches

The addition and the permit to use starch in the EU are regulated for many specific meat products. Regulations can differ from country to country, so one must always check the local law of the country where the product is to be sold.

Why use starch in the meat industry?

No other food ingredient compares with starch regarding the sheer versatility of application in the food industry *(Eliasson, 2004)*. Starch is a common ingredient in the meat industry for its water-binding capabilities, and it has been used as a meat filler from ancient times forward. Starches are used as water binders in processed meats to increase yields, reduce cooking losses, improve texture, sliceability and succulence, and extend shelf life. However, the magnitude of these effects differs according to several factors, such as the botanical origin of the starch, modification, concentration, the matrix in which it is acting, the technological process, cooking temperatures, salt concentration and other processing steps *(Skrede, 1989; Li and Yeh, 2002; Zhang and Barbut, 2005)*. Starch can be incorporated and distributed in the meat matrix by either injection, tumbling, or simply by direct addition (in a bowl chopper or blender).

Many meat products are on the market, made with different ingredients, processing conditions, shapes, shelf-life, etc. However, from the “starch perspective”, meat products can be divided into ground-type products where the addition of brine (during injection and/or tumbling) is common, emulsified type products (usually produced in a bowl chopper or other emulsifying equipment) and fresh products, such as burgers and fresh sausages.

Fat Mimicry

Meat products with high-fat content rich in saturated fatty acids are often assumed to contribute predominantly to the increased risk of obesity, heart diseases and stroke *(WHO, 2003)*. With consumers becoming more health conscious, there is a trend toward producing food with decreased fat, cholesterol and calorie levels. This has influenced the meat industry to redesign products that traditionally have high fat.

The fat content of meat products can vary considerably, depending on the proportion of lean and fat from the meat source and the level of added fat as an ingredient. Traditional meat products such as sausages and hams are high in fat – even up to 50%. Fat is an essential component of meat products influencing flavour, succulence, tenderness, juiciness, mouthfeel, and heat transfer, and it also serves as the discontinuous phase of sausage emulsions *(Pearson and Gillet, 1999)*.

Consequently, reducing fat can lead to severe quality and technological issues in meat processing because products tend to become rigid, dry and rubbery, have an additional shrinkage during cooking and have an excess accumulation of purge in packages.

The commonly used starches are the ones that will create a fat-like structure inside the meat matrix. In emulsified meat systems, maltodextrins are generally used because of their thin-viscosity and fat-mimetic properties. Another possibility is to use rice starches, which offer some "fatty behaviour," such as a soft gel structure and a creamy mouthfeel, due to their small particle size, the smallest of all commonly used starches *(Chen et al., 2020)*.

In ground-type meat products, where the goal is to replace visible fat-like particles, the first step should be making gel by mixing and heating starch and water, which will be incorporated as a "fat source" into the mixture. For this specific application, gelling starches are the preferable choice.

How to choose the "correct" starch?

Choosing the correct starch for the application is essential to maximize starch performance and improve product quality. There is a great variety of native and modified food starches available on the market. Every starch company has a preselected range for meat applications, each with different product names. Generally, it is always good advice to contact the application specialist from the selected company and ask for a recommendation.

When formulating a new product, various conditions that could affect the selection of the starch can be summarized in the following questions:

- Why do you want to use starch in your product? This way, you can identify whether you require one starch, a blend of starches or a combination of starch, other hydrocolloids and/or proteins.
- Are you going to cook the starch? In other words, will you use the cook-up, cold-water swelling starch, or a combination? The final temperature (pasteurization or sterilization) and cooking time are critical.
- What amount of free water will be present? Starch requires minimum levels of water to be fully functional. Besides meat proteins, other ingredients will bind water and could interfere with starch gelatinization.
- How will the product be stored? With this information, we can ensure the best stability during storage. An example is a product to be frozen and kept in the freezer longer.

- How will the product be labelled? Will you choose the starch with or without E-number (clean label starch)?

To choose the suitable modification, the formulator must think of the desired attributes of the final product to be created, which cannot be achieved using native starch sources. These attributes often include firmer texture, survival of higher cooking temperatures (sterilization) or shear, better freeze-thaw stability (storage conditions), lower or higher viscosity, emulsification and various economic aspects (low cook-loss, low drip loss, price and usage level).

Table 1. Some general functionalities of modified starches in meat systems (adapted from *Tarté, 2009*)

Modification type	Functionality in meat product
Stabilization	Decrease gelatinization temperature Freeze-thaw stability Shelf life Lower syneresis
Cross-linking	Process tolerance (high temperature, low pH, high shear) Delay in viscosity development
Acid hydrolysis	Firmer texture after cooling (high gel strength)
Oxidation	Unique rheology (fat mimetic) Create softer and stable gels (less syneresis)
Dextrinization (maltodextrins)	Better solubility Film formation (fat mimetic)
Enzyme hydrolysis	Fat mimetic Thermoreversible gelling
Lipophilic substitution	Emulsion stability Decrease fat loss
Pregelatinization	Cold water thickening properties
Thermal treatment	Improve process tolerance to heat, acidity and shear Delay in viscosity development

*Combination treatments are also commonly used to achieve desired objectives. The most important one is cross-linking/stabilization.

As seen in Table 1, every modification gives starch some unique properties in meat systems, many of which are related to water management within the meat matrix. Water management can be expressed by yields and cooking losses, by purge and drip loss over storage, and, finally, by finished product texture, flavour, and colour, all of which are influenced, to varying degrees, by the amount of water in the formula and how it is bound.

Although most of the starches used in meat are cook-up starches because they should bind the water when the meat is being heated (as the meat proteins release water when denaturing), as well as additional free water coming from the brine, cold-water swelling (or pregelatinized) starches can also be used. The primary function of pregelatinized starches in meat systems is to help form a ground meat product or bind water up front – used in injected meats for particle suspension in brine, where they can help avoid sedimentation of cook-up starches and other ingredients insoluble in cold water.

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EFFECT OF FAT LEVEL ON QUALITY CHARACTERISTICS OF TRADITIONAL SUCUK SAUSAGES. PART 1: PHYSICO-CHEMICAL CHANGES DURING PRODUCTION

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Invited paper

Abstract: This trial aimed to investigate changes in pH, proximate composition and fat rancidity parameters during the production of traditional Sucuk sausage (dry fermented sausage) with different fat levels. Three groups of sausages were produced from beef meat and fat: LF (low fat, with 10% added fat), MF (medium fat, with 20% added fat) and HF (high fat, with 30% added fat). The production was carried out in a traditional smoking house, and fermentation and ripening lasted 28 days. Samples were analysed after stuffing (day 0) and on days 7, 14, 21 and 28 of production. The higher fat content of the HF group probably influenced the lowest pH value at the beginning of production (day 0) ($p<0.05$). The HF group also had the mildest drop in pH ($p<0.05$), while the MF and LF groups had a similar trend in pH decline. Fat content had a significant effect on the proximate composition of the products, best reflected at the end of the production process, where the HF group had significantly higher fat and lower moisture and protein content ($p<0.05$). A higher total free fatty acids (FFA) content was obtained in the LF group, showing greater lipolysis than in MF and HF groups. Interestingly, sausages with higher fat content had lower peroxide values during production ($p<0.05$).

Key words: sucuk sausage, fat level, proximate composition, peroxide value, free fatty acid value

Introduction

Three types of traditional fermented sausages are most popular in Serbia: Sremski kulen, Sremska sausage and sucuk. Sucuk is a Turkish-style sausage, very popular in several Middle East countries, as well as Europe *(Erkmen, 1997; Stanišić et al., 2012)*.

Sucuk and sausages of a similar type are spicy, typically dry fermented sausages made of beef, water buffalo meat or mutton *(Gökalp, 1986)*. In Serbia, they are traditionally produced in small plants or village households in autumn and winter when weather conditions (temperature and relative humidity) are favourable. They are made of smaller pieces of beef or sheep meat and tail fat, with meat:fat ratio around 75:25. The mixture is filled into natural beef casings and tied with rope. The product is then smoked and dried for approx—30 days under local weather conditions *(Stajić et al., 2013)*.

Due to a high-fat content rich in saturated fatty acids and cholesterol, consumption of meat products is associated with the risk of heart problems, obesity and various blood diseases *(WHO, 2003)*. Dry fermented sausages are high-fat and rich in saturated fatty acids. Sucuk usually contains approx-30% fat content in the form of small visible cubes. This high-fat content is essential for proper fermentation and ripening and also plays an important role in the overall sensory quality of the finished product *(Kayaardı and Gök, 2003)*. For these reasons, reducing this type of product's fat content to improve nutritional characteristics is very difficult *(Bloukas et al., 1997)*.

The scientific knowledge of traditionally produced sucuk sausages with reduced fat content is limited *(Kargozari et al., 2014)*. Additionally, due to the natural fermentation and uncontrolled climatic conditions, there is little uniformity in traditionally produced products' recipes and processing conditions, which makes published trials challenging to repeat. In order to improve the nutritional quality of dry fermented sausages, this trial was set to investigate the effect of different fat levels on a change in the quality characteristics of sucuk sausages during production.

Materials and Methods

Sausages were produced in a meat processing plant of the Institute for Animal Husbandry (Belgrade, Serbia) from February to March 2022. For the production of sausages, beef, ham meat and tail fat were used. Three groups of sausages with different fat levels were produced: LF (low fat) group was made of beef meat with the addition of 10% fat, MF (medium fat) group was made of beef meat with the addition of 20% fat and HF (high fat) group was made of beef meat with the addition of 30% fat. In all variants, the same amounts of the following ingredients were added: 1.8 % salt, 0.011% $NaNO_2$, 0.4% sucrose, 0.3% powdered black pepper, 0.2% garlic and 0.2% powdered red paprika.

The production process was as follows: beef ham meat and tail fat were frozen at -4°C and then minced in a meat grinder (Seydelman 114, Germany) to about 1 cm in diameter. Cutting and mixing with the rest of the dry ingredients was carried out in the cutter (Seydelman K60, Germany) to 3 mm particle size. The mixture was then filled in natural beef casings of 38 mm. Sausages were tied with rope and made into characteristic horseshoe shapes. After stuffing (day 0), the sausages were drained in a cold store (4 ± 1°C) for 12 h for the surface to dry, after which they were hung in a traditional smoking house (without the possibility to control the temperature or humidity), with the parameters varying between 5–10°C and RH 75–90%. They were then occasionally smoked for 28 days.

Sampling of all three variants of sucuk was carried out after filling (day 0) and on production days 7, 14, 21 and 28. Nine sausages were taken from each group to determine pH value and conduct chemical analyses. All samples were analysed after removing the outer casing and grounding in the mixer (Ultra Turrax T18, IKA, Germany).

pH value was measured by pH-meter Hanna, HI 83141 (Hanna Instruments, USA), equipped with a puncture electrode. The pH meter was calibrated using standard phosphate buffers *(ISO 2917, 1999).*

The proximate composition of sausages was determined in the following manner: moisture content by drying samples at 105°C *(ISO 1442, 1997)*; protein content by Kjeldahl method and multiplying by factor 6,25 *(ISO 937, 1978)*; total fat content by Soxhlet method *(ISO 1443, 1973),* and ash content by mineralisation of samples at 550 ± 25°C *(ISO 936, 1998).*

Peroxide value was determined by the method described in *ISO 3960 (1977)*, and peroxide values were expressed as milliequivalents of active oxygen per kg of fat (mEq O_2/kg).

Free fatty acid content was expressed as g oleic acid/100 g fat after titration with 0.1N NaOH and determining the total acidity, as described in *ISO 660 (2011).*

The results of pH value, proximate composition, peroxide and FFA value were processed by single factor analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SPSS 20.0 software (IBM SPSS Statistics Version 20, IBM Corp, USA). The differences between individual averages were tested using Tukey’s method, and significant differences were considered for $p<0.05$.

Results and Discussion

Changes in the pH values during the 28 days of production of three groups of sucuk sausages are presented in Fig.1.

During the production of dry sausages, as a product of bacterial fermentation, lactic acid is formed, which results in a pH drop *(Lücke, 1994)*. Multiple factors influence this drop in pH, with the most important being present microflora and the amount and type of added sugars *(Hiero et al., 1997)*. pH values of sausages from the MF and LF groups followed a similar pattern during the entire production process, while the ones from the HF group slightly differed. Initial pH values (day 0) were different between groups, with HF having the lowest values ($p<0.05$) compared to MF and LF sausages, with no significant difference between the latter. These findings disagree with *Gómez and Lorenzo (2013)*, who reported no significant differences in initial pH values for chorizo sausages produced with different fat levels. However, other authors have observed a similar effect of fat level on the initial pH of various dry fermented sausages *(Olivares et al., 2010; Lorenzo et al., 2011; Lorenzo and Franco, 2012)*.

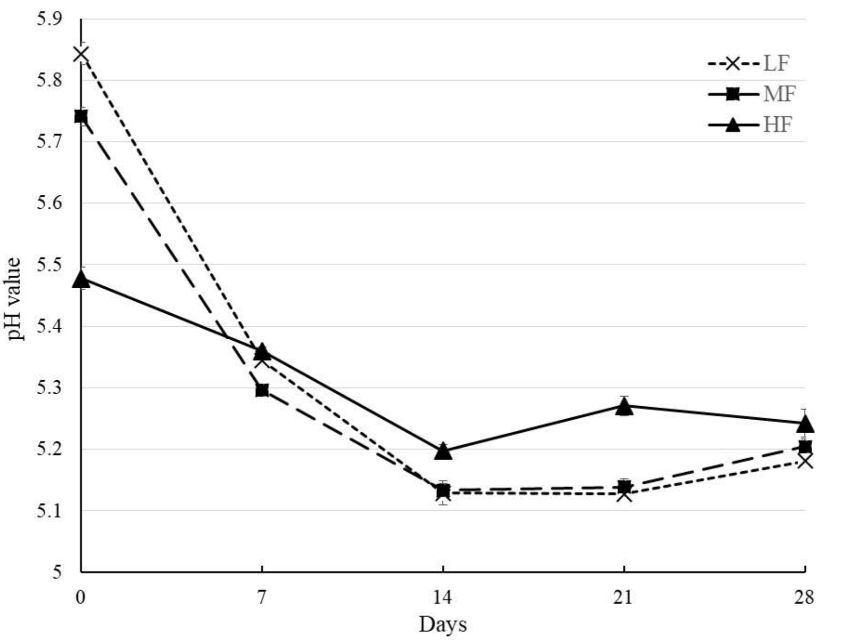

Figure 1. Changes in pH of the three groups of sucuk sausages during the 28 days of production. LF: low fat (10%); MF: medium fat (20%); HF: high fat (30%). Each symbol represents the mean value ± standard deviation.

The highest pH drop for all three groups of sausages was recorded on days 7 and 14, where all three groups had similar values. *Kayaardi and Gök (2003)* reported similar dynamics in pH drop for traditionally produced sucuk. The organic acids production, due to the microflora activity, was probably the reason behind the fast pH decline in the first two weeks of production. Although after day 14, pH values slightly increased, they were similar at the end of ripening (day 28) and did not significantly differ between groups. As stated by *Spaziani et al. (2009)*, the traditional production of fermented sausages is characterised by a slow drop in pH (due to low temperatures and the absence of starter cultures), with the minimal values reached between the 20th and 40th days of production. In this trial, the fat reduction significantly impacted a pH drop, indicating that the medium and low-fat sausages will probably have a different flavour pattern compared to high-fat ones. This is in agreement with *Olivares et al. (2010)* and *Lorenzo and Franco (2012)*, who reported a more significant decrease in pH values in low-fat sausages. However, *Liaros et al. (2009)* did not find a significant difference in pH drop during the production of sausages with different fat levels. Similarly, investigating the quality of Greek sausages, *Papadima and Bloukas (1999)* reported no effects of fat level on final pH.

At the end of the production process, the pH of all three groups of sausages increased slightly and had similar value (around 5.2), which was lower compared to the ones reported by *Comi et al. (2005)* for naturally fermented dry sausages that ranged from 5.2 to 6.4. This increase in pH at the end of production was probably due to ammonia and amine production due to enzymatic activity *(Lücke, 1994)*.

The changes in proximate composition during the production process of sucuk are shown in Table 1. As expected, they are characterised by a decline in the moisture content and an increase in fat, protein and ash content during the 28 days of fermentation and ripening in all three groups ($p<0.05$). As stated by *Živković et al. (2011)*, the changes in the chemical composition of dry fermented sausages during ripening are characterised by a tendency to increase dry matter content.

In all groups of sausages, moisture content was constantly falling during the entire production process ($p<0.01$) due to temperature and humidity conditions. The initial differences between sausages in moisture content (day 0) were greater than at the end of the production process (day 28), probably due to more rapid water evaporation from sausages with lower fat content. In finished products, moisture content varied from 28.07% (HF) to 33.29% (LF). The moisture content of dry fermented sausages, around 30%, is typical for these products in Hungary, Greece and Croatia *(Kozacinski et al., 2008)*.

The fat content of sucuk significantly differ among groups ($p<0.01$), with mean values of 23.12% in LF, 32.71% in MF and 43.48% in HF sausages at the end of the production process, as expected. Sausages from the HF group had higher fat and lower protein content, followed by MF sausages initially and during the entire production process. Fat content at the end ripening of HF and MF groups was within the range for traditional sucuk *(Yaman et al., 1998)*.

Table 1. Changes in the proximate composition (%) of the three variants of sucuk sausage during the 28 days of the production process (means ± standard deviation)

Parameter / Group		Day					Sig.
		0	7	14	21	28	
Water	LF	67.12 ± 1.63^{aA}	60.17 ± 1.06^{bA}	52.02 ± 0.80^{cA}	37.34 ± 1.32^{dA}	33.29 ± 2.33^{eA}	**
	MF	60.82 ± 1.39^{aB}	53.95 ± 1.07^{bB}	42.45 ± 0.95^{cB}	32.97 ± 0.36^{dB}	31.49 ± 2.48^{eAB}	**
	HF	54.48 ± 1.36^{aC}	43.01 ± 0.87^{bC}	39.15 ± 1.14^{cC}	32.36 ± 0.62^{dB}	28.07 ± 1.05^{eB}	**
Sig.		**	**	**	*	*	
Protein	LF	20.69 ± 3.95^{dA}	26.14 ± 1.99^{cA}	28.97 ± 2.85^{bA}	34.30 ± 2.78^{aA}	36.90 ± 2.09^{aA}	**
	MF	18.09 ± 0.75^{dB}	21.82 ± 1.01^{cB}	23.12 ± 1.56^{cB}	29.51 ± 1.34^{bB}	32.48 ± 2.54^{aB}	**
	HF	15.20 ± 0.94^{cC}	19.77 ± 1.08^{bC}	20.24 ± 1.70^{bC}	27.76 ± 2.98^{aB}	30.94 ± 2.76^{aB}	**
Sig.		**	**	**	*	**	
Fat	LF	8.77 ± 0.20^{dC}	10.47 ± 0.49^{cC}	14.82 ± 0.48^{b}C	21.88 ± 1.14^{aC}	23.12 ± 0.91^{aC}	**
	MF	18.58 ± 0.39^{dB}	20.94 ± 1.00^{cB}	26.96 ± 0.91^{bB}	33.94 ± 1.16^{aB}	32.71 ± 1.53^{aB}	**
	HF	26.66 ± 1.58^{eA}	33.02 ± 0.95^{dA}	36.53 ± 1.58^{cA}	39.93 ± 1.53^{bA}	43.48 ± 1.39^{aA}	**
Sig.		**	**	**	**	**	
Ash	LF	3.45 ± 0.04^{eA}	4.30 ± 0.04^{dA}	4.76 ± 0.04^{cA}	5.65 ± 0.02^{bA}	6.36 ± 0.08^{aA}	**
	MF	3.42 ± 0.02^{eA}	3.87 ± 0.03^{dB}	4.88 ± 0.04^{cA}	5.48 ± 0.04^{bAB}	5.62 ± 0.04^{aB}	**
	HF	3.37 ± 0.02^{eA}	3.89 ± 0.03^{dB}	4.80 ± 0.04^{cA}	5.19 ± 0.04^{aB}	5.10 ± 0.02^{aB}	**
Sig.		ns	*	ns	*	*	

LF: low fat (10%); MF: medium fat (20%); HF: high fat (30%)
$^{a-e}$ Different letters within the same row denote significant differences between means
$^{A-C}$ Different letters within the same column denote significant differences between means
^{ns} non-significant ($p \geq 0.05$); * significant at $p<0.05$; ** significant at $p<0.01$

The ash content significantly decreased during ripening in all three groups of sausages ($p<0.01$). These findings disagree with *Salgado et al. (2005)*, who found no changes in ash content during the fermentation and ripening of Chorizo, a traditional Spanish fermented sausage. At the end of production, ash values were 6.36, 5.62 and 5.10 (for LF, MF and HF sausages, respectively), which is in the range reported by *Coppola et al. (1997)*, *Gimeno et al. (2000)*, *Lorenzo et al. (2000)* and *Franco et al. (2002)* for similar types of sausages.

The changes in fat rancidity indicators during fermentation and ripening of sucuk sausages were evaluated by peroxide and free fatty acid (FFA) values (Fig 2.)

The initial values for both indicators were approximately the same for all sausage groups. However, after seven days of production, the most significant drop in peroxide value was recorded for the HF group ($p<0.05$), while MF and LF groups had similar values. After day 7, the LF group had a stip increase in peroxide values, with the biggest differences between groups recorded for days 14 and 21, where the LF group had the highest values and the HF group the lowest, while the MF group was intermediary ($p<0.05$). At the end of the production process, the MF group had the lowest peroxide value ($p<0.05$), while HF and LF were similar. In general, peroxide values of sausages in this trial were much lower compared to the ones reported by *Franco et al. (2002)* of 16 and 28 meq O_2/kg and *Salgado et al. (2006)* of 12.85 meq O_2/kg of fat.

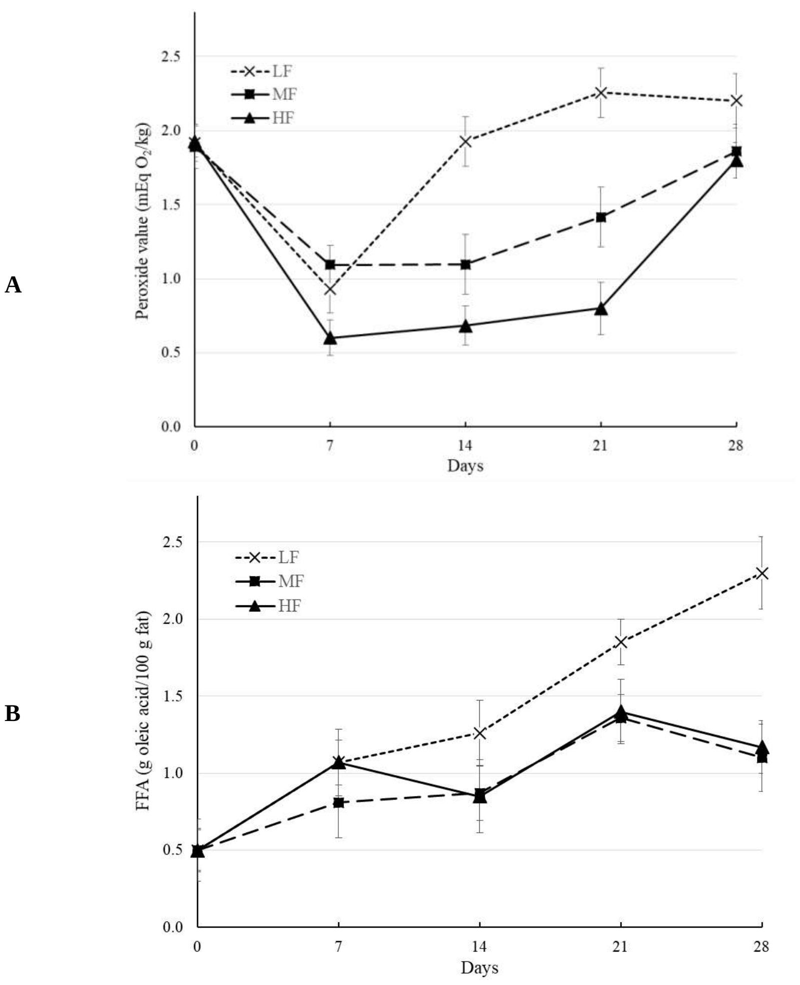

Figure 2. Changes in peroxide (A) and FFA (B) values of the three variants of Sremska sausage during the production process. LF: low fat (10%); MF: medium fat (20%); HF: high fat (30%). Each bar represents the mean value ± standard deviation.

As a result of lipolysis, free fatty acids are released during the production process of dry-fermented sausages *(Gandemer, 2002).* Although the initial values were similar, the FFA (expressed as g of oleic acid per 100 g of fat) had the highest increase in the LF group of sausages ($p<0.05$). The increase in the FFA content of MF and HF groups followed a similar pattern and did not differ significantly. These results are in disagreement with the ones reported by *Soyer and Ertas (2007), Liaros et al. (2009), Olivares et al. (2011)* and *Lorenzo and Franco (2012),* who found higher peroxide and FFA values in sausages with the higher fat content. However, these studies are based on pork subcutaneous fat, which is reacher in polyunsaturated fatty acids and more subject to oxidative changes than present beef tail fat *(Marco et al., 2006; Gómez and Lorenzo, 2013)*.

Conclusion

Decreasing the fat level in traditionally produced sucuk sausages significantly affected the proximate composition and lipolytic parameters during the 28 days of production. Sausages containing higher fat content had significantly lower water, protein and ash content throughout and at the end of the production process.

Fat content significantly affected peroxide and free fatty acid values. Interestingly, sausages containing higher fat content had lower lipolytic changes during and at the end of production. This can be correlated with a higher degree of saturated fatty acids in beef fat, which are less prone to oxidative changes.

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EFFECT OF FAT LEVEL ON QUALITY CHARACTERISTICS OF TRADITIONAL SUCUK SAUSAGES. PART 2: TEXTURE, COLOUR AND SENSORY QUALITY

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Abstract: Traditional dry fermented sucuk sausages were made from beef and formulated with 10, 20 and 30% of added fat (LF, MF and HF group, respectively). The production was carried out in a traditional smoking house, and fermentation and ripening lasted 28 days. After the end of the production process, colour (CIE L*a*b*), texture TPA parameters and sensory quality were analysed. An increase in fat content significantly affected the reduction in TPA values for hardness, springiness, cohesiveness and chewiness ($p<0.01$). Data obtained in this study indicate that sausages with higher fat content were less firmly bound (lower cohesiveness) and less elastic (lower springiness). Additionally, fat content significantly influenced an increase in lightness (L*) and yellowness (b*) and a decrease in redness (a*) of sucuk sausages ($p<0.05$). The lowest sensory scores were obtained for HF sausages, especially for consistency, cut appearance and taste.

Key words: sucuk sausage, fat level, colour, texture, sensory quality

Introduction

There is a great variety of dry-fermented sausages, and almost every European country has at least several characteristic products from this group *(Casaburi et al., 2007)*. Three types of these sausages are most popular in Serbia: Sremski kulen, Sremska sausage and sucuk. Sucuk is a Turkish-style sausage traditionally made of beef, water buffalo, or mutton *(Gökalp, 1986)*. Sucuk has traditionally been produced without the addition of starter cultures and with natural processing conditions, usually in the autumn and winter *(Soyer et al., 2005)*.

The dietary regime with lower fat content, saturated fatty acids and cholesterol have been promoted by various health organizations worldwide. In the last decades, due to a negative association of high-fat consumption, especially the ones rich in saturated fatty acids, with various health issues, the meat industry started to produce products with reduced fat content *(Colmenero, 2000; Mendoza et al., 2001)*. As fat is an important ingredient that, besides nutritional profile, influences sausage texture, flavour, colour and overall acceptability, its reduction can have adverse technological and sensory effects *(Choi et al., 2010; Stanišić et al., 2012)*.

In this regard, dry fermented sausages, as the products that traditionally have a high amount of fat, are unsuitable for fat reduction. Traditionally they contain approx. 30% fat in the recipe, which after drying, due to the loss of moisture, rises to more than 40% in the finished product *(Wirth, 1988)*. Consequently, lowering the fat content in these products may have a negative effect on their technological and sensorial characteristics *(Muguerza et al., 2003; Stajić et al., 2020)*. Fat reduction can lead to a too-dry product, with a hard and rubbery texture, due to a higher moisture loss *(Keeton, 1994)*. This can also lead to decreased sausage appearance due to a wrinkled surface and casing *(Muguerza et al., 2002)*.

The main objectives of this study were to determine the effect of different fat levels on the texture, colour and sensory quality characteristics of sucuk sausages produced traditionally, with natural fermentation and ripening.

Materials and Methods

Sausages were produced in a meat processing plant of the Institute for Animal Husbandry (Belgrade, Serbia) from February to March 2022. For the production of sausages, beef, ham meat and tail fat were used. Three groups of sausages with different fat levels were produced: LF (low fat) group was made of beef meat with the addition of 10% fat, MF (medium fat) group was made of beef meat with the addition of 20% fat and HF (high fat) group was made of beef meat with the addition of 30% fat. In all variants, the same amounts of the following ingredients were added: 1.8 % salt, 0.011% $NaNO_2$, 0.4% sucrose, 0.3% powdered black pepper, 0.2% garlic and 0.2% powdered red paprika.

The production process was as follows: beef ham meat and tail fat were frozen at -4°C and then minced in a meat grinder (Seydelman 114, Germany) to about 1 cm in diameter. Cutting and mixing with the rest of the dry ingredients was carried out in the cutter (Seydelman K60, Germany) to 3 mm particle size. The mixture was then filled in natural beef casings of 38 mm. Sausages were tied with rope and made into characteristic horseshoe shapes. After stuffing (day 0), the sausages were drained in a cold store (4 ± 1°C) for 12 h for the surface to dry, after which they were hung in a traditional smoking house (without the possibility to control the temperature or humidity), with the parameters varying between 5–10 °C and RH 75–90%. They were then occasionally smoked for 28 days.

A sampling of all three variants of sucuk was carried out after the production process. Nine sausages were taken from each group for colour, texture and sensory analyses.

The colour was measured using Chromameter CR-400 (Minolta Co. Ltd, Tokyo, Japan). The instrument was configured with the following parameters: D65 light source, 10° observer, and 8 mm aperture size and calibrated using a white ceramic tile. The measurements were done according to the CIE L*a*b* system: lightness (L*), redness (a*) and yellowness (b*). C* (chroma) and h (hue angle) were calculated using the available software *(CIE, 1976)*. The colour measurements were performed at room temperature (20 ± 2°C) immediately after cutting the sausages.

Texture analyses were done using a universal testing machine (Instron model 4301, Instron Ltd., England). After cutting into slices of 1-1-2 cm (width-height-length), nine readings were taken for each sausage variant. The samples were held for equilibration to room temperature and compressed twice to their original height, with a compression aluminium platen of 75 mm (P/75) and a 250 kg load cell. The pre-test speed was 3 mm/s, the test speed was 1 mm/s, and the post-test speed was 1 mm/s. The following parameters were obtained: hardness, springiness, cohesiveness, and chewiness.

Sensory evaluation was done by a panel of five trained evaluators using a quantitative–descriptive test *(SRPS ISO 6658/2002)*. Scores were given on a scale from 1 to 5, where score 1 was unacceptable, and score 5 was optimum. Sensory properties investigated were surface appearance, cut appearance, colour, smell, taste and consistency.

The results of texture, colour and sensory analyses were processed by single factor analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SPSS 20.0 software (IBM SPSS Statistics Version 20, IBM Corp, USA). The differences between individual averages were tested using Tukey’s method, and significant differences were considered for $p<0.05$.

Results and Discussion

Results from the TPA analysis showed a significant effect of fat level on all analysed characteristics (Table 1). It has been reported that moisture and fat content at the end of the fermentation and ripening are the main factors affecting the binding and rheological properties of dry fermented sausages *(González-*

Fernández et al., 2006). Hardnesses, springiness, cohesiveness and chewiness values significantly decreased with increased fat content (p<0.01).

Table 1. Texture parameters of the three variants of sucuk sausages (means ± standard deviation)

Parameter	LF	MF	HF	Sig.
Hardness (N)	157.55 ± 8.62^{a}	128.22 ± 6.91^{b}	107.83 ± 7.08^{c}	**
Springiness (mm)	0.51 ± 0.04^{a}	0.40 ± 0.02^{b}	0.38 ± 0.04^{b}	**
Cohesiveness	0.44 ± 0.02^{a}	0.34 ± 0.02^{b}	0.29 ± 0.02^{c}	**
Chewiness (N/mm)	35.14 ± 4.30^{a}	24.62 ± 2.04^{b}	16.44 ± 2.25^{c}	**

LF: low fat (10%); MF: medium fat (20%); HF: high fat (30%)
$^{a\text{-}e}$ Different letters within the same row denote significant differences between means
** Significant at p<0.01

Several authors found that dry-fermented sausages with reduced fat content have higher values for hardness and chewiness *(Salazar et al., 2009; Liaros et al., 2009; Mendoza et al., 2001; Muguerza et al., 2002)*. Additionally, hardness values are negatively correlated to moisture content *(Lorenzo and Franco, 2012)*. In this trial, LF sausages had significantly higher hardness, springiness, cohesiveness and chewiness values than the other two groups (p<0.01). In contrast, MF sausages had significantly higher hardness, cohesiveness and chewiness values than the HF group (p<0.01). Low chewiness values in HF groups indicate that it takes less effort to prepare food for swallowing compared to LF and MF sausages *(Stajić et al., 2014)*. Data obtained in this study indicate that sausages with higher fat content were less firmly bound (lower cohesiveness) and less elastic (lower springiness). Similar values for TPA were also reported by several authors *(González-Fernández et al., 2006; Olivares et al., 2010; Beriain et al., 2011; Lorenzo and Franco, 2012)*. The results of this trial are in disagreement with *Liaros et al. (2009)*, who reported no significant differences (p>0.05) between fermented sausages with different fat levels for springiness, cohesiveness and chewiness.

Of all the quality characteristics of sucuk, colour seems to be the most important one *(Yildiz-Turp and Serdaroğlu, 2008)*. Fat content significantly affected the CIEL*a*b* colour parameters of sausages (Table 2). As expected, LF sausages, with the lowest fat content, had the lowest L* values compared with the other two groups (p<0.01). These findings are in agreement with previous trials of *Hand et al. (1987), Papadima and Bloukas (1999), Soyer et al. (2005), Bozkurt and Bayram (2006)* and *Olivares et al. (2010)*, who reported increasing of sausage lightness with increasing the fat content. Although HF sausages had a higher fat content compared to the MF group, there were no significant differences in L* values between them.

Table 2. Colour parameters of the three variants of sucuk sausages (means ± standard deviation)

Parameter	LF	MF	HF	Sig.
L*	40.18 ± 7.59^{a}	45.99 ± 4.68^{b}	50.66 ± 5.61^{b}	**
a*	14.29 ± 2.41^{a}	12.14 ± 2.35^{b}	11.23 ± 1.60^{b}	*
b*	7.77 ± 1.30^{a}	9.07 ± 1.12^{b}	9.05 ± 0.82^{b}	**
C*	16.00 ± 2.31	14.88 ± 2.47	15.63 ± 1.84	ns
h	28.68 ± 6.39^{a}	36.46 ± 4.09^{b}	35.88 ± 4.80^{b}	**

LF: low fat (10%); MF: medium fat (20%); HF: high fat (30%)
$^{a-e}$ Different letters within the same row denote significant differences between means
^{ns} non-significant ($p \geq 0.05$); * significant at $p<0.05$; ** significant at $p<0.01$

Papadima and Bloukas (1999) found that increasing the fat level of dry fermented Greek sausages significantly increased the lightness (L*) and yellowness (b*) and decreased redness (a*). The redness (a*) values significantly differ only between LF and the other two groups (p<0.05), with no significant differences between MF and HF groups. It has previously been reported that sausages with lower fat and higher lean content are redder *(Candoğan and Kolsarici, 2003)*, which agrees with this trial. The faster formulation of nitrosyl myoglobin in low-fat sausages, as *Soyer et al. (2005)* reported, also contributes to a higher redness value.

Low-fat sausages had the lowest degree of yellowness (b*) compared to MF and HF groups (p<0.01), which is also confirmed by other researchers *(Soyer and Ertas, 2007)*. The b* value positively correlates to the amount of sausage lipids *(Soyer et al., 2005)*. *Muguerza et al. (2002)* and *Olivares et al. (2010)* also reported increasing yellowness due to a higher fat level.

Different fat levels of sausages did not have a significant effect on the chroma values (C*), indicating that there were no differences in colour saturation between groups. Larger values for hue angle (h) indicate a less red product, defined as the development of colour from red to yellow *(Tapp III et al., 2011)*. LF sausages had significantly lower h colour values than the other two groups (p<0.01). The hue angle values of sausages from the HF and MF groups were similar.

Sensory characteristics of fermented sausages are strongly correlated to the length and rate of fermentation and drying, breakdown of lipids, smoking and the quality of raw materials used *(Berdagué et al., 1993; Johansson et al., 1994)*. In this trial, fat content significantly affected the sensory scores for consistency, cut appearance and taste ($p<0.05$). At the same time, there were no differences in surface appearance, surface colour and smell between LF, MF and HF groups of sucuk sausages (Fig. 1). MF and LF sausages had similar sensory scores for all tested attributes, except cut appearance. Higher scores for consistency, taste and cut appearance for lower-fat sausages (LF and MF groups) compared to high-fat sausages (HF group) are reported by other authors *(Lorenzo and Franco, 2012).*

These results agree with the research of *Papadima and Bloukas (1999)* that traditionally dried Greek sausages with 30% of fat had the lowest sensory scores for appearance and consistency, while sausages containing 20% fat had the highest. Additionally, the same authors found no differences in odour between sausages. In general, in this trial, all sausages were characterised by a specific flavour and texture of this type of product *(Bozkurt and Erkmen, 2002).*

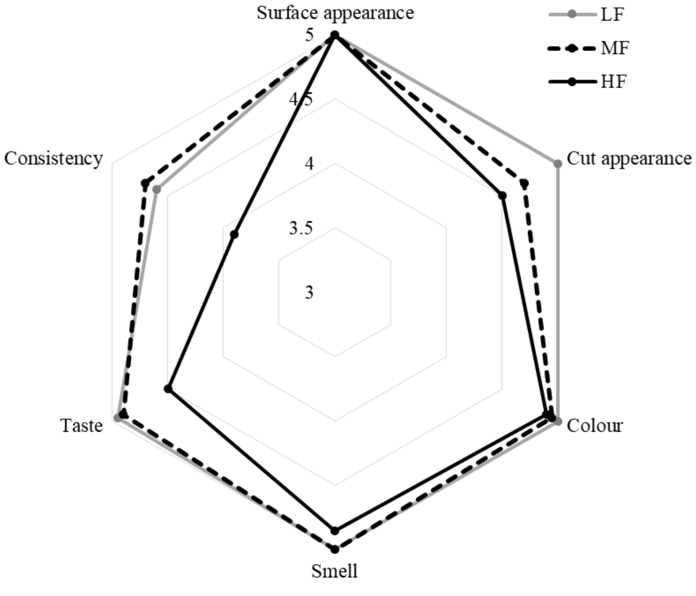

Figure 1. Results of the sensory evaluation (quantitative–descriptive analysis) on three groups of sucuk sausages. LF: low fat (10%); MF: medium fat (20%); HF: high fat (30%).

Conclusion

The findings of this study showed that it is possible to reduce the fat content of traditionally produced dry fermented sucuk sausages, but considerably affecting the TPA parameters and instrumental colour at the end of processing. Sausages containing 20 and 30% of fat had lower hardness, chewiness, and higher lightness values than sausages with 10% fat. Fat level significantly affected the sensory attributes of sausages, showing high-fat sausages had lower scores for consistency, taste and cut appearance compared to mid- and low-fat ones.

Based on the obtained data, it can be concluded that producing naturally fermented sucuk sausages with 10 and 20% fat has some advantages, like a firmer texture and better sensory scores. However, the production of sausages with 30% fat content resulted in some defects, such as soft texture, pale appearance and lower scores for taste and consistency.

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MEAT OF NATIVE PIGS BREEDS AS A RAW MATERIAL FOR TRADITIONAL PRODUCTS OBTAINED IN SERBIA AND POLAND

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Invited paper

Abstract: The global tendency to increase the productivity of livestock leads to the displacement of native animal breeds by modern, selected breeds or production lines that grow faster and are characterized by better meat yield. However, with an increase in productivity, resistance to adverse environmental conditions decreases, the morbidity of animals increases. An alternative are native breeds that are adapted to local (often difficult) climatic conditions, are an important element of the landscape and constitute an invaluable genetic resource for the population and genetic variability. An example can be the native breeds of Serbian (Mangalica, Moravka, Resavka) or Polish (Puławska, Złotnicka White, Złotnicka Spotted) pigs. The aim of the study was to analyze the quality of meat and cold cuts from pigs of the Mangalica and Moravka, as well as Puławska, Złotnicka White and Złotnicka Spotted breeds. The meat of pigs of native breeds was characterized by very good culinary and processing quality. Particularly noteworthy is the higher content of intramuscular and intermuscular fat, which has a positive effect on the taste of cold cuts and marbling of meat. The fatty acid profile of the fat of breeds kept in Serbia was more favorable compared to Polish breeds - higher content of essential fatty acids. Serbian and Polish cured meats differed in taste, color parameters and the level of polycyclic aromatic hydrocarbons. The Carpathians are the limit of the variety of cold cuts, especially sausages.

Keywords: pigs, native breeds, meat, traditional products

Introduction

The pursuit of profit, ever higher production results led to the industrialization of agricultural production. More and cheaper goods is the main goal of modern agriculture, including animal husbandry. Forecasts indicate that over the next 30 years the demand for milk and milk products will increase by almost a half and for meat by 76%. Therefore, the world is dominated by the industrial production model, which is the greatest threat to the diversity of genetic resources of farm animals. The conflict between the increase in production and its negative effects on human health as well as on nature is becoming more and more visible. As a result of selection, man turned farm animals into production machines. The rearing of such animals takes place in large rooms, with the possibility of movement limited to a minimum. However, as performance increases, resistance to adverse environmental conditions decreases. Over the past 30 years, animal disease has increased by 600%. The causes of many diseases are: the intensification of production and the use of high-protein feeds, hormones, antibiotics and growth stimulants. In addition, modern, selected breeds, production lines or varieties that have better performance values, lay more eggs, grow much faster and have higher milk yield, displace native breeds. Native breeds are not suitable for breeding on large-scale farms, they are not adapted to life in constant confinement. In agriculture, and especially in animal husbandry, biological diversity (biodiversity, genetic variability) is needed, i.e. the presence in a given population of as many different genes and their combinations as possible.

Biodiversity is essential for both people and the environment as an indispensable factor in the fight against climate change. It is the basis of the functioning of nature. However, it is decreasing at an alarming rate - 200 species die out every day in the world *(https://www.consilium.europa.eu/pl/policies/biodiversity/)*. It is estimated that there are almost 8,000 livestock breeds. Of these, 18% have the status of not endangered, 17% endangered, endangered - protected or in a critical condition, 7% are extinct animals and about the remaining 58% we can only say that they exist and we do not know what their condition is. Protecting the genetic resources of local breeds is an investment in the future of future generations. Old breeds are the source of unique genetic combinations, they are well adapted to breeding. They are part of our culture and tradition. Preserving native breeds gives consumers access to healthy, tasty, traditional, local, unprocessed and high-quality products. The need to protect native, local varieties of plants and animal breeds results not only from their production features, used or possible to be used in the future, but they are also a testimony to the material culture of the community, they perform natural and landscape, ethnographic and socio-cultural functions *(Szyndler-Nędza, 2006; FAO, 2007; 2006; Krupiński and Ptak, 2018; Krupiński et al. 2017)*.

The biodiversity created over the years of work of breeders and farmers is the cultural and scientific achievement of the entire society, and we are all responsible for its preservation. The most appropriate method of protecting the genetic resources of crop plants and farm animals is their preservation in situ - in the natural environment, i.e. in farms and home gardens. Extensive or medium-intensive farming combined with nature protection can bring income with relatively small outlays. Local animal breeds and plant varieties are particularly useful for use on organic farms, agritourism farms or those located in protected areas. In-situ conservation of livestock genetic diversity includes all activities aimed at maintaining viable populations of farm animals, including these under active breeding programs, in the agrosystems where they were produced or currently occur normally, including breeding activities undertaken to ensure the continuous contribution of these resources to sustainable production food and agriculture, now and in the future.

During the Earth Summit in Rio de Janeiro (June 3–14, 1992), the United Nations Convention on Biological Diversity was adopted, which formulated and adopted commitments regarding: protection of biodiversity, sustainable use of its elements, fair sharing of benefits resulting from the use of genetic resources *(Convention on biological diversity, 1992)*. The threat to biodiversity is particularly visible in animal production. In 2014, 8,774 breeds were registered in the global DAD-IS (Domestic Animal Diversity Information System) database, including 7,718 local breeds. Of these breeds, only 18% (about 1,600 breeds) were considered not endangered, while as much as 7% (647 breeds) were considered extinct. According to FAO criteria; 150 breeding-age cows of a given breed, 300 ewes (sheep), 300 mother goats, 200 mares, 100 sows and 100 female poultry give the breed the status of "critical condition" *(https://www.fao.org/dad-is/regional-national-nodes/efabis/en; Krupiński and Ptak, 2018)*. Only between 2000 and 2005 years, 66 breeds of livestock became extinct in the world. Such a high rate of extinction of breeds proves the increasing threat of loss of livestock biodiversity *(FAO, 2015; Krupiński and Ptak, 2018)*. Poland ratified above Convention in December 1995, which imposed on our state the obligation to preserve biodiversity in its own country, not only in protected areas, but also in areas used economically, mainly for agriculture. In 1999, work began on the National Program for the Protection of Animal Genetic Resources, the overarching goal of which was to ensure that the genetic diversity of farm animals necessary for food production and agriculture will be preserved now and in the future *(Krupiński et al., 2018)*.

In May 2000, the Minister of Agriculture and Rural Development approved breeding programs for the protection of genetic resources of individual populations, including programs for the Polish Konik and the Hucul horse. These programs contain information on the history of the origin of a given breed and the justification for the need to protect it, specify the goals and schedule of activities, as well as the scope of in-situ and ex-situ conservation. The programs also define the principles and methods of breeding work and indicate the organizations responsible for their implementation *(Krupiński et al., 2018)*. Poland is currently one of the countries most involved in the protection of genetic resources of farm animals. There is currently implemented 32 protection programs cover 92 breeds, lines or varieties, including 42 populations of mammals, 35 birds, 5 bees and 10 lines or strains of fish *(Krupiński and Ptak, 2018)*.

Programs for the protection of genetic resources are carried out mainly with the in-situ method, which enables protection through use while improving the specific and valuable features of a given breed. The genetic resources protection program in Poland covers, among others, cattle: Polish Red cattle, White-back, Polish Black and White, Polish Red and White; horses - Polish horses - Polish Primitive Horses, Hucul horses, Malopolski horses, Silesian horses, Wielkopolska horses, Sokolski horses, Sztumski horses: Sheep's: Pomeranian, Kamieniecka, Wrzosówka, Black-headed, Żelaźnińska, Uhruska, Corriedale, Świniarka, Colored Mountain Sheep , Polish Mountain Sheep, Podhale Zackel, Olkuska, Polish Pogórza sheep, Wielkopolska, White-headed meat sheep, Old-type Polish Merino, Colored Merino; Carpathian goat or: Złotnicka spotted, Złotnicka white, and Puławska pigs *(Krupiński et al.,2017; Krupiński et al., 2018)*.

In Serbia there are two main organization (Agricultural university in Novi Sad and Institute for animal husbandry) which acts as Central breeders organization. One of the aims of these organisations is conservation of native animals and their implementation in modern agricultural life in Serbia. Ministry of agriculture have found the way to stimulate breeders in form of some money for endengered species. Serbia also ratified international conventions relevant to protection of biodiversity, including the Convention on the Conservation of European Wildlife and Natural Habitats, Bern, 1979 and the Convention on Biodiversity, Rio de Janeiro, 1992. The Republic of Serbia has become a signatory of the Rio Declaration on Environment and Development by succession. Namely, the Federal Government of the then FR Yugoslavia adopted its Resolution on Biodiversity Conservation Policy in FR Yugoslavia on 31st December 1993, while the Law on Ratification of the Convention on Biological Diversity was adopted in 2001. Since that moment this normative act has represented the basis and the framework for adequate action and development towards biodiversity conservation on the territory of Serbia. In Serbia houses some of the most important biodiversity centers in Europe. The Republic of Serbia possesses unique breeds and varieties of domestic animals created through long-term selection processes conducted by humans and influenced by natural conditions present in particular regions *(Ministry of Environment and Spatial Planning, Republik of Serbia, 2010; 2011).* The List of indigenous breeds of domestic animals and endangered indigenous breeds (Official Gazette of the Republic of Serbia, Issue 38/10) indicates that the following autochthonic breeds of domestic animals in the Republic of Serbia are still in existence: domestic turkey, domestic duck, Danubian goose, domestic guinea fowl, Serbian High-flyer pigeon and the Serbian Sarplaninac (Sar Mountain) Shepherd dog. The Republic of Serbia also has an autochthonic breed of bee, *Apis melifera carnica*, with its varieties. It is one of most valuable breeds of honeybee in the world. Between 400 and 500 individual farms, entrepreneurs, state institutions and public enterprises maintain endangered breeds of pets *(Regulation on the List of Domestic Animal Genetic Reserves, 2017).*

These include Podolsko goveče (Podolian), Buša (Busha) cattle; horses Domaci Brdski konj (Domestic-Mountain Pony), Nonius (Nonius); Balkanska goat (Balkan goat), Domaća bela goat (Serbian white goat); sheeps Bardoka (Bardoka), Vlaško-vitoroga ovca (Vlashko - Vitoroga sheep), Karakačanska ovca (Karakachan sheep), Krivovirska ovca (Krivovirska sheep), Lipska ovca (Lipska sheep), Pirotska ovca (Pirotska sheep), Svrljiška ovca (Svrljig sheep), Cigaja (Tsigai sheep), Čokanjska cigaja (Chokanj sheep), Šarplaninska ovca (Sharmountain sheep); pigs Mangulica (Mangalica), Moravka and Resavka.

Šiška (Shishka) and Šumadinka (Sumadinka pig) no data available about population and number these breeds are assumed to be extinct *(Ministry of Environment and Spatial Planning, Republic of Serbia, 2011; Grittner et al., 2021).*

Despite the existing subsidies for farmers keeping animals of conservative breeds, the most reliable method of keeping them or increasing the number of animals is the profitability of production. It can be increased by products made of raw materials obtained from animals of conservative breeds, products with a unique taste and smell. From the meat of Złotniki pigs (spotted and white) and pigs of the Puławska breed, excellent traditional cold cuts are made in Wielkopolska, Mazovia, the Lublin region and Podkarpacie *(Migdał et al., 2019a; 2019b).* The meat of Mangalica, Moravka and Resavka pigs is used to make cured meats popular in Serbia.

The aim of the research was to characterize the meat and popular cold cuts made from the meat of native pig breeds in Poland and Serbia.

Materials and Methods

The aim of the study was to analyze the quality of meat and cold cuts from pigs of the Mangalica (n=6) and Moravka (n=6), as well as Puławska (n=10), Złotnicka White (n=10) and Złotnicka Spotted (n=10) breeds. There were analyzed raw meat and traditionally smoked meat products obtained from meat of native breeds bred in a traditional way. The products were purchased from different regions of Poland and Serbia where the raw materials were obtained from homeland breeds.

The raw meat (*m. longissimus dorsi lumborum* between 13th and 14th rib) were minced and average samples obtained were subjected to following chemical analyses:

- Water content according to the standard PN-ISO 1442:2000 *(Polish Committee for Standardization, 2013a).*
- Fat content according to the standard PN-ISO 1444:2000 *(Polish Committee for Standardization, 2013b).*
- Protein content by Kjeldahl method PN-75/A-04018 *(Polish Committee for Standardization, 2002).*
- Total ash content according to the standard PN-ISO 936:2000 *(Polish Committee for Standardization, 2013c).*
- Total carbohydrates content was calculated assuming that the all total solids and water stand for 100%.

Fatty acid profile - samples were extracted with chloroformmethanol (2 : 1, v/v), according to the method developed by Folch et al. *(1957)*. Then, 1 g of meat samples was mixed with 15 mL of chloroform-methanol mixture, and homogenized for 10 min at 5,000 rpm, and after a 5-minute pause – for another 5 min, at 1,000 rpm, using homogeniser MPW-120. The mixture was then filtered through filter paper to a regular cylinder, and completed with extraction mixture up to 15 mL. Next, 3 mL of 0.74% KCl solution was added to 15 mL of the filtrate. The alcohol–water phase was removed, and the chloroform phase was washed three times using 2 mL solution of chloroform : methanol : 0.74% KCl (3 : 48 : 47, v/v/v). Subsequently, the chloroform phase was recovered, dehydrated with anhydrous sodium sulphate (Na2SO4), and dried using nitrogen at 45°C. Further, 0.5 ml 0.5 N KOH in methanol was added to the sample (about 10 mg), and heated at 85°C. Next, 1 ml 12% BF3 in methanol was added, and the sample was again heated at 85°C. After cooling in room temperature, 1 ml hexane and 5 ml saturated solution of NaCl were added. Fatty acid methyl esters profile in one ʒl samples at the split ratio of 10 : 1 were separated by gas chromatography on a TRACE GC ULTRA gas chromatograph, equipped with 30 m capillary column SUPELCOWAX 10 of 0.25 mm inner diameter and coating thickness of 0.25 ʒm

(30 m × 0.25 mm × 0.25 um). Operating conditions were as follows: helium was used as the carrier gas, flow of 1 ml/min, split flow of 10 ml/min, injector temperature of 220°C, detector temperature of 250°C, and initial column temperature of 160°C.

Meat products were minced and average samples obtained were subjected to following chemical analyses: water, fat, protein, total ash, total carbohydrates content and

- NaCl content by the Mohr's method (Polish standard PN ISO 1841-1:2002 *(Polish Committee for Standardization, 2013d).*
- polycyclic aromatic hydrocarbons (PAHs) (benzo(a) pyrene and sum of benzo(a)pyrene, benzo(a)antracene, benzo(b)fluorantene and chrysene) according to the HRGC-HRMS method (CZ_SOP_D06_06_180 - except chap. 11.3.3.1 - 11.3.3.7, 11.3.3.9 l, 11.3.4 (US EPA 429, ISO 11338); PAH16: Determination of polyaromatic hydrocarbons by isotope dilution method using HRGC-HRMS).

Results and Discussion

Pigs of native Polish and Serbian breeds, unlike the popular, commercial breeds of pigs, are perfectly adapted to the environmental conditions in the country of origin: to their typical climate, feed resources and rearing conditions. They are long-lived and sows have a well-developed maternal instinct. Native breeds owe their features to specific genetics, resulting from long-term breeding in local conditions. They are ideal for extensive and backyard farming. Even growing them on a small scale can be profitable due to the growing interest in local products *(Szyndler-Nędza, 2006)*. In recent years, an increase in their population can be observed - Table 1.

The European Farm Animal Biodiversity Information System (EFABIS) is the European information system for animal genetic resources. EFABIS serves as the platform for the exchange of national data on animal genetic resources, provided by the European National Coordinators. EFABIS is the source of European breed-related data for the Domestic Animal Diversity Information System (DAD-IS). https://www.fao.org/dad-is/regional-national-nodes/efabis/en/.

Mangulica - Mangalitsa - The first description for Mangalitsa found in scientific literature is from 1886 in the journal *Težak*. Mangalitsa is improved Šumadinka breed, that was crossed with a Bakonian pig cultivated in Hungary. Mangalitsa is a late breed of pigs, a fatty type with more strains within the breed (Swallow Belly, White and Red Mangalitsa). The occurrence of inbreeding is one of the main causes of poor production performance of this breed and has a negative impact on the condition of the population *(Petrović et al., 2007b; Radović et al., 2020).*

Table 1. Number of total stock and breeding sows of native pig breeds in Serbia and Poland [head] [according to Domestic Animal Diversity Information System (DAD-IS)] (https://www.fao.org/dad-is/browse-by-country-and-species/en/)

Year	Serbia			Poland		
	Mangulica Mangalitsa	Moravka Morava	Resavka Resava	Puławska Pulawska	Złotnicka biała Zlotniki White	Złotnicka pstra Zlotniki Spotted
2004	1000/200	1000/30	100/8	500/460	250/230	150/114
2014	1000/247	500/18	100/6	1700/537	940/696	650/491
2015	2000/698	500/95	100/15	2100/655	1090/844	580/476
2016	4000/1344	1000/257	200/31	1005/944	1300/992	750/490
2017	4000/1480	1000/265	200/20	5430/1450	1208/1022	730/577
2018	4000/2051	1000/377	200/40	4569/1611	1452/1201	1070/813
2019	4000/1438	1000/182	200/39	4937/1762	1356/1320	1105/1072
2020	4000/2813	1500/884	200/59	5335/2165	1369/1314	1140/1097
2021	4000/2808	4000/2899	500/218	6507/2260	1179/903	1026/861
2022	4000/2438	5500/4864	500/199	-/2196*	-/1335*	-/1167*
2023	4000/2349	5500/3824	500/204	-/2460*	-/1056*	-/1054*

*Number of the basic herd of sows as of March 15 of a given year according to http://www.bioroznorodnosc.izoo.krakow.pl/swinie/liczebnosc

Moravka - Morava is a domestic pig breed cultivated mainly in the region of Svilajnac from which it spread throughout Serbia. It is the product of an unplanned crossing of Šumadinka and Berkshire pig breeds. In the years 1953-1958, imported Cornwall pigs were used to improve Moravka. Resavka - Resava was created in the same area of Serbia but in much lower numbers than Moravka. It was reared especially in the valley of the river Resava. It was also formed by the non-systematic crossing of domestic breeds (Šumadinka and Mangalitsa) with Berkshire breed. The production characteristics of Resavka are similar to properties of the Moravka breed *(Živković and Kostić, 1952; Petrović et al., 2007a; Radović et al., 2020)*.

The Puławska breed, formerly known as the pigeon breed, was bred at the beginning of the 20th century in the Lublin province. It was created on the basis of hybrids of the primitive Polish Eeyore pig with the Berkshire breed. It is suspected that the tamworth breed could also have been involved in its creation. The Puławska breed has intermediate characteristics between the meat-fat type and the meat type. It is characterized by a rapid increase in body weight and excellent slaughter performance *(Babicz et al., 2019).*

Złotniki breeds were bred in Poland in the years 1946-1949, as a result of work carried out at the Agricultural University in Poznań. They were created on the basis of hybrids of primitive long-eared and short-eared pigs, with an admixture of the Great White English breed. Currently, we distinguish two varieties of Złotniki pigs. Złotnicka pigs of the white variety are medium-sized, with a harmonious body structure. The color is white, sometimes with small dark patches. They represent the meat type, mature late and have an average growth rate. Pigs of the Złotnicka spotted breed are medium-sized, harmoniously built, with a spotted black and white coat. They are in the meat-fat type, in the meat direction. They mature quite late and have an average growth rate. Złotnicka spotted pigs use feed slightly less efficiently and have lower carcass meat content. Złotnicka spotted meat is devoid of PSE defects, has an excellent texture and taste. It was entered on the list of regional products under the name Wielkopolska Wieprzowina Złotnicka *(Buczyński et al., 2009; Szyndler-Nędza et al., 2012)*. It should be noted that pigs of the Moravka and Resavka breeds as well as the Puławska breed were crossed with the British Berkshire breed.

Native breeds, despite a lower level of utility, are characterized by such valuable features as resistance to diseases, high fecundity and prolificacy, good maternal characteristics, longevity and adaptability to extreme environmental conditions and low-quality fodder. In many cases, the products obtained from these animals have a special, unique quality. Table 2 shows the chemical composition of meat (*m. longissimus dorsi lumborum*) from Moravka, Swallow-Belly Mangalitsa, Złotnicka spotted, Złotnicka white, and Puławska pigs.

The meat of pigs of native breeds was characterized by very good culinary and processing quality. Particularly noteworthy is the higher content of intramuscular and intermuscular fat, which has a positive effect on the taste of cold cuts and marbling of meat. Meat of Mangalica and Moravka pigs contained higher amounts of fat from 6.45 to 7.53%, respectively. Similar results for above two breeds were obtained by *Petrović et al. (2010).* Their research revealed that was less total fat and cholesterol contents in *musculus longissimus dorsi* of Moravka than in Mangalitsa. It is generally accepted that traditional, local breeds produce a higher IMF content *(Serra et al., 1998; Florowski et al., 2006; Park et al., 2007; Pugliese and Sirtori, 2012)*. As *Serrano et al. (2008)* indicated, muscles of the Spanish breed Iberico are characterised by a particularly high content of fat (8.8%). Intramuscular fat considerable ranging from 3.32 to 4.27% in the *m. longissimus dorsi* was reported for the native Italian breed of Nero Siciliano *(Pugliese et al., 2004)*. On the other hand, *Čandek-Potokar et al. (2003)* determined the content of IMF in the Slovenian breed of Krškopolje at 3%. The fat content above 2.5% can influence on lower consumers’ score because of meat high marbling *(Czarniecka-Skubina et al., 2007).*

Table 2. Chemical composition of meat (*m. longissimus dorsi lumborum* from Moravka, Mangalitsa, Złotnicka spotted, Złotnicka white, and Puławska pigs.

Chemical composition (%)	Serbia		Poland		
	Mangulica Mangalitsa	Moravka Moravka	Puławska Pulawska	Złotnicka biała Zlotniki White	Złotnicka pstra Zlotniki Spotted
Dry matter	29.21±2.62	29.82±3.15	27.70±2.47	26.70±3.28	28.51±3.95
Protein	21.27±1.12	20.74±2.41	22.88±3.68	23.01±2.12	23.53±2.89
Fat	6.45±0.45	7.53±0.95	3.45±0.78	2.19±0.37	3.45±0.64
Ash	1.11±0.09	1.09±0.07	1.10±0.08	1.11±0.02	1.08±0.03
Carbohydrates	0.41±0,03	0.46±0.03	0.27±0.01	0.39±0.10	0.45±0.15
Fatty acids = 100%					
SFA – saturated fatty acids	41.96±1.42	42.17±0.98	33.62±2.53	40.97±2.87	36.57±2.59
UFA – unsaturated fatty acids	58.04±2.54	57.83±1.75	66.38±3.57	59.03±3.65	63.43±3.52
PUFA n-6	9.41±0.84	9.85±0.47	7.07±0.85	3.55±0.38	5.12±0.64
PUFA n-3	0.71±0.06	0.84±0.04	0.37±0.06	0,26±0.04	0.29±0.03
PUFAn-6/PUFAn-3	13.25±1.04	11.73±0.95	19.11±1.59	13.65±1.26	17.66±1.32
UFA/SFA	1.384±0.01	1.37±0.01	1.98±0.02	1.44±0.01	1.74±0.02

The differences in IMF's fatty acids profiles were caused by different ways of breeding. Polish fatteners were bred with full portion mixtures with barley, triticale, corn middlings, post-extraction soya and post-extraction rapeseed meals, whereas the fatteners of Mangalitsa and Moravka were fed with corn silage, corn meal, feed flour, soybean oil meal, sunflower oil meal. It should be pointed out the high levels of C16:0 and C18:0 in IMF of Mangalitsa and Moravka. In the *m. longissimus dorsi lumborum* fat of pigs of the Pulawska and Złotniki Spotted breeds, observed wide, adverse from nutritional point of view, ratio of PUFA n6/n3 was found, 19.11 and 17.66 respectively. Therefore, the n-6/n-3 ratio was higher than dietary recommendations in all cases *(British Nutrition Foundation, 1994). Ruiz et al. (1998)* concluded that free-reared pigs fed on pasture and acorns showed higher levels of MUFA than those fed on concentrates. *Parunović et al. (2012)* found that free-range Mangalitsa pigs showed a higher PUFA content in the musculus longissimus than pigs reared indoors and fed conventionally. *Galián et al. (2008)* pointed out that the PUFA levels should not be higher than 12–14% in meat intended for the production of sausages. The varying fatty acid composition of adipose tissue and muscle have profound effects on meat quality *(Wood et al., 2008; Parunović et al., 2013). Cameron et al. (2000)* showed that C18:2, C20:4 and C22:6 polyunsaturated fatty acids had a positive correlation with the flavor of meat. Consumers prefer traditional, local pork products made from meat of native breeds, because such products are quality products *(Pugliese and Sirtori, 2012;*

Pugliese et al., 2013). Pigs of native breeds can be used for the production of heavier fatteners, because even after reaching a higher body weight, the carcass is characterized by good parameters and the meat remains of very good quality *(Babicz et al., 2009; Martyniuk, 2010; Szyndler-Nędza, 2012)*. The advantage of domestic native breeds' meat is the favorable structure of muscle fibers and the content of the optimal amount of intramuscular fat, which has a positive effect on the marbling of muscle tissue and sensory properties, mainly the taste and smell of meat. Thanks to these properties, meat of these breeds can be used to produce high-quality regional products, meats, traditionally smoked *(Hammermeister and Blicharski, 2007; Szyndler-Nędza et al., 2011)*. Table 3 presents the chemical composition of selected cold cuts made from the meat of pigs native breeds.

Table 3. Chemical composition of selected cold cuts produced of native breeds swine's meat

Kind of cold cut/ Pig breed	Dry matter %	Protein %	Lipid %	Ash %	Salt %	Energy value kcal/100 g	Polycyclic aromatic hydrocarbons PAHs (μg/kg)	
							Benzo[a] pyrene	Sum of 4 PAHs* Benzo[a]pirene, Benzo[a[antracene, Benzo[b[fluorantene, Chrysene
Polish pork sausages, n=5								
Puławska breed Nadwieprzańska sausage	38.64 ± 2.45	20.61 ± 1.02	14.81 ± 1.25	2.82 ± 0.01	2.20 ± 0.01	215.73 ± 10.2	<0.57	<3.1
Puławska breed Juniper (Jałowcowa) sausage	44.19 ± 2.34	21.85 ± 1.54	19.35 ± 2.42	2.61 ± 0.02	2.20 ± 0.01	267.07 ± 9.12	0.7±0.14	9.00±1.80
Breakfast sausage (złotniczki) Złotniki White breed	44.54 ± 2.95	14.36 ± 0.62	27.55 ± 1.14	2.36 ± 0.05	2.10 ± 0.02	305.90 ± 13.15	<0.6	<2.51
Złotnicka sausage Złotniki Spotted breed	50.98 ± 3.11	19.48 ± 1.16	27.18 ± 1.32	3.58 ± 0.05	2.20 ± 0.02	334.70 ± 13.50	<0.50	11.00±2.2
Country style sausage złotnicka	55.79 ± 3.12	20.91 ± 1.13	30.96 ± 1.41	3.70 ± 0.04	2.30 ± 0.02	362.95 ± 16.36	<0.57	8.4±1.68
Złotniki Kabanos (sausage) Złotniki Spotted breed	52.26 ± 2.84	27.19 ± 0.91	20.46 ± 1.74	4.01 ± 0.05	2.20 ± 0.02	297.70 ± 12.12	<0.50	4.90±0.98

Table 3. (continue)

Serbian pork sausages, n=3								
„Kulen” Mangalitsa breed	54.89 ± 1.23	24.63 ± 0.67	25.67 ± 1.08	4.13 ± 0.04	2.32 ± 0.02	329.55 ± 13.53	<0.50	1.60±0.40
„Sremska kobasica” Mangalitsa breed	48.63 ± 1.85	21.06 ± 1.28	22.26 ± 1.56	4.64 ± 0.05	2.26 ± 0.02	285.58 ± 9.20	<0.50	2.12±0.50
„Domaca kobasica” Moravka breed	60.97 ± 2.12	23.09 ± 1.01	32.21 ± 2.05	5.03 ± 0.06	2.65 ± 0.03	382.25 ± 18.32	0.9±0.12	3.5±0.90
Polish pork cold cuts (hams), n=5								
Nadwieprzańska Ham Puławska breed	29.29 ± 1.46	21.88 ± 0.68	4.85 ± 1.96	2.26 ± 0.04	2.20 ± 0.02	132.39 ± 9.54	3.5±0.70	14.9±1.60
Raw ripening pork loin Puławska breed	43.19 ± 2.54	35.03 ± 1.46	16.88 ± 0.93	4.02 ± 0.06	2.40 ± 0.02	292.28 ± 10.30	<0.50	<2.1
Bacon Puławska breed	42.61 ± 2.32	21.11 ± 0.76	19.55 ± 1.16	1.95 ± 0.05	1.80 ± 0.01	260.39 ± 18.34	<0.54	<3.75
Złotnicka ham Złotniki White breed	38.90 ± 2.23	22.41 ± 0.95	13.45 ± 1.12	3.04 ± 0.06	2.10 ± 0.03	210.60 ± 14.26	0.95±0.19	6.43±1.30
Bacon Złotniki Spotted breed	75.16 ± 6.32	16.95 ± 1.14	55.47 ± 4.19	2.42 ± 0.05	2.05 ± 0.02	568.31 ± 40.21	<0,20	<2.00
Złotnicka cold cut Złotniki Spotted breed	48.93 ± 2.06	25.09 ± 1.21	19.46 ± 0.74	4.26 ± 0.05	2.40 ± 0.02	275.98 ± 13.41	<0.20	<2.00
Serbian pork cold cuts (hams), n=3								
“Pršut” dry-cured ham Mangalitsa breed	60.34 ± 2.62	28.43 ± 0.64	26.83 ± 0.85	4.73 ± 0.05	5.35 ± 0.02	356.19 ± 12.42	<0.4	<0.8
Dry-cured ham Moravka breed	59.96 ± 2.21	33.64 ± 0.46	20.32 ± 0.83	5.43 ± 0.06	6.74 ± 0.03	318.56 ± 10.32	<0.4	<0.8
Dry sirloin Mangalitsa breed	46.44 ± 2.23	32.79 ± 0.74	7.63 ± 0.23	5.62 ± 0.05	4.26 ± 0.02	201,12 ± 8.43	<0.4	<0.6

In Serbia the most common traditional meat products are fermented dry sausages, home-made “Kulen” and “Srem” sausages, fermented dry sausage, “Peglana” sausage, “Petrovska” sausage and “pršut” dry-cured ham. Those products have high acceptability in Balkan’s region, which proves the high quality of these products. In Poland, the most popular traditional products are country style sausage, juniper (jałowcowa) sausage, bacon, traditional ham. The analyzed

Serbian and Polish cured meats were characterized by very good quality and chemical composition. Sausages and bacon were characterized by a high fat content. *Džinic et al. (2016)*, showed that the level of fat between sausages of “čajna” ranged from 36.77% to 48.31%. *Petrović et al. (2011)*, found similar values in “Petrovska klobasa”, traditional fermented sausage, where fat content ranged from 34.09% to 46.01%. Also similar results were obtained by *Ikonić et al. (2010)*. The vast majority of analyzed traditional pigs’ meat products was in accordance with new requirements of the European Union concerning maximum levels PAH in the selected foodstuffs as considered in the Commission Regulation (EC) 835/2011 *(2011)*. Only one pork ham (“Nadwieprzańska” ham) did not meet the limits set by EU regulation 835/2011 *(2011)*, but met the limits set by EU *Regulation 1327/2014 (2014)*. Serbian and Polish cured meats differed in taste, color parameters and the level of polycyclic aromatic hydrocarbons. The Carpathian Mountains are the specific boarder for the variety of cold cuts, especially sausages. On the southern side of The Carpathians, ripening, fermented sausages are produced, while on the northern side they are intensively smoked and dried. On the southern side, paprika is the main seasoning for sausages, on the northern side - garlic and pepper. The varied feeding of animals (maize, soybean and sunflower on the southern side of The Carpathians - rye, barley and rapeseed on the northern side) also determines the varied fatty acid profile of the meat fat, and thus also the fat of sausages. There are also differences in the method of smoking (hot or cold in the south of Europe - hot in the north) and the type of smoking wood used.

Marketing promotion activities in Hungary, Croatia, Romania and Serbia caused the Mangalica pig and meat products from the meat of this breed became the most recognizable brand. However, the practical protection of native breeds depends on their market position, including meat quality, traditional or regional products made from that meat and promotional and marketing activities. On July 14, 2005, the Regional Association of Breeders and Users of Puławska Pigs was established "PUŁAWIAK", while the culmination of marketing activities was placing on May 27th 2009 "Puławska pig" at the Minister of Agriculture and Rural Development List of Traditional Products *(Szulc and Skrzypczak, 2015)*. In 2010, cooperation with the hypermarket chain, allowed to include meat of Puławska pigs, to the shops offer, as a delicatessen product. In addition, the campaign "Regional pork - appreciate the taste of tradition" is being conducted increasing the awareness of restaurateurs regarding Polish native breeds of pigs and expanding knowledge about sensory qualities and possibilities of use culinary meat cuts from pigs of the Puławska, Złotnicka White and Złotnicka Spotted breeds.

The basis for the promotion of native breeds of farm animals and raw materials and products obtained from them can be an appropriate certification and labeling system. In Poland, as part of the project "Directions of use and protection of animal genetic resources in conditions of sustainable development", assumptions for certification and the logo of the PARENTAL BREED (NATIVE BREED) were developed *(Krupiński et al., 2017)*.

The developed system of using the logo is not a quality system within the meaning of EU regulations, but it meets similar requirements. The assumption of the logo is to promote on local markets products of a specific native breed kept in natural systems of maintenance and feeding. The main purpose of certification and logo for producers of native breeds is to disseminate and raise public awareness of the role and importance of native breeds as an important part of national heritage. Consumers' interest in traditional products made from raw materials originating from native breeds and subsidies for farmers keeping these animal breeds will contribute to the preservation of biodiversity and can stop the extinction of native animal breeds.

The system of using the logo is not a quality system within the meaning of EU regulations, but it meets similar requirements. In principle, this logo will promote on local markets products of a specific Polish native breed kept in traditional (natural) housing and feeding systems. The main purpose of using the logo for producers of native breeds is to disseminate and raise public awareness of the role and importance of native breeds as an important part of our national heritage. It will be applicable to all types of products (carcasses, meat and meat products, milk and milk products, eggs, honey, wool, skins, etc.) from animals covered by programs for the protection of farm animal genetic resources coordinated by the National Research Institute of Animal Production. It should also be noted that the logo is compatible with other quality marks. The Polish Native Breeds logo is the property of the National Research Institute of Animal Production. The Foundation of the National Research Institute of Animal Production, Patronus Animalium, acting on behalf of the Institute, determines the rules and conditions for granting the rights to use the logo of Polish Native Breeds or a specific Polish Native Breed with the name of the breed distinguished. The Foundation, on the basis of separate agreements, establishes logo operators, authorizing breed associations or regional breed associations to participate in the certification program. The right to mark products with the " Polish Native Breed" logo on the basis of an agreement with the Foundation is granted to breeders of conservation breeds participating in the Program for the Protection of Genetic Resources of Farm Animals and processing plants producing products from conservation breeds *(Krupiński et al., 2017; Radomski et al., 2019; https://ksb.izoo.krakow.pl/site/certification)*. In 2020, the National Association of Breeders and Producers of Native Breeds "Native Breeds" was established in Krakow, which goals are promotion of: traditional, slow farming of native breeds, the use of traditional processing methods based on traditional recipes and food from native farm animals.

Conclusion

Native breeds can be a way for small farms for functioning and increase profits, because from animals of native breeds we can obtain and process products of unique quality, with a long tradition of production. An example can be the native pig breeds of Serbia (Mangalica, Moravka, Resavka) or Polish (Puławska, Złotnicka White, Złotnicka Spotted). However, it should be noted that the popularity and availability of cold cuts from Mangalitsa and Moravka pigs in Serbia is much greater than the availability of cold cuts from Puławska, Złotnicka White and Złotnicka Spotted pigs in Poland. Preserving native breeds in the natural environment, i.e. on family farms, often organic, agritourist or located in protected areas is the best method of conservation. Certification system of raw materials and processed products from animals included in conservation programs and kept in traditional production systems with the brand “Native Breed”, worked out as a result of the research conducted in the National Research Institute of Animal Production, can contribute to the development and strengthening of local markets, influencing the improvement of economic efficiency of farm animals keeping these breeds.

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ASSESSMENT OF WATER-HOLDING CAPACITY IN DIFFERENT MEATS USING EZ-DRIPLOSS METHOD: A REVIEW OF KEY METHODOLOGICAL FACTORS

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Abstract: The water-holding capacity (WHC) of meat is the ability of the *post-mortem* muscle to retain moisture/water even when external influences are applied to it. The quality of different meats depends largely on their WHC, which is related to processing technology and consumer acceptance. The WHC of meat is influenced primarily by intrinsic and extrinsic factors, and secondarily by specific methodological factors that should not be neglected when a specific treatment/method is used for its determination. EZ-DripLoss is a gravimetric method for measuring the drip loss of meat and is widely used in the industry and science for quantifying meat quality. In this method, meat is suspended in a special airtight EZ container for 24 hours, with gravity applied to the meat sample. This review summarises the specific methodological factors associated with the EZ-DripLoss method, i.e., muscle and sampling area position, sample core fiber orientation, weighed/non-weighed sampling procedure, dabbing of the sample, and the storage period/measurement interval. Future research should aim to provide more in-depth information and standardization of the EZ-DripLoss methodology.

Key words: water-holding capacity, meat quality, methodology, EZ-DripLoss

Introduction

The ability of meat to retain water is a complex attribute that is a consequence of muscle structure and the biochemical changes that occur when muscle is converted into meat *(Bowker and Zhuang, 2015)*. According to *Huff-Lonergan and Lonergan (2005)*, the water-holding capacity (WHC) of meat is the ability of *post-mortem* muscle to retain moisture/water despite external influences (e.g., gravity, freezing, heating, or cooking) are applied to it. Released water can be described as drip, purge, weep, exudate, or cook loss, and these are inversely related to WHC *(Warner, 2014)*. Water accounts for approximately 75% of the weight of meat, and the ability of muscle to retain moisture is key to maintaining many meat quality attributes that are highly required in processing and by consumers *(Huff-Lonergan and Lonergan, 2005).* This water is mainly found in the myofibrils, between the myofibrils and the connective tissue coat (endomysium), between the muscle fiber bundles and the surrounding connective-tissue coat, and between the individual bundles of muscle fibers, while the remaining 15% of the water is found in the extracellular space *(Kudryashov and Kudryahova, 2023).*

One of the most important quality characteristics of fresh meat, which is related to consumer acceptance and processing aptitude, in addition to colour, is its ability to retain water *(Huff-Lonergan and Lonergan, 2005; Kaić et al., 2020).* A reduced ability of meat to retain water is unattractive to consumers at the time of purchase and leads to economic losses and losses of valuable water-soluble proteins and minerals. In addition, WHC also affects the processing characteristics of meat, as meat with low WHC has inferior yields and lower quality, regardless of whether it is fresh or processed *(Prevolnik Povše et al., 2015).* Therefore, the industry needs methods that can easily and precisely determine the WHC of meat and meat products *(Filho et al., 2017).* Currently, there are a variety of methods for measuring WHC in different meats, and within each method there are some modifications (equipment used, sample preparation, measurement interval, sample weight, etc.). Nevertheless, the principles for WHC determination within methods are generally the same. The meat sample is weighed before and after a certain treatment (e.g., centrifugation, cooking, freezing) and the result is then expressed as a weight difference in comparison to the initial sample weight *(Kaić et al., 2023).* In general, the widely used methods for WHC determination can be divided into three basic groups: gravimetric methods, methods using external forces, and thermal methods *(Honikel, 1994).* Gravimetric methods include the measurement of drip from suspended samples during hanging in a bag, beaker, or container (1 to several days) with gravity as the only force that is exerted to the sample (bag method, EZ-DripLoss method, tray method). Methods with external forces (such as pressure, centrifugal force, capillary, or vacuum) are applied to the samples to accelerate the drip loss (usually minutes to an hour). Thermal methods are based on the application of thermal processes and measure the cooking loss of meat. Aside of afore mentioned methods for WHC determination, technological progress has led to the development of new methods for WHC determination such as the electrical conductivity method, the microwave spectroscopy method, the video image method, the nuclear magnetic resonance method, infrared spectroscopy, and the color difference method. These methods are described in detail by *Kudryashov and Kudryahova (2023).*

The EZ-DripLoss method is a gravimetric method for the determination of WHC in meat. The method was first proposed by *Rassmussen and Anderson (1996)* and subsequently developed and described in detail by the Danish Meat

Research Institute *(DMRI, 2018).* Briefly, EZ-DripLoss is a method in which meat is suspended in a specialized airtight EZ container for 24 hours, with gravity applied to the meat sample. According to *Rasmussen and Anderson (1996)*, the EZ-DripLoss method has a high sensitivity, is easier to perform in a reproducible way, requires less space than the other conventional methods, and is easier to use under abattoir conditions. The EZ-DripLoss method was first developed for the determination of drip loss in pork *(DMRI, 2018)* and subsequently used in various pork quality studies *(Correa et al., 2007; Žilić et al., 2016; Filho et al., 2017).* However, this methodology is also well documented for other meats, such as poultry *(Kaić et al., 2021; Kaić et al., 2023)*, lamb *(Holman et al., 2020)*, mutton *(Kaić et al., 2020)*, alpaca *(Logan et al., 2019)*, deer *(Raizmaité et al., 2017)*, and horse *(Raizmaité et al., 2021).*

Meat WHC is a complex attribute influenced by numerous intrinsic (e.g., genotype, feeding of animals, muscle type, chemical composition) and extrinsic (e.g., rearing, factors related to pre- and post-slaughter handling) factors *(Cheng and Sun, 2008).* However, it should not be neglected that within the specific treatment/methodology, methodological factors also play a role in measuring WHC. Literature reports have shown the importance of methodological factors (muscle and sampling area position, sample core fiber orientation, weighed/non-weighed sampling procedure, dabbing of the sample, and the storage period/measurement interval) in the context of the EZ-DripLoss method *(Correa et al., 2007; Filho et al., 2017; Logan et al., 2019; Holman et al., 2020; Kaić et al., 2021; Kaić et al., 2023).* Therefore, this paper focuses on and summarizes the main methodological factors that need to be considered, detailed described, and finally standardized when using the EZ-DripLoss method for WHC determination.

EZ-DripLoss method

The EZ-DripLoss method is carried out in such a way that the muscles are removed from the carcass the day after slaughter for analysis *(Rasmussen and Andersson, 1996).* A muscle core (sample) with a diameter of 25 millimetres and a height of 25 millimetres is removed from the meat in duplicate with a standardized circular knife, paying attention to the orientation of the muscle fibers (perpendicular to the circular knife). The sample is then placed (without weighing) directly into a pre-weighed special EZ container fitted with a lid to prevent loss of meat juice through evaporation outside the container *(Warner, 2014).* The container with the sample is then kept in a refrigerator under controlled conditions (at a temperature of 4-6 ˚C) for 24 hours. After the predetermined measurement interval, each EZ container is weighed (on a chemical scale with 3 decimal places), including the muscle core and drip loss, and one more time after removing the muscle core, for drip loss only *(DMRI, 2018).*

According to *DMRI (2018),* samples are not dabbed before final weighing, and EZ-DripLoss is calculated as follows:

$$EZ_S = [(W_I - W_c)/(W_t - W_c)] * 100$$

where:

W_c = weight of the empty EZ-DripLoss container

W_t = weight of the EZ-DripLoss container with meat sample and drip loss, and

W_I = weight of the EZ-DripLoss container with drip loss.

As introduced by *Rasmussen and Andersson (1996)* and *DMRI (2018)*, the mean of the muscle cores taken in duplicate is used for each EZ-DripLoss assessment.

Methodological factors that influence on EZ-DripLoss assessment

Muscle and sampling area position

EZ-DripLoss method was first introduced for the determination of drip loss in porcine loin muscles *(DMRI, 2018)*. Literature reports on the application of this method with poultry in *pectoralis* muscle *(Kaić et al., 2021)*, with mutton and alpaca in the *longissimus thoracis et lumborum* muscle *(Logan et al., 2019; Kaić et al., 2020)*, with deer and lamb in the *longissimus dorsi* and *semimembranosus* muscles *(Raizmaité et al., 2017; Holman et al., 2020)*, and with horse in the *pectoralis profundus* muscle *(Raizmaité et al., 2021)*. Differences in the used muscles need to be considered because their fiber characteristics, extra-cellular spacing, myofibrillar dimensions or fat content are reported to have an impact on water distribution, mobility, and thus level of drip loss *(Holman et al., 2020)*. Furthermore, the differences in drip loss are also found due to used different sampling position. Namely, with porcine meat *Christensen et al. (2003)* and *Otto et al. (2004)* found significantly higher drip loss in the ventral part of the longissimus dorsi muscle than in the dorsal part. In addition to the within-slice variance, *Christensen et al. (2003)* add that regardless of the visual homogeneity of the *longissimus dorsi* muscle, drip loss can also vary significantly between its cranial and caudal ends. This clearly demonstrates the importance of detailed investigation and standardization of the muscle and sampling position within the muscle.

Sample core fiber orientation

Within the EZ-DripLoss method, the fiber orientation of the sample core should be vertical *(Rasmussen and Andersson, 1996; DMRI, 2018)*. However, different information on the fiber orientation of the sample core can be found in the literature. Literature reports show discrepancy regarding the sample core fiber orientation. With alpaca meat, *Logan et al. (2019)* did not explain the sample core fiber orientation when using the EZ-Driploss method. With pork meat, *Christensen et al. (2003)* and *Otto et al. (2006)* described the EZ-DripLoss sampling method used, according to which the core fiber orientation of the sample could only be assumed, and no direct information was provided. *Filho et al. (2017)* used sample cores with a fiber orientation of 45° and recommend the methodology used for the accuracy of EZ-DripLoss in pork meat. With lamb meat, *Holman et al. (2020)* investigated differences in drip loss with vertical and horizontal fiber orientation measured over 4-day intervals. The authors found that fiber orientation had no effect on the drip loss values in lamb meat. Contrary to these results, *Kaić et al. (2021)* found that fiber orientation significantly influenced EZ-DripLoss in chicken breast meat. The authors found that the samples with vertical fiber orientation had a 0.50% higher drip loss than the samples with horizontal fiber orientation. According to *Filho et al. (2017)* and *Holman et al. (2020)* this difference could be attributed to structural elements in the muscle where ‘free water’ expelled from meat as drip is associated with vertical fiber orientation and its movement is accelerated in vertical samples due to the greater gravimetric forces.

Weighed versus not weighed sampling procedure

The EZ-DripLoss method was introduced as a simple and robust method that does not require muscle samples to be weighed before being placed in the containers *(Rasmussen and Andersson, 1996; DMRI, 2018).* As such, it has been widely used in the assessment of drip loss of pork *(Correa et al., 2007; Žilić et al., 2016; Filho et al., 2017)*, chicken *(Kaić et al., 2023)*, beef *(Mergeduš et al., 2022)*, and deer meat *(Raizmaté et al., 2017)*. However, the literature reports that EZ-DripLoss assessment is also performed by the weighing the meat samples before placing them in the containers. Drip loss is then calculated as the change in sample weight. This approach has been used to assess drip loss in pork *(Correa et al., 2007; Filho et al., 2017)*, poultry *(Kaić et al., 2021)*, lamb *(Holman et al., 2020)*, mutton *(Kaić et al., 2020)*, alpaca *(Logan et al., 2019)*, and horse meat *(Raizmaté et al., 2017)*. Due to different handling procedures, *Correa et al. (2007), Filho et al. (2017)*, and *Kaić et al. (2023)* investigated the differences in the EZ-DripLoss methodology used (weighed samples versus not weighed samples). These authors found that the weighed meat samples had a greater average drip loss than the not weighed meat samples at the same measurement interval. *Correa et al. (2007)* indicated that these differences were due to the dabbing procedure used for weighed samples, which helps to remove exudate from the core surface, while *Kaić et al. (2023)* argued that these differences could also be due to greater manipulation procedures associated with sample weighing.

Sample dabbing

Rasmussen and Andersson (1996) and *DMRI (2018)* introduced the EZ-DripLoss method, in which muscle cores are not dabbed before final weighing. Dabbing before weighing is not performed because the weight of the sample was not considered in the EZ-DripLoss determination *(Correa et al., 2007).* Although in a study on pork *(Otto et al., 2004),* and in a study on mutton *(Kaić et al., 2020)* the EZ-DripLoss values were expressed as a percentage of the initial weight (i.e., the samples were weighed) it was pointed out that the EZ-DripLoss method does not require dabbing of the muscle surface sample. In a study on pork quality, *Filho et al. (2017)* reported that sample dabbing did not improve the reliability of the EZ-DripLoss method (weighed versus not weighed samples). In addition, *Christensen (2003)* reported that the absence of dabbing procedure helps to reduce the influence of the sampler on the measurement. However, in a study on pork, *Correa et al. (2007)* found that the average drip loss value for dabbed samples measured after 24 hours was significantly higher (5.4%) than for non-dabbed samples (3.5%). *Correa et al. (2007)* pointed out that the absence of dabbing affects the reliability of the measurement and may lead to an underestimation of the drip that is excluded from the sample during storage.

Storage period (measurement interval)

According to the instructions for use, EZ-DripLoss should be evaluated after a 24-hour measurement interval *(Rasmussen and Andersson, 1996; DMRI, 2018).* However, it must be considered that the exudation is a slow process in which water is expelled from the myofibrils, and over time is drained out of the muscle *(Den Hertoh-Meischke et al., 1997).* This is consistent with literature reports in which the measurement interval showed a positive linear relationship with drip loss. With pork, *Žilić et al. (2016)* found that the average drip loss was 1.40% within 24 hours, and 1.94% within 48 hours. The average drip loss of pork samples reported by *Correa et al. (2007)* was 3.54% within 24 hours and 4.66%

within 48 hours, while for pork samples reported by *Filho et al. (2017)* was 3.10% within 24 hours and 4.40% within 48 hours. With deer meat, *Raizmaté et al. (2017)* found that the average drip loss in free-living animals was 1.80% within 24 hours, while the average drip loss in farmed animals was 3.42% within 24 hours. With chicken breast meat, *Kaić et al. (2023)* found that the average drip loss was 0.95% within 24 hours, 1.30% within 48 hours, and 1.38% within 72 hours. *Filho et al. (2017)* explained that the differences in EZ-DripLoss values between studies could be related to the different water loss rates in meat. Namely, in pork *Filho et al. (2017)* found that about 70% of the drip loss occurred in the first 24-hour measurement interval, while *Correa et al. (2007)* found 76% of the drip loss for the same measurement interval. With chicken breast meat, *Kaić et al. (2023)* found that 73% of the drip loss occurred in the first 24-hour measurement interval and pointed out that assessing drip loss after 24 hours may underestimate the drip loss of different meats, so a longer measurement interval should be recommended when using this method. A similar result was previously reported by *Otto et al. (2004)* and *Filho et al. (2017)* who recommended the use of a 2-day measurement interval for the assessment of drip losses in pork. *Kilgannon et al. (2018)* suggested that drip loss should be determined with using a 3-day measurement interval for beef, while *Holman et al. (2020)* recommended longer measurement intervals (more than 4 days) for lamb.

Conclusion

Following the previously published reports on this topic, this paper focuses on the most important methodological factors for a reliable EZ-DripLoss assessment. The paper gives a deeper insight into the inconsistency of methodological factors that can have a major impact on the assessment of drip loss in different meats when applying the EZ-DripLoss method (muscle and sampling area position, sample core fiber orientation, weighed/non-weighed sampling procedure, dabbing of the sample, and the storage period/measurement interval). From the factors discussed it is obvious that current knowledge about the suitability of the EZ-DripLoss method for different meat analysis is still insufficient. Therefore, future research should provide detailed methodological information, which are key factors for deeper insight and further steps towards standardization of the EZ-DripLoss method.

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PATH COEFFICIENT ANALYSIS BETWEEN BODYWEIGHT AND SOME REAL-TIME BODY MEASUREMENTS OF GRAZING CATTLE ON DIFFERENT ARTIFICIAL PASTURES

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Invited paper

Abstract: This study was conducted to determine characters effecting bodyweight in different artificial pastures by using simple correlation coefficient and path analysis. Two different artificial pastures were established, each covering 1.5 ha area during the first year of the research. Twenty Holstein male calves with 6 months old were assigned to the experimental areas randomly, each pasture containing 10 animals. Each group was weighed and monitored on a fortnightly basis, using electronic weighing scale. Body weight (BW) and some body measurements were determined on the animals. The results showed that there were significant association between bodyweight measurements and the bodyweight. Positive and significant relationships were found between the bodyweight and Body Length (BL) (0.890**), Wither Height (WH) (0.877**), Heart Girth (HG) (0.929**), Hip Height (HH) (0.834**) and Hip Width (HW) (0.839**). Several traits affected the liveweight directly or indirectly. Standardized correlation coefficients for BL and HH were found non-significant and removed from regression model. The optimum multiple regression equation for animals was obtained with a determination coefficient (R^2 of 0.93). The results indicated that the traits (HG, WH, HW and BD) were statistically significant as direct effects on BW of cattle. Most of the indirect contributions of the variables were realized by HG. It could be concluded that HG is important parameter and meausuring HG would allow small scale farmers to predict bodyweight of cattle with a high degree of accuracy even on grazing conditions.

Keywords: bodyweight, beef cattle, path analysis, body measurements

Introduction

Body weight (BW) is one of the most economically important traits in especially beef prodcution systems, whereby breeders want to select the best animals for breeding purposes (*Dekhili and Aggoun, 2013; Bila et al., 2021*). *Bozkurt* (2006), *Ozkaya and Bozkurt* (2009), *Nosike et al. (2017)* and many more studies in different farm animal species stated that linear body measurements are important parameters for predicting BW.

Small scale farming in developing world is characterised by poor resources and investments. Managerial decisions are mainly based on trials and errors at smallholder level. Since feed rationing is based on animal's live weight this should be known for this purpose. Body measurements of beef cattle are used for several purposes, including prediction of body weight, growth rate, body condition and conformation (*Wilson et al., 1997; Fourie et al., 2002*).

Animal marketing between farmers is mostly dependent on visual evaluation. The majority of veterinary medications are administered based on body weight parameters. However, drug assessment is typically done using estimations. The use of live weight criteria in ration formulation, medication estimate, body condition score, and marketing necessitates sophisticated facilities that are costly and beyond of reach for many small-scale farmers.

A technique called path analysis is frequently used to explore the indirect and direct effects of interacting predictor variables on dependent variables (*Norman and Streiner, 2003*). Animals' morphological features have been utilized to determine body weight (*Yakubu, 2010*). Simple correlation coefficients between body weight and morphometric parameters or regression of body weight on a number of body measurements have been used to estimate body weights (*Ozkaya and Bozkurt 2009; Bozkurt et al, 2016*).

According to *Keskin et al.* (2005), during the selection process of certain traits for breeding purposes, some traits may be affected directly while others indirectly. *Ogah et al.* (2009) stated that a simple correlation between independent and dependent variables may not be adequate for defining the association between traits. Path analysis, on the other hand, is a mathematical method used to investigate the cause-effect relationship between dependent and independent variables (*Yakubu and Salako, 2009*).

Path analysis has been shown in studies to be a beneficial approach in animal breeding for estimating body weight using biometric features in different farm animal species *(Yakubu and Salako, 2009; Egena et al., 2014; Mendes et al., 2005; Tyasi et al. 2020).* Path analysis is a multiple regression model extension that identifies explanatory factors that have the greatest influence on the response variable. This study demonstrates that utilizing live body weight and morphometric features as prediction indicators, BW can be estimated with a high degree of accuracy. This will aid in the selection of animals for breeding purposes based on real-time body measurements taken on different rearing conditions. There is limited literature documented about the estimation of BW from biometric traits using path analysis technique. Therefore, the goal of this study was to discover direct and indirect causal relations between BW and body traits in European type male cattle kept grazing on artificial pastures.

Materials and Methods

Experimental location

This study was carried out in the Lake District, in Isparta Province (37°45′N, 30°33′E, elevation 1035 m) located in the Mediterranean region of Turkey on three consecutive years of 2010 and 2012.

Animal and pasture management

A total of 20 Holstein male cattle with an average 6 months old were included and allocated evenly to two artificially established pastures in the experiment which lasted for 90 days in 2011 and 2012, each year 20 Holstein beef animals were used and conducted at University Research Farm. Animals were initially weighed at the beginning of the experiments and were randomly divided according to their weights into two grazing groups. Each group was weighed and monitored on a fortnightly basis, using electronic weighing scale (True-Test 2000 SmartUnit). The free access of the animals to water was available throughout the experimental period.

For the establishment of artificial grazing land, 3 ha pasture land was chosen adjacent to the university farm and cultivated in March 2010 with two different botanical compositions. Pasture 1 (P1) was composed of *Medicago sativa L.* (20%) + *Bromus inermis L.* (40%) + *Agropyron cristatum L.* (30%) + *Poterium sanguisorba* (10%); and Pasture 2 (P2) had mixtures of *Medicago sativa L.* (15%) + *Onobrychis sativa Lam.* (15%) + *Agropyron cristatum* L. (35%) + *Bromus inermis* L. (35%), respectively.

Body measurements and data collection

Body measurements were taken while animals were standing in a crush before weighing. A plastic tape marked in centimetres (cm) was used for the measurement of most body traits except wither height, which was measured by measuring stick.

1-Heart Girth (HG) - was measured as the minimal circumference around the body immediately behind the front shoulder,

2-Wither Height (WH) - was the distance from the ground beneath the animal to the top of the withers directly above the centre of the shoulder,

3-Body Length (BL) - was the distance from the point of the shoulders to the ischium; in other words, from the sternum (manubrium) to the aitchbone (tuber ischiadicum),

4-Hip Width (HW)- was the widest point at the centre of the stifle,

5-Body Depth (BD) - from sternum area immediately caudal to the forelimbs to top of the thoracic vertebra.

6- Hip Height (HH) - was the distance from the ground beneath the animal to the top of the hips

Body weight was measured in kilograms and the body measurements in centimetres. Body measurements data were taken during the experimental period, composed of 120 records for each parameter in total in two grazing periods.

Statistical Analysis

The best linear regression equations for BW from other traits as independent variables, including heart girth (HG), body length (BL), wither height (WH), hip width (HW), body depth (BD) and Hip Height (HH) were determined. Descriptive statistics, including Means, Standard Deviations (SD) and Coefficients of Variation (CV) of body weight and morphometric body traits were performed, using (MINITAB V. 18, 2017).

Pairwise correlations among body weight and morphological traits were also determined. Path coefficients were calculated using SPSS (2001). This was to allow direct comparison of values to reflect the relative importance of independent variables in explaining variation in the dependent variable. The path coefficient from an explanatory variable (X) to a response variable (Y) as described by Mendes et al. (2005) as shown below was used:

$$P_{Y.Xi} = b_i \, S_{Xi}/S_Y$$

where: $P_{Y.Xi}$= path coefficient from X_i to Y (i= BL, WH, HG, HH, BD and HW)

b_i = partial regression coefficient,

S_{Xi} = standard deviation of X_i

S_Y = standard deviation of Y

The following multiple linear regression model was adopted:
$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + ... + e$
where:
Y = endogenous variable (body weight),
a = intercept,
b‘s = regression coefficients,
e = error term.

Results and Discussion

Descriptive statistics of BW and body measurements (BW, HG, WH, BL, HW, BD, HH) of animals grazed in different pastures are shown together with means, standard deviations (SD), coefficients of variation (CV) , minimum and maximum values in Table 1.

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. Descriptive statistics of BW (kg) and body measurements (cm) by pasture types

Variables	Pasture Type	Mean	StDev	CoefVar	Minimum	Maximum
BW	1	224.05	39.04	17.42	156	318
	2	222.53	35.96	16.16	149	284
HG	1	135.43	8.29	6.12	117	154
	2	136.67	8.78	6.42	119	152
WH	1	110.57	5.23	4.73	101	121
	2	111.03	5.17	4.66	101	120
BL	1	116.13	7.16	6.16	101	131
	2	117.17	6.44	5.50	106	127
HW	1	34.2	2.82	8.26	28	39
	2	33.7	2.20	6.54	30	38
BD	1	58.93	4.12	6.99	51	67
	2	59.00	2.63	4.47	55	66
HH	1	116.27	4.42	3.80	107	127
	2	116.02	3.70	3.19	109	124

Table 2. Overall performance comparisons of animals by years and pasture types. *

Performance (kg)	Years			
	2011		2012	
	Pasture types			
	1	2	1	2
IW	184.6 (8.9)	182.6 (9.1)	227.1 (18)	240.3 (20)
FW	284.7 (12)	280.5 (9.8)	326.8 (16)	316.6 (17)
TWG	100.1 (4.0)	97.90 (2.9)	86.5 (6.2)	89.6 (6.4)
DLWG	1.112 (0.044)	1.066 (0.032)	0.961 (0.069)	0.994 (0.071)

*The standard error values of the means are shown in parentheses. All values are not statistically significant within the same year (P >0.05).
IW= Initial weight, FW= Final weight, TWG= Total weight gain, DLWG= Daily live weight gain.

There were no significant (P >0.05) differences between pasture types in final weight (FW), total weight gain (TWG) and daily live weight gain (DLWG). In respect to performance of animals in pasture types, the final weights were 284.7 and 280.5 kg for P1 and P2 in 2011, 296.2 and 307.4 kg for P1 and P2 in 2012, respectively. The average total weight gains 100.1 and 95.9 kg for P1 and P2 in 2011, 69.1 and 68.1 kg for P1 and P2 in 2012 and finally daily live weight gains of 1.112 and 1.066 kg for P1 and P2 in 2011, 0.768 and 0.746 kg for P1 and P2 in 2012, respectively (Table 2).

As can be seen from Tables 1 and 2 both performance and development of body measurements between pasture types and within the same years are not statistically significant (P >0.05). The data collected in all years were combined for path analysis.

Pair-wise correlations among body measurement traits and BW of the animals are presented in Table 3.

Table 3. Pearson correlation coefficients between BW and body measurements. *

Traits	BW	HG	WH	BL	HW	BD
HG	0.929					
WH	0.877	0.835				
BL	0.890	0.896	0.905			
HW	0.839	0.768	0.705	0.780		
BD	0.864	0.780	0.789	0.805	0.755	
HH	0.834	0.797	0.851	0.787	0.705	0.781

*Significant at P<0.01 for all correlation coefficients except

The correlation coefficient among body measurement traits and BW ranged between 0.705–0.929, respectively. The highest correlation coefficient was between body weight and heart girth (r=0.929), which was slightly less than those found by *Bozkurt (2006)* and *Ozkaya and Bozkurt (2009)* and the lowest was between HW and WH and similarly between HW and HH (r=0.705) respectively. However, the results showed a highly significant and positive correlation between BW and all the body measurement traits (P<0.01), respectively. These results were in line with *Heinrichs et al. (1992), Wilson et al. (1997), Ulutas et al. (2001)* and *Bozkurt et al. (2016).* Our findings were similar to *Bozkurt (2006) and Ozkaya and Bozkurt (2009)* but in disagreement with *Tyasi et al. (2020)* who found that hip height and body length were good predictors, which might be due to breed variation. According to *Bozkurt et al. (2016),* body measurement traits plays an important role to predict the body weight of beef cattle; however, the correlation coefficient does not provide how much each body measurement trait contribute to body weight. Hence, it is important to use path coefficient analysis to investigate the direct and indirect effects of each body measurement traits on BW of animals.

Prelimenary Regression analysis

Regression Equation

$$BW= -445.2+ 2.148HG + 1.772WH - 0.630BL + 3.096HW + 2.307BD + 0.105HH \quad R^2=94.1\%$$

Table 4. Significance of regression coefficients of each parameter in preliminary regression analysis

Coefficients

Term	**Coef**	**SE Coef**	**T-Value**	**P-Value**	**VIF**
Constant	-445.2	40.2	-11.08	0.000	
HG	2.148	0.367	5.85	0.000	5.93
WH	1.772	0.698	2.54	0.014	7.93
BL	-0.630	0.594	-1.06	0.293	9.86
HW	3.096	0.892	3.47	0.001	3.09
BD	2.307	0.720	3.21	0.002	3.72
HH	0.105	0.672	0.16	0.876	4.51

VIF= variance Inflation Factor

As can be seen from Table 4, since BL and HH were insignificant (P >0.05) these parameters were removed from the equation as predıctors and standardized regression analysis were performed again.

Optimum Standardized Regression Analysis

Regression Equation

BW = - 435 + 1.97 HG + 1.43 WH + 2.89 HW + 2.26 BD
R^2=93.6%

Table 5. Significance of regression coefficients of each parameter in optimum regression analysis

Coefficients

Term	**Coef**	**SE Coef**	**T-Value**	**P-Value**	**VIF**
Constant	-435.0	27.8	-15.62	0.000	
HG	1.974	0.312	6.32	0.000	4.35
WH	1.429	0.489	2.92	0.005	3.94
HW	2.889	0.855	3.38	0.001	2.88
BD	2.260	0.691	3.27	0.002	3.47

Eventhough 2 non-signicant variables were removed from the model it did not caused a great change in determination of coefficent (R^2=93.6 %).

Path coefficient analysis was utilized to investigate the effect of body measurement traits on body weight. Path correlation coefficients between body measurement traits and BW, direct effect and indirect effects of body measurement traits on BW are presented in Table 6.

Table 6. Path coefficient analysis on direct effect and indirect effects of body measurement traits on BW

Traits	**Correlation Coef. on BW**	**Direct Effects**	**Indirect Effects**				
			HG	**WH**	**HW**	**BD**	**Total**
HG	0.929	0.490^{**}	-	0.175	0.130	0.134	0.439
WH	0.877	0.246^{**}	0.379	-	0.117	0.135	0.631
HW	0.839	0.210^{**}	0.346	0.144	-	0.139	0.629
BD	0.864	0.213^{**}	0.353	0.166	0.132	-	0.651

**: significant at P<0.001

The results indicated that the traits (HG, WH, HW and BD) were statistically significant as direct effects on BW of cattle. Path analysis allows the partitioning of correlation coefficients into component parts (*Topal and Esenboga, 2001; Woods et al., 2003*). The first component is the path coefficient (beta weight) that measures the direct effect of the predictor variables on the response variable (BW). The second component estimates the indirect effect of the predictor correlation coefficients between traits and body weight. The highest path correlation of HG on BW was of 0.929 (Table 6). Its direct effect was was also the highest (path coefficient=0.490; P<0.001). This implies that one unit change in standard deviation of HG results in approximately 0.50 unit change in standard deviation of body weight (Table 6). However, Heart Girth (HG) made the lowest total indirect influence (0.439) on the BW.

Most of the indirect contributions of the variables were realized by HG. It could be concluded that WH and HG are important parameters for predicting bodyweight of cattle with a high degree of accuracy.

The common measures of prediction of BW such as simple correlation coefficients between BW and morphometric measures or regression of BW on a different of body measurements have been used by *Ajayi et al. (2008); Ige (2014)* and *Nosike et al. (2018).* These, on the other hand, fall short of describing the complex biological relationships that exist between body weight and morphological characteristics. Simple correlation coefficients and regression do not capture the causal effects of biologically associated variables well.

Furthermore, researchers (*Adenaike et al., 2015; 2018*) have shown that regression procedures fail to account for multicollinearity between independent variables, resulting in skewed results. Multicollinearity occurs when the association between variables is very high, making it difficult to obtain accurate estimates of their individual regression coefficients. In this case, certain variables essentially measure the same phenomenon and provide similar data. These variables may have a negative impact on regression outcomes. As a result, path analysis has been found to be more effective in determining the direct and indirect effects of one variable on another, as well as separating the correlation coefficients of the variables into components of direct effect, indirect effect and compound path (*Yakubu and Mohammed, 2012*).

Conclusion

Correlation coefficients observed between BW and body measurements were positive and significant at P<0.01, implying that the bodyweight of cattle may be predicted even on grazing conditions, particularly situations where scales are not readily available. The path analysis showed that HG had the highest direct contribution to BW. The implication is that measuring HG could be useful for selection, breeding and feeding programs for monitoring grazing performance.

Moreover, it was concluded that Heart girth could be used for management decisions and as indirect selection criteria for selection on BW due to HG had the highest direct and the lowest total indirect effect on bodyweight.

The relationships between BW and some body measurements might be misleading when path analysis is not taken into consideration. Owing to this, the path analysisis very important for determining factors affecting BW. The path analysis result showed that the Heart girth can be used as a predictor variable with high confidence.

This study also showed that it will provide more reliable and accurate results in studies to be conducted in more controlled conditions.

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EFFECT OF CONDENSED TANNINS CONCENTRATIONS ON PROTEIN DEGRADABILITY OF RED CLOVER, ITALIAN RYEGRASS AND THEIR MIXTURES

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Abstract: Growing grasses in a mixture with legumes leads to more profitable production, better quality of forages, an increase in soil biogenicity and fixation of a significant amount of nitrogen. The experiment was established as a two factorial trial by the method of randomized complete block design in three replications. Italian ryegrass – monocrop (IR), red clover – monocrop (RC) and their mixtures – IR:RC (15:5 kg ha^{-1}), IR:RC (15:10 kg ha^{-1}), IR:RC (20:5 kg ha^{-1}) and IR:RC (20:10 kg ha^{-1}) were planted in November 2016, with the first cutting in the spring 2017 – on May the 9th, and the second cutting in the early summer – on June the 22nd. The aim of this study was to investigate the concentrations of phenolic compounds such as condensed tannins in Italian ryegrass, red clover and their mixtures, as well as their concentrations impact on protein degradability in the rumen depends on the seeding rate in the mixtures and harvested in the spring and early summer. Results obtained in this study showed that higher concentrations of non-protein nitrogen and soluble protein in forages harvested in the spring influenced higher rapidly degradable protein concentration in investigated mixtures. The highest rumen undegradable protein was determined in Italian ryegrass monocrop harvested in early summer, and we assume that high condensed tannins concentration influenced the slower protein degradability. Our recommendation for plant breeders is that cultivars with higher content of condensed tannins should be created and introduced in animal nutrition.

Key words: protein degradability, condensed tannins, red clover-Italian ryegrass mixtures

Introduction

Red clover is the basic source of protein for ruminants, and it is one of the main constituents of the diet of these animals. *Marshall et al. (2017)* found out that some legumes such as alfalfa, red clover and white clover are important species for sustainable livestock production systems, because in mixture with grasses, they can fix, on average 150 kg N ha^{-1} yr^{-1}, and some of that N subsequently becomes available to the companion grass. Grass-legume mixtures are acknowledged to have higher crude protein concentrations, as well as lower fiber concentrations compared to pure grass stands *(Ball et al., 2001). Brown et al. (2018)* observed that in grass-legume mixtures crude protein content increased, as well as dry matter yield. In the results of the investigation reported by *Albayrak and Ekiz (2005)* the authors indicated that planting legumes with grasses improves forage nutritive value and increases the dry matter yield of pastures compared to pastures with pure grasses.

Italian ryegrass is a forage crop that represents excellent forage quality. In animal nutrition, it can be used fresh, as green forage, for grazing, for preparing hay, and for preparing silage and haylage. According to *Simić et al. (2011)* it is an ideal species for growing in a mixture with red clover. It seems possible that some of the positive effects of Italian ryegrass on ruminant performance may be due not only to high digestibility and high carbohydrate concentrations but also to benefits provided by molecules such as phenolic compound. However, relatively little is known about the soluble phenolic compound composition of Italian ryegrass, making it difficult to determine which compounds might positively influence animal performance. Among the numerous benefits of ruminant nutrition, it is important to mention that some forage species may contain compounds that can reduce protein degradability. Condensed tannins prevent bloat by binding to and precipitating proteins, which reduces protein concentration in the rumen and increases rumen bypass or undegradable protein *(Barry and Manley, 1986)*. The condensed tannins expressed by many forages can bind to salivary proteins, reducing palatability and intake, and they can also form indigestible complexes with rumen microbes and cell wall carbohydrates, reducing the rate of rumen digestion and intake *(Reed, 1995)*.

There is an increased interest in studying those leguminous species that reduce proteolytic processes and reduce protein degradation in the rumen. The aim of this study was to investigate the concentrations of phenolic compounds such as condensed tannins in Italian ryegrass, red clover and their mixtures, as well as their concentration᾽s impact on protein degradability in the rumen depends on the seeding rate in the mixtures and harvested in the spring and early summer.

Materials and Methods

The study was carried out at the experimental field of the Institute for forage crops Kruševac, Serbia. The experiment was established as a two factorial trial by the method of randomized complete block design in three replications. The study area was situated at an altitude of 166 m above sea level in Central Serbia. The mean annual temperature and the total precipitation for the region are 12.6º C and 653.2 mm, respectively. Italian ryegrass – monocrop (IR), red clover – monocrop (RC) and their mixtures – IR:RC (15:5 kg ha^{-1}), IR:RC (15:10 kg ha^{-1}), IR:RC (20:5 kg ha^{-1}) and IR:RC (20:10 kg ha^{-1}) were planted in November 2016, with the first cutting in the spring 2017 – on May the 9th, and the second cutting in the early summer – on June the 22nd. Plants were harvested in full flowering stage.

The dry matter content was determined on a sample weighing 1 kg, by drying at a temperature of 60° C to a constant weight. The samples were ground on a mill with a sieve of 2 mm, and then on a laboratory cyclone mill with a diameter of 1 mm. The samples obtained in this way were dried at 105° C to a constant weight. All results related to chemical composition and amount of nutrients are expressed in absolute dry matter. All chemical analyzes were performed in duplicate.

The CP (Crude Protein) of the samples was determined by Kjeldahl method. The NPN (Non-Protein Nitrogen), NDICP (Neutral detergent Insoluble Crude Protein), ADICP (Acid Detergent Insoluble Crude Protein), SolP (Soluble Protein), TP (True Protein) and IP (Insoluble Protein) were determined by *Licitra et al. (1996)*. The CNCPS (Cornell Net Carbohydrate and Protein System) crude protein fractions of the samples, PA, PB$_1$, PB$_2$, PB$_3$ and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to *Fox et al. (2004)*: PA = NPN; PB$_1$ = SolCP – NPN; PB$_2$ = CP – SolCP – NDICP; PB$_3$ = NDICP – ADICP; PC = ADICP. Where PA refers to the non-protein nitrogen (g kg^{-1} DM); PB$_1$ the rapidly degraded crude protein (g kg^{-1} DM); PB$_2$ the intermediately degraded crude protein (g kg^{-1} DM); PB$_3$ slowly degraded crude protein (g kg^{-1} DM) and PC the bound crude protein (g kg^{-1} DM).

The CNCPS (Cornell Net Carbohydrate and protein System) is a mathematical model designed to evaluate the nutrient requirements and supply of cattle over a wide range of environmental, dietary, management and production situations. Rumen-degradable CP (RDP) was calculated based on CNCPS subfractions using fractional rate of degradation (Kd) values given for legume pasture (*Grabber, 2009*). Rumen degradable protein (RDP) was calculated as follows:

$$\text{RDP} = \sum \text{CP sub-fractions} \times \text{Kd} / (\text{Kd} + \text{Kp})$$

where Kp is the fractional rate of passage which is assumed to be 0.045 h^{-1}. Fractional degradation rates of CP sub-fractions adapted from legume pasture values reported in the CNCPS v_6.1 feed library (www.cncps.cornell.edu) are as follows: Kd (PA) = 2.00 h^{-1}; Kd (PB_1) = 0.20 h^{-1}; Kd (PB_2) = 0.15 h^{-1}; PB_3 = 0.08 h^{-1}. Rumen-undegradable CP (RUP) was calculated by subtracting RDP from total CP.

The sampled plant material was prepared for the drying process in laboratory conditions for phenolic compound determination. The plant material was dried in a thin layer in a drafty and dark place where the temperature ranged from 18-22° C, and the air humidity ranged from 55-65%. The drying process was regularly controlled, where damaged specimens and those that had changed colour were removed. After a 7-day drying process, the dry plant material was crushed and packed in dark glass containers until the extraction process.

About 2.0000 g of dried homogenized plant material was weighed and 20 ml of methanol:water:HCl (80:19:1) extraction solution was added. After standing for 2 hours at room temperature, the solution was decanted, and the solid residue was extracted 2 more times in the manner already described (20 ml and 10 ml of the extraction solution). The filtrate obtained by squeezing the collected extracts through a Buchner funnel was transferred to a normal 50 ml vessel, and diluted with a solvent to the line. The obtained extract was stored in a dark and cold place.

The determination of total polyphenols (TPP) of sample extracts was performed with the Folin-Ciocalteu reagent according to the method of *Singleton and Rossi (1965)* with some modifications. The absorbance is measured at 765 nm by UV-Vis spectrophotometer (HALO RB-10, Dynamica). The results are expressed in g gallic acid equivalent / 100 g plant dry matter (gE GA / 100 g DM) by reference to the calibration curve of gallic acid. The total flavonoids (TF) determination of sample extracts was performed according to the method described by *Jia et al. (1999)* with some modifications. The absorbance is measured at 415 nm using a UV-Vis spectrophotometer (HALO RB-10, Dynamica). The results are expressed in g quercetin equivalent / 100 g plant dry matter (gE Qu / 100 g DM) with reference to the quercetin calibration curve. The condensed tannins (CT) content of sample extracts were determined using the vanillin assay described by *Makkar and Becker (1993)* with some modifications. The absorbance at 550 nm was measured against a blank using a UV-Vis spectrophotometer (HALO RB-10, Dynamica). The total concentration of condensed tannins was expressed in mg of catechin equivalents / 100 g plant dry matter (mgE C / 100 g DM) with reference to the catechin calibration curve.

The experimental data were analyzed by a two-way analysis of variance for samples using a model that accounted for the main effects of Italian ryegrass – red clover mixtures and cut. Effects were considered significant at $p < 0.05$ level.

The significance of differences between arithmetic means was tested by Tuckey test (STATISTICA 6, Stat. Soft. 2006).

Results and Discussion

Crude protein concentration and primarily protein fractions of red clover, Italian ryegrass and their mixtures harvested in the spring and early summer are presented in Table 1. Crude protein concentration was different between harvest seasons and was lower in the spring (mean value was 146.9 g kg^{-1} DM) and greater in the early summer (155.7 g kg^{-1} DM). Red clover and Italian ryegrass monocrops differed significantly ($p< 0.05$) in CP concentration, whereas similar concentrations between the mixtures were observed with an average of 156.8 to 170.2 g kg^{-1} DM. Red clover monocrop was characterized by the highest CP concentration, followed by the IR:RC (20:5) mixture.

Statistical analysis showed that harvest date and seeding rate of IR and RC in mixtures significantly affected concentrations of primarily protein fractions: TP, NPN, IP, SolP, NDICP and ADICP. Significantly greater NDICP, ADICP, ICP and TP concentrations were recorded in the mixtures harvested in early summer (Table 1), whereas only SolP and NPN concentrations were higher in IR:RC mixtures harvested in the spring than harvested in the early summer (treatments differed by 13.7 and 26%, respectively). The lowest NDICP concentration was recorded in IR:RC (15:5) mixture, whereas the mean values of this protein fraction recorded in other three IR:RC mixtures were similar, and ranged from 20.03 to 21.80 g kg^{-1} DM. On the other hand, ADICP concentration was the highest in IR:RC (20:10) mixture, but ADICP concentrations were similar in the other three mixtures, ranging from 14.73 to 15.15 g kg^{-1} DM and did not differ significantly. The lowest ICP and NPN concentrations were recorded in 20:10 IR:RC mixture, and the mean values were 78.65 and 56.88 g kg^{-1} DM, respectively. On the other hand, this mixture was the highest in SolP and TP (mean values were 85.75 and 102.5 g kg^{-1} DM, respectively). Higher CP concentration in RC monocrop influenced higher ($p< 0.05$) protein fractions content in RC monocrop than in IR monocrop, except NDICP concentration. The concentration of this protein fraction was similar in IR and RC monocrop, and did not differ significantly.

Table 1. Crude protein content and primarily protein fractions of red clover, Italian ryegrass and their mixtures harvested in the spring and early summer

Parameter	Cut	IR	RC	IR:RC 15:5	IR:RC 15:10	IR:RC 20:5	IR:RC 20:10	Mean
CP, g kg^{-1} DM	I	76.36^{e}	179.5^{a}	163.6^{c}	149.4^{d}	170.8^{bc}	141.8^{d}	**146.9^{B}**
	II	81.80^{e}	180.3^{a}	149.9^{d}	175.5^{b}	169.5^{bc}	177.1^{b}	**155.7^{A}**
	Mean	**79.08^{D}**	**179.9^{A}**	**156.8^{C}**	**162.5^{C}**	**170.2^{B}**	**159.4^{C}**	
NDICP, g kg^{-1} DM	I	12.20^{g}	16.00^{ef}	19.80^{bcd}	21.76^{abc}	15.90^{ef}	18.93^{cde}	**17.43^{B}**
	II	16.96^{de}	13.23^{fg}	16.06^{ef}	21.83^{abc}	24.16^{a}	22.76^{ab}	**19.17^{A}**
	Mean	**14.58^{C}**	**14.61^{C}**	**17.93^{B}**	**21.80^{A}**	**20.03^{AB}**	**20.85^{A}**	
ADICP, g kg^{-1} DM	I	9.40^{e}	16.03^{bc}	13.23^{d}	13.63^{cd}	13.20^{d}	18.90^{a}	**14.06^{B}**
	II	12.26^{d}	16.16^{b}	16.23^{b}	16.60^{ab}	17.10^{ab}	16.16^{b}	**15.75^{A}**
	Mean	**10.83^{C}**	**16.10^{AB}**	**14.73^{B}**	**15.11^{B}**	**15.15^{B}**	**17.53^{A}**	
ICP, g kg^{-1} DM	I	30.13^{h}	83.86^{cde}	76.70^{ef}	74.90^{f}	79.56^{def}	70.80^{f}	**69.32^{B}**
	II	56.46^{g}	106.4^{a}	89.23^{bc}	95.90^{b}	90.23^{bc}	86.50^{cd}	**87.45^{A}**
	Mean	**43.30^{D}**	**95.13^{A}**	**82.96^{BC}**	**85.40^{B}**	**84.90^{B}**	**78.65^{C}**	
SolCP, g kg^{-1} DM	I	46.20^{e}	95.66^{a}	86.86^{ab}	74.53^{c}	91.30^{a}	70.93^{cd}	**77.58^{A}**
	II	25.33^{f}	73.90^{c}	60.66^{d}	79.66^{bc}	79.30^{bc}	90.56^{ab}	**68.23^{B}**
	Mean	**35.76^{C}**	**84.78^{A}**	**73.76^{B}**	**77.10^{B}**	**85.30^{A}**	**85.75^{AB}**	
TP, g kg^{-1} DM	I	32.10^{g}	92.80^{d}	85.83^{e}	82.73^{e}	81.70^{e}	91.70^{d}	**77.81^{B}**
	II	55.30^{f}	111.4^{b}	103.6^{c}	119.3^{a}	102.1^{c}	113.3^{b}	**100.8^{A}**
	Mean	**43.70^{C}**	**102.1^{A}**	**94.73^{B}**	**101.0^{A}**	**91.91^{B}**	**102.5^{A}**	
NPN, g kg^{-1} DM	I	44.23^{g}	86.73^{ab}	77.76^{bc}	66.66^{d}	89.20^{a}	50.03^{fg}	**69.10^{A}**
	II	26.50^{h}	68.93^{cd}	46.26^{g}	56.23^{ef}	67.40^{d}	63.73^{de}	**54.84^{B}**
	Mean	**35.36^{C}**	**77.83^{A}**	**62.01^{B}**	**61.45^{B}**	**78.30^{A}**	**56.88^{B}**	

CP – crude protein; NDICP – neutral detergent insoluble crude protein; ADICP – acid detergent insoluble crude protein; ICP – insoluble crude protein; SolCP – soluble crude protein; TP – true protein; NPN – non-protein nitrogen, DM – dry matter; I – plants harvested in the spring 2017 – on May the 9th; II – plants harvested in the early summer – on June the 22nd; IR – Italian Ryegrass; RC – Red Clover; Different letters denote significance different means ($p < 0.05$).

The association of protein solubility with degradability in the rumen and corresponding ruminant performances has been observed by some authors *(Grubić et al., 1996; Grubić and Adamović, 2003; Grubić et al., 2003).* Solubility of proteins is one of the main factors that determines the protein degradability in the rumen. At the same time, the solubility of proteins indicates their accessibility to the action of proteolytic enzymes. Soluble crude proteins are usually degradable very fast in the rumen, but some part of this fraction can have a significant amount of crude protein whose degradability proceeds at a slower rate.

In addition to the fact that legumes are characterized by high nutritive value, their proteins are subject to rapid degradation in the rumen. Consequently, the rate of protein degradation in the rumen directly affects the efficiency of nitrogen utilization by the animal *(Broderick, 1995). Kingston-Smith et al. (2003)* indicated that both rumen microorganisms and proteases in plant material cause inefficient utilization of nitrogen in animal organisms. Generally, the non-utilization of nitrogen is the result of an imbalance between protein and carbohydrates in the diet, which increases the risk of nitrogen loss and its excretion into the environment *(Tamminga, 1996)*. In order to avoid these losses and environmental contamination, a diet must be formulated to satisfy, but not to exceed the amount of nitrogen that is necessary for the growth of microorganisms, as well as a sufficient amount of amino acids that are necessary for the normal function of the animal organism *(Schwab et al., 2005)*. Modern feeding systems, in order to minimize nitrogen losses imply knowledge of the amount and ratio of degradable and undegradable protein in the rumen, as well as the necessary amounts of nitrogen for sufficient development of microorganisms *(Lanzas et al, 2007; 2008)*.

Protein fractions calculated by the CNCPS system of analysis, RDP, RUP and DMD are presented in Table 2. These protein fractions are mainly affected by the content of primarily protein fractions in the DM of IR, RC monocrop and their mixtures. Results obtained in this study showed that the highest protein fractions were PA and PB_2. The higher content of PA fraction was estimated in the DM of forages harvested in the spring than in the DM of forages harvested in early summer by 36.55% ($p< 0.05$). Concentrations of PB_3 fraction were similar and did not differ between harvest dates (26.21 g kg^{-1} CP in the DM of forages harvested in the spring *vs* 29.60 g kg^{-1} CP in the DM of forages harvested in early summer). Significantly higher concentrations of PB_1, PB_2 and PC were estimated in the DM of forages harvested in early summer than those harvested in spring. The highest value of PB_3 and PC protein fraction was determined in IR monocrop, and influenced the highest RUP concentration. On the other hand, IR:RC 20:5 mixture was characterized by the highest PA and PB_1 protein fractions influenced by the highest RDP concentration (Table 2).

The protein value of feeds for ruminants is based on an estimation of the quantity of dietary and microbial protein absorbed in the small intestine. Dietary nitrogen that escapes degradation in the rumen is, therefore, an important factor in determining the protein value *(Aufreŕe et al., 2002)*. PA fraction served as the main indicator for proteolysis. Usually, extensive protein hydrolysis after harvest and during fermentation is characteristic of forage legumes preserved as silage. Differences in proteolysis among IR:RC mixtures have been observed, but plant characteristics associated with these differences have not been well defined *(Albrecht and Muck, 1991)*. *Coblentz et al. (1998)* suggested that the undegradable fraction of CP was considerably larger in some plants due to the heavy lignification of the stem tissue in plants, especially in the summer.

We noted significant differences ($p< 0.05$) in DMD between the different harvest times of IR and RC monocrops and their mixture, as well as significant differences between IR and RC and their mixtures. DMD was higher in the spring than in early summer. The highest DMD was observed in RC monocrop, higher by 31% than DMD of IR monocrop. DMD in investigated IR-RC mixtures ranged from 657.8 to 686.1 g kg^{-1} DM, and the highest DMD was noted in 15:5 IR:RC mixture, but the lowest DMD noted in 20:10 IR:RC mixture.

Table 2. Protein fractions concentration determined by CNCPS system of analysis or red clover, Italian ryegrass and their mixtures harvested in the spring and early summer

Parameter	Cut	IR	RC	IR:RC 15:5	IR:RC 15:10	IR:RC 20:5	IR:RC 20:10	Mean
PA, g kg^{-1} CP	I	579.3^{a}	482.4^{c}	475.4^{c}	446.3^{c}	521.8^{b}	352.7^{efg}	**476.3^{A}**
	II	323.7^{fgh}	382.3^{de}	308.7^{h}	320.0^{gh}	397.7^{d}	359.9^{ef}	**348.8^{B}**
	Mean	**451.5^{AB}**	**432.4^{B}**	**392.0^{C}**	**383.3^{C}**	**459.8^{A}**	**356.4^{D}**	
PB$_1$, g kg^{-1} CP	I	26.26^{de}	50.06^{cde}	55.73^{cde}	52.46^{cde}	12.43^{e}	147.4^{a}	**57.38^{B}**
	II	27.20^{de}	27.63^{de}	95.96^{bc}	133.7^{ab}	69.90^{cd}	151.4^{a}	**84.29^{A}**
	Mean	**26.73^{C}**	**38.85^{C}**	**75.85^{B}**	**93.08^{B}**	**41.16^{C}**	**149.4^{A}**	
PB$_2$, g kg^{-1} CP	I	234.9^{e}	371.2^{cd}	348.1^{d}	355.5^{d}	372.7^{cd}	366.3^{d}	**341.5^{B}**
	II	448.4^{ab}	483.8^{a}	485.9^{a}	421.6^{bc}	389.7^{cd}	359.9^{d}	**431.6^{A}**
	Mean	**341.7^{D}**	**427.5^{A}**	**417.0^{AB}**	**388.6^{BC}**	**381.2^{C}**	**363.1^{CD}**	
PB$_3$, g kg^{-1} CP	I	36.30^{ab}	10.56^{cd}	39.90^{ab}	54.53^{a}	15.73^{cd}	0.23^{d}	**26.21^{NS}**
	II	50.93^{a}	16.46^{cd}	1.10^{d}	29.80^{bc}	41.90^{ab}	37.43^{ab}	**29.60^{NS}**
	Mean	**43.61^{A}**	**13.51^{C}**	**20.50^{BC}**	**42.16^{A}**	**28.81^{B}**	**18.83^{BC}**	
PC, g kg^{-1} CP	I	123.2^{bc}	89.36^{efg}	80.90^{fg}	91.20^{efg}	77.30^{g}	133.0^{b}	**99.16^{B}**
	II	149.8^{a}	89.80^{efg}	108.3^{cd}	94.63^{def}	100.8^{de}	91.33^{efg}	**105.8^{A}**
	Mean	**136.5^{A}**	**89.58^{C}**	**94.6^{C}**	**92.9^{C}**	**89.1^{C}**	**112.2^{B}**	
RDP, g kg^{-1} CP	I	791.9^{bcd}	804.9^{ab}	803.6^{abc}	787.6^{cde}	817.2^{a}	747.3^{h}	**792.1^{A}**
	II	716.2^{i}	778.9^{def}	754.6^{gh}	765.6^{fg}	772.5^{ef}	776.3^{def}	**760.7^{B}**
	Mean	**754.0^{C}**	**791.9^{A}**	**779.1^{B}**	**776.6^{B}**	**794.8^{A}**	**761.8^{C}**	
RUP, g kg^{-1} CP	I	208.1^{fgh}	195.1^{hi}	196.4^{ghi}	212.4^{efg}	182.8^{j}	252.7^{b}	**207.9^{b}**
	II	283.8^{a}	221.0^{def}	245.4^{bc}	234.4^{cd}	227.5^{de}	223.7^{def}	**239.3^{A}**
	Mean	**245.9^{A}**	**208.1^{C}**	**220.9^{B}**	**223.4^{B}**	**205.2^{C}**	**238.2^{A}**	
DMD, g kg^{-1} DM	I	628.6^{e}	722.1^{a}	692.9^{b}	680.3^{bc}	693.8^{b}	665.3^{cd}	**680.5^{A}**
	II	452.0^{f}	694.2^{b}	679.2^{bc}	653.5^{d}	655.3^{d}	650.2^{d}	**630.7^{B}**
	Mean	**540.3^{E}**	**708.1^{A}**	**686.1^{B}**	**666.9^{CD}**	**674.5^{BC}**	**657.8^{D}**	

PA – instantaneously solubilised protein; PB$_1$ – rapidly degradable protein; PB$_2$ – intermediately degradable protein; PB$_3$ – slowly degradable protein; PC – completely undegradable protein; RDP – Rumen Degradable Protein; RUP – Rumen Undegradable Protein; DMD – Dry Matter Digestibility; I – plants harvested in the spring 2017 – on May the 9th; II – plants harvested in the early summer – on June the 22nd; IR – Italian Ryegrass; RC – Red Clover; Different letters denote significance different means ($p < 0.05$).

Concentrations of total polyphenols, total flavonoids and condensed tannins of IR, RC and their mixtures harvested in spring and early summer are presented in Table 3. Mean values for the concentrations of TPP, TF and CT were significantly higher in samples harvested in spring than in early summer ($p < 0.05$).

Table 3. Concentration of Total Polyphenols, Total Flavonoids and Condensed Tannins in red clover, Italian ryegrass and their mixtures harvested in the spring and early summer

Parameter	Cut	IR	RC	IR:RC 15:5	IR:RC 15:10	IR:RC 20:5	IR:RC 20:10	Mean
TPP, gE GA / 100 g DM	I	10.00^{de}	13.01^{b}	11.29^{c}	13.49^{b}	16.20^{a}	10.64^{cd}	**12.44^{A}**
	II	12.50^{b}	12.71^{b}	9.80^{de}	9.22^{ef}	10.02^{de}	8.48^{f}	**10.44^{B}**
	Mean	**11.25^{B}**	**12.86^{A}**	**10.54^{C}**	**11.35^{B}**	**13.11^{A}**	**9.56^{D}**	
TF, gE Qu / 100 g DM	I	7.21^{d}	10.45^{b}	7.29^{d}	8.79^{c}	14.47^{a}	7.30^{d}	**9.26^{A}**
	II	7.42^{d}	8.90^{c}	6.93^{d}	6.67^{de}	8.80^{c}	5.69^{e}	**7.42^{B}**
	Mean	**7.36^{C}**	**9.67^{B}**	**7.11^{CD}**	**7.73^{C}**	**11.67^{A}**	**6.49^{D}**	
CT, mgE C / 100 g DM	I	463.9^{c}	490.1^{c}	391.6^{d}	548.4^{b}	542.9^{b}	476.8^{c}	**485.8^{A}**
	II	955.2^{a}	406.5^{d}	381.0^{d}	327.9^{e}	382.9^{d}	260.5^{f}	**452.5^{B}**
	Mean	**710.6^{A}**	**448.3^{BC}**	**386.3^{D}**	**438.1^{C}**	**462.9^{B}**	**368.6^{E}**	

TPP – Total Polyphenols; TF – Total Flavonoids, CT – Condensed Tannins; I – plants harvested in the spring 2017 – on May the 9th; II – plants harvested in the early summer – on June the 22nd; IR – Italian Ryegrass; RC – Red Clover; gE GA / 100 g DM - g gallic acid equivalent / 100 g plant dry matter; gE Qu / 100 g DM - g quercetin equivalent / 100 g plant dry matter; mgE C / 100 g DM - mg of catechin equivalents / 100 g plant dry matter; Different letters denote significance different means ($p< 0.05$).

Italian ryegrass monocrop was characterized by the highest content of CT (58.5% higher than in RC monocrop). In investigated IR:RC mixtures, CT concentration ranged from 368.6 mgE C / 100 g DM (IR:RC 20:10) to 462.9 mgE C / 100 g DM (IR:RC 20:5). We assume that the highest CT concentration in IR monocrop harvested in early summer (955.2 mgE C / 100 g DM) influenced the highest RUP concentration (283.8 g kg^{-1} CP). *Waghorn (2008)* indicated that condensed tannins bind to protein in the rumen, reduce protein degradation, and when dietary crude protein concentrations exceed animal requirements for CP, these effects can improve performances. Although IR was superior in CT content related to red clover, this investigation showed that it is not well adapted to being grown in mixed crops with red clover regarding supporting high content of RUP in forages. RUP concentration in mixed IR:RC crops ranged from 182.8 g kg^{-1} CP (IR:RC 20:5) mixture harvested in spring to 252.7 g kg^{-1} CP (IR:RC 20:10) harvested also in spring (Table 2). RC had a slightly higher content of CT than IR harvested in spring, but the difference was not significant. In the summer harvest content of CT in RC was significantly lower than in IR, but it did not affect the high difference between RUP content in these forages. *Albrecht and Muck (1991)* concluded that lower non-protein nitrogen that has been found in red clover results from the action of polyphenol oxidase. This enzyme system reacts with O_2 and phenols normally present in red clover to produce quinones that inhibit the plant proteases that influence the protein degradability of forages *(Broderick et al., 2001; Wilkins and Jones, 2000).*

Conclusions

This study confirmed that forage legumes provide high protein feed for ruminants, either in single species swards such as red clover or in mixed species swards containing Italian ryegrass and red clover. The lowest Soluble CP and non-protein nitrogen were determined in IR monocrop. As a consequence, the highest RUP content was also determined in IR monocrop. The results of this study indicate the potential importance of using Italian ryegrass forage in order to increase the content of rumen undegradable protein (RUP) in the ration of ruminants. We can conclude that the positive effect of high CT content in IR on reducing the CP degradability compared to RC monocrop and their mixtures may be used for the improvement of N utilization in ruminant nutrition. There are further investigations required in order to determine the optimal seeding rate of IR and RC in the mixtures and to determine the optimal harvest date of these mixtures regarding achieving the optimal RUP and RDP content for ruminant nutrition.

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TREND OF MILK YIELD TRAITS OF BULL MOTHERS OF THE HOLSTEIN-FRIESIAN BREED

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Invited paper

Abstract: The research was conducted on 175 cows of the Holstein-Friesian breed that were selected as bull dams and achieved 400 lactations. Animals are reared on 5 farms of the "Belgrade" Agricultural Corporation. Bull dams are the offspring of 32 bulls calved between 2007 and 2014. The research included the following characteristics of milk yield in standard lactation (305 days): milk yield (kg), milk fat yield (kg), milk fat content (%), protein yield (kg), protein content (%). The variability of traits and the influence of factors (farm, lactation in order, season and year of calving, bull-sire) were evaluated by the method of least squares using the GLM (General Linear Model) procedure in the SAS software package. The average milk yield of bull dams is 9617.11 kg, with 3.44% milk fat and 3.21% protein. The average yield of milk fat and protein is 329.56 kg and 308.65 kg, respectively. The largest positive deviation of milk yield from the general average is 363.64 kg, while the largest negative deviation is -1021.36. The largest negative deviation of milk fat yield and protein yield is -21.74 kg and -36.09 kg, respectively. The largest positive deviation of milk fat yield is 18.35 kg and protein yield is 9.46 kg. The influence of the farm is present in the variability of all the observed traits of milk production except milk yield. Lactation in turn had a statistical effect ($p<0.05$) on all traits included in the research, except for protein content. The influence of the calving season is present in the variability of milk yield and protein yield, while it had no effect on the other traits included in the research. Calving year statistically ($p<0.05$) had a significant effect on the variability of all observed milk yield traits. The sire bull influenced the phenotypic expression of milk yield traits, while his influence on the variability of milk fat and protein content was not significant.The results of the research indicate that the established variability of milk yield traits in the population of the Holstein-Friesian breed provides enough room for their further improvement through selection.

Key words: bull dams, milk traits, variability, genetic trend, Holstein-Frisian breed

Introduction

Milk yield is one of the most important criteria for defining economic profit in dairy cattle breeding. The previous selection of Holstein-Friesian cattle was mainly aimed at improving milk yield characteristics (*Lazarević, 2019*). Genetic improvement is possible by selecting parents with higher breeding values (*Kosgey et al., 2006*) that can make positive genetic contributions to the next generation.

The first step is to identify the potential cows, candidates to become a bull dam. Bull dams represent a group of elite females that are selected based on EBV or GEBV. These cows are mated with elite bulls for the purpose of producing bull calves (*Schefers and Weigel, 2012*). The best cows are selected from the mother herd, which as a rule represent about 1% of the population, primarily in terms of milk and milk fat production, and then also in body structure, udder structure, milking characteristics and reproductive properties. These cows should be at least two standard deviations above the average of the parent population in milk production (*Main Cattle Breeding Program, Holstein-Friesian Breed, 2019*).

When mating between animals is planned, it is necessary to examine the changes in the average breeding value of the population as a result of selection and determine the effectiveness of that breeding program. Estimation of genetic and environmental trends in a population makes it possible to evaluate selection methods (*Mohammaddiyeh and Behmaram, 2023*).

One of the most important indicators of achieved selection success in cattle populations intended for milk production is the genetic trend of milk yield traits (*Beskorovajni, 2014*).

Breeding objectives need to be outlined and regularly updated to address the changes in the consumer environment. Breeders have to plan ahead because genetic choices made today will improve profits only in future generations, and a review of past selection objectives may be of use in determining new selection goals (*Ombura et al., 2007).*

The objectives of this study were to determine the variability and genetic trend of milk traits in the nucleus dairy herd of Holstein-Frisian bull dams by using the linear method of evaluation, and to determine their importance in the selection of cattle.

Materials and Methods

The research was carried out on 175 head of the Holstein-Friesian breed, which were selected as bull dams and achieved 400 lactations. Goats were grown on 5 farms of the Agricultural Corporation "Belgrade" (table 1). Bull dams are the offspring of 32 bulls that calved in the period from 2007 to 2014 (table 4). Bulls with a minimum of 5 daughters were included in the survey.

Within each year, four seasons were observed (table 3):

1. winter: includes cows calved in December, January and February;
2. spring: includes cows calved in March, April and May;
3. summer: includes cows calved in June, July and August;
4. autumn: includes cows calved in September, October and November.

Table 2 shows the distribution of concluded lactations by lactation order. Due to the small number of lactations, all lactations after the third were considered as one group.

Table 1. Distribution of concluded lactations by farms

Farm	Number of lactations
1	138
2	143
3	103
4	4
5	12
Total	400

Table 2. Distribution of concluded lactations by parity/lactation order

Lactation order	Number of lactations
1	175
2	115
3	67
>3	43
Total	400

Table 3. Distribution of concluded lactations by calving season

Calving season		Number of lactations
1	(winter)	102
2	(spring)	72
3	(summer)	112
4	(autumn)	114
	Total	400

The research included the following milk yield traits in standard lactation (305 days):

- milk yield (kg),
- yield of milk fat (kg),
- milk fat content (%),
- protein yield (kg),
- protein content (%).

Descriptive statistical analysis was performed with the statistical program SAS (SAS Institute Inc. 9.3, 2012). The variability of traits was assessed by the method of least squares using the GLM (General Linear Model) procedure in the SAS software package. The following model was used to analyse the influence of genetic and non-genetic sources of variability:

$$Y_{ijklmn} = \mu + F_i + L_j + S_k + C_l + S_m + e_{ijklmn}$$

where:

- Y_{ijklmn}: studied trait,
- μ: population average for given trait,
- F_i: fixed effect of i farm (i=1,…,5),
- L_j: fixed effect of j lactation order (j=1,2,3,>3),
- S_k: fixed effect of k calving season (k=1,2,3,4),
- C_l: fixed effect of l calving year (l=1,…,8),
- S_m: fixed effect of m sire (m=1,…,32),
- e_{ijklmn}: random error with features N (0,σ2).

Results and Discussion

Table 4 shows the average milk yield per calving year and the general average for all observed traits. The average milk yield of bull dams is 9617.11 kg, with 3.44% milk fat and 3.21% protein. The average yield of milk fat and protein is 329.56 kg and 308.65 kg, respectively.

Observing the milk yield per calving year, significant variability is observed. The lowest milk yield of 8720.50 kg was achieved in the first observed year (2007). After the increase in milk yield in 2008, the milk yield decreased in 2009 and 2011. The increase in milk yield was observed in 2010, then in 2012 and 2013, when the milk yield was the highest (9980.75 kg).

Table 4. Distribution of concluded lactations and average milk yield in standard lactation (305 days) per calving year

Calving year	Number of lactations	Milk yield, kg	Milk fat yield, kg	Milk fat content, %	Protein yield, kg	Protein content, %
2007	2	8720.50	319.92	3.67	302.14	3.46
2008	7	8954.71	317.90	3.55	278.03	3.10
2009	16	8595.75	307.85	3.59	272.56	3.17
2010	30	9521.30	347.94	3.65	311.04	3.27
2011	61	9246.77	325.36	3.52	302.03	3.27
2012	98	9549.00	333.29	3.50	309.48	3.24
2013	123	9980.75	331.05	3.33	318.11	3.19
2014	63	9778.78	323.49	3.32	306.96	3.14
Overall mean	400	9617.11	329.59	3.44	308.65	3.21

Animals selected for bull dams achieved the highest milk fat yield of 347.94 kg in 2010, while the protein yield was the highest in 2013 (318.11 kg).

The content of milk fat (3.67%) and protein (3.46%) was the highest in 2007, when the lowest milk yield was achieved.

In the research of *Lazarevic et al., (2018)* 575 standard lactations of Holstein-Friesian cows selected as bull dams and their progeny were observed. On average they produced 9239.84 ± 1607.64 kg of milk, with a milk fat content of 3.44 ± 0.20 and protein content of 3.21 ± 0.12.

Kawahara et al., (2006) for the population of the Holstein-Friesian breed in Japan reported average values of milk yield, milk fat, dry matter without fat, protein and content of fat, matter without fat, and protein of 7899 kg, 301 kg, 689 kg , 253 kg, 3.83 %, 8.74 % and 3.18 %, respectively.

Stanojević et al. (2013) state that in the Holstein-Friesian breed, the average milk yield in the first three standard lactations is 8179 kg of milk with 3.46% milk fat and 280.38 kg of milk fat.

In a study by *Campos et al. (2015)* the mean and standard deviation for milk yield, milk fat and protein for standard lactation Holstein cows in Brazil were 8415.22 ± 1910.17 kg, 276.89 ± 66.63 kg and 253.56 ± 57.21 kg, respectively.

Pantelić et al. (2010) conducted research on 292 Simmental cows selected as bull dams in the territory of the Republic of Serbia. The average milk yield of individual animals selected for bull dams is 5754.49 kg, with 3.98% milk fat and 230.24 kg milk fat. Bull dams of Holstein Friesian breed achieved an average milk production of 10,245.98 kg, with a variation interval ranging from 6,514 kg to 13,251 kg. The average milk fat production was 361.95 kg, and the milk fat content was 3.53% (*Pantelić et al., 2012*).

In the Israeli Holstein population, average milk yield in standard lactation was 10.281 kg with 3.23% milk fat and protein content of 3.04% (*Weller and Ezra, 2004).*

Table 5 shows the deviation of milk yield traits from the general average observed by year of calving.

Table 5. Deviation of milk yield traits from the general average observed by year of calving (standard lactation, 305 days)

Calving year	Milk yield, kg	Milk fat yield, kg	Milk fat content, %	Protein yield, kg	Protein content, %
2007	-896.61	-9.67	0.23	-6.51	0.25
2008	-662.40	-11.69	0.11	-30.62	-0.11
2009	-1021.36	-21.74	0.15	-36.09	-0.04
2010	-95.81	18.35	0.21	2.38	0.06
2011	-370.34	-4.23	0.08	-6.62	0.06
2012	-68.11	3.70	0.06	0.83	0.03
2013	363.64	1.46	-0.11	9.46	-0.02
2014	161.67	-6.10	-0.12	-1.69	-0.07

The largest positive deviation of milk yield from the general average was recorded in 2013 (363.64 kg), while in 2009 the largest negative deviation was recorded (-1021.36). A negative deviation of milk yield from the general average was recorded from 2007 to 2012. In the last two years of the observed period, there is a positive deviation from the general average.

Deviation of milk fat content is in contrast with deviation of milk yield, which is the result of negative genetic correlation of these two traits. In the period from 2007 to 2012, there was a positive deviation from the general average, and a negative deviation in the last two years of the observed period.

The biggest negative deviation of milk fat yield and protein yield was in 2009 (-21.74 kg, respectively -36.09 kg). The largest positive deviation of milk fat yield was in 2010 (18.35 kg) and protein yield was in 2013 (9.46 kg).

The largest positive deviation of the protein content was in 2007 (0.25%), contrary to this result, the largest negative deviation from the general average was recorded already the following year (-0.11%).

The production year has its own special characteristics, which are manifested through climatic factors, available food, farm management, economic conditions that affected the entire business, so the phenotypic manifestation of milk yield characteristics had oscillations during the observed period. These results were also contributed to by the uneven number of concluded lactations by observed age.

As stated by *Beskorovajni (2014),* in the population of the improved Black and White breed, the largest positive deviation of milk yield compared to the general average was achieved in 2009 (1590.10 kg). Contrary to this result, the largest negative deviation was recorded in the year 2000 (-1590.60 kg) and the differences in the amount of milk yield, milk fat, 4% MCM and milk fat content per calving year were statistically highly significant

Table 6 shows the influence of factors (farm, lactation order, season and year of calving, bull-sire) on the variability of observed milk yield characteristics in standard lactation.

The influence of the farm is present in the variability of all the observed traits of milk production except milk yield. Lactation in turn had a statistical effect ($p<0.05$) on all traits included in the research, except for protein content. As the average milk yield increases from the first to the following lactations, it is necessary to include this factor in the models for evaluating breeding value (*Beskorovajni, 2014).*

The calving season, that is, the differences between individual seasons, can have a significant impact on milk production. The difference between individual seasons is reflected in specific climatic conditions and differences in the way cows are fed and housed (*Pantelić et al., 2014*). The influence of the calving season is present in the variability of milk yield and protein yield, while it had no effect on the other traits included in the research.

Calving year statistically ($p<0.05$) had a significant effect on the variability of all observed milk yield traits. The sire bull influenced the phenotypic expression of milk yield traits, while his influence on the variability of milk fat and protein content was not significant.

Table 6. Effect of factors on the variability of milk yield characteristics in standard lactation (305 days), F-test

Factor	F- test (standard lactation)					
	d.f.$_1$	Milk yield, kg	Milk fat yield, kg	Milk fat content, %	Protein yield, kg	Protein content, %
Farm	4	2.44^{ns}	4.56^{*}	3.14^{*}	3.61^{*}	9.12^{*}
Lactation order	3	13.40^{*}	11.76^{*}	2.71^{*}	13.47^{*}	0.48^{ns}
Calving season	3	4.26^{*}	2.63^{ns}	2.62^{ns}	4.48^{*}	0.62^{ns}
Calving year	7	6.39^{*}	2.44^{*}	23.47^{*}	5.48^{*}	12.68^{*}
Bull-sire	31	2.21^{*}	2.26^{*}	1.01^{ns}	2.24^{*}	0.79^{ns}

$p>0.05^{ns}$, $p<0.05^{*}$

In the research of *Lazarević et al. (2018)* the impact of bull-sire, year of birth, lactation order, farm, year and calving season was present at different levels of statistical significance on yield traits, while the genetic group had no influence on any of the milk traits. Bull-sire, year of birth, lactation order and calving season did not influence the variability of milk fat and protein content.

According to *Lazarević (2019)* the influence of lactation order, farm, year and calving season is present at a very high level of statistical significance ($p<0.001$) in the variability of all observed milk yield traits. The sire had a highly significant influence ($p<0.001$) on all milk yield traits both in the whole and in standard lactation, except for the protein content in standard lactation where the statistical significance of this factor is lower ($p<0.01$).

Conclusion

The results of the research indicate that the established variability of milk yield traits in the population of the Holstein-Friesian breed provides enough room for their further improvement through selection. At evaluated years, many fluctuations were observed in the genetic trend of traits. It seems there was not a strict plan for breeding and genetic improvement of milk traits. Therefore, to improve the breeding status of the population animals should be selected based on their breeding values.

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FARMER EXPERIENCE IN TRANSITION FROM CONVENTIONAL TO ROBOTIC MILKING

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Invited paper

Abstract: The aim of the study was to present some of the farmers experience in transition from conventional to robotic milking. Survey were conducted with dairy farmers in the Republic of Croatia witch had transition from conventional to robotic milking. The survey had 21 questions, separated in two main sections: personal data's and about experiences during using milking robots. Based on the conducted research, it can be concluded that farmers had positive experiences and fulfilled expectations in transition from conventional to robotic milking. Most of the examined farmers (95 %) managed to improve quality of life and improve health of cows regarding to: reproduction, mastitis, hooves, etc. About 90 % of them doesn't have any negative experiences with milking robots. The vast majority of respondents (84 %) increased milk production per cow and the quality of milk also increased in amount of 58 %. Farmers pointed out that certain education could be helpful for them for better using milking robots, for example in the selection of bulls suitable for robotic milking and for the interpretation of data from the robotic software.

Key words: farmer experience, transition, conventional milking, robotic milking

Introduction

Today's milk production has become a very intensive animal husbandry, requiring a large investment in technology. Due to its economic sensitivity and complexity, milk production is accompanied by various innovations. *Havranek and Rupić (2003)* amounts that milking is a very delicate and demanding job, accounting for approximately 50 percent of the farm's time each day.

Automated milking systems (AMS), i.e. milking robots, are increasingly being introduced on dairy farms in order to facilitate milking, and to increase production efficiency. The use of milking robots with accompanying computer and software programs enables complete automation of the milking process and can be achieved the following: 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*). According to *Bijl et al. (2007)* the first robots began to be used in the Netherlands in 1992, and since then their number has been steadily growing. It is not possible to say exactly the current number of milking robots in the world, but roughly estimates is around 50.000 milking robots *(Mijić and Bobić, 2019*). The Netherlands is currently the leader in robotic milking, with about 22% of farmers owning 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 global population of milking robots could double in the next five years. Milking robots have proven to be particularly suitable on free stall farms, but also on farms where cows are kept on pastures (*Woodford et al., 2015*). The study of the *Rodenburg (2017)* also found that the proportion of labour involved in milking is declining, while the proportion of labour involved in farm management is increasing.

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 that the number of milking robots in Croatia is constantly increasing *(Mijić and Bobić, 2020*).

The aim of the study was to present some of the farmers experience in transition from conventional to robotic milking.

Materials and Methods

For the purposes of this research survey were conducted with dairy farmers in the Republic of Croatia witch had transition from conventional to robotic milking. The survey was conducted with 19 dairy farmers that own 24 robots. The survey had 21 questions, separated in two main sections:

- about personal data’s (age, education) and farm data‘s (number of cows, number of robots, etc.),
- about using milking robots (motivation to purchase of a milking robots, investment costs, experiences, etc.).

Results and Discussion

All respondents owned from less than 50 to more than 300 cows. From all 19 dairy farmers that own in total 24 robots, the most present robot were Lely (17 milking robots), bought from 12 dairy farmers, who own from the least to the most cows per farm, for example some of them have less than 50 cows on the farm and some over 300 cows (Table 1.). Robots BauMatic and GEA were the list present on investigated farms, just with 1 and 2 milking robots, respectively.

Table 1. Basic datas on the study farms

Number of farms	Robot manufacturer	Number of robots	Number of cows
1	BauMatic	1	51 - 100
4	DeLaval	4	51 - 100
2	GEA	2	≤ 50 51 - 100
12	Lely	17	≤ 50 51 – 100 101 - 200 >300

After analyses of the answers regarding to questions in first main section it was found that examined farmers were mostly (53%) between 41 and 50 years old, and between 31 and 40 years old (21%) (Figure 1.). While those which are the oldest or the youngest than that were present with the smallest percentage (16%; 10%, respectively). The majority of the respondents have completed high school, of which 24% Agricultural, 12% Veterinary high school and 41% of some other high school. Just 12% of the farmers had finished Agriculture faculty as a higher education (Figure 2).

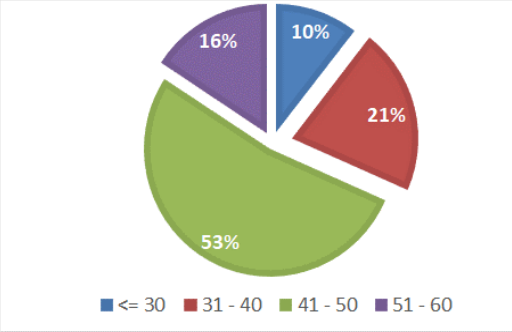

Figure 1. Farmers' age

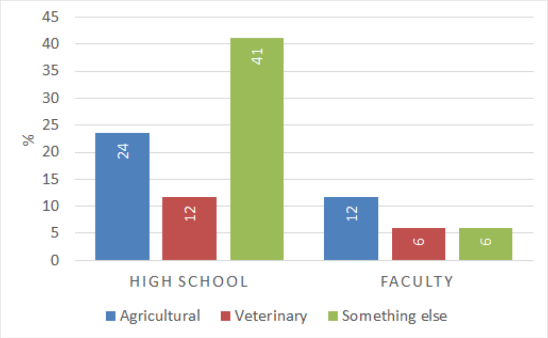

Figure 2. Farmers' level of education

Regarding to questions in second main section relating to using milking robots, it was found that examined farmers invested over 1 million Kuna's to purchase of a milking robots. In Figure 3. is visible that 58 % of the respondents invested 1 million Kuna's, 26% less than 1 million, and 16 % more than 1 million, respectively. The most of them in amount of 79 % managed to found finance from various resources (their own resources, banks, EU founds, etc.), and just 21 % of them used only their own resources (Figure 4.).

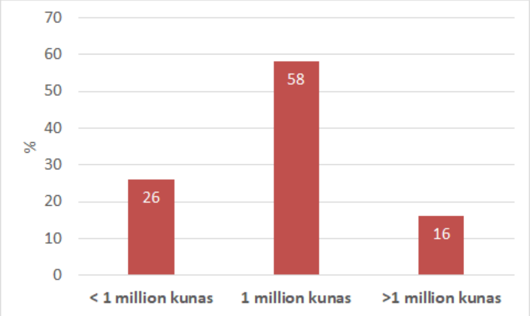

Figure 3. Approximate investment costs for the purchase of a milking robots

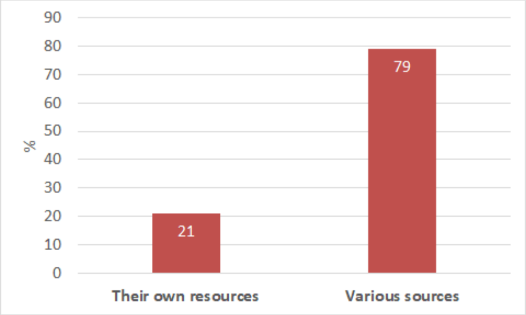

Figure 4. Resources for the purchase of a milking robots

The main two reasons or motivation to purchase and use the milking robots were reducing the human labour (47 %) and improving their quality of life (26 %) (Figure 5.). Furthermore, 11 % of the respondents wanted to increase the quantity and quality of milk and wanted to improve the farm management. Only 5 % of them wanted to improve the health of the cows (Figure 5.). Besides those main reasons, farmers wanted to motivate the younger member of their family, to involve them more in farm tasks and to keep them in dairy business.

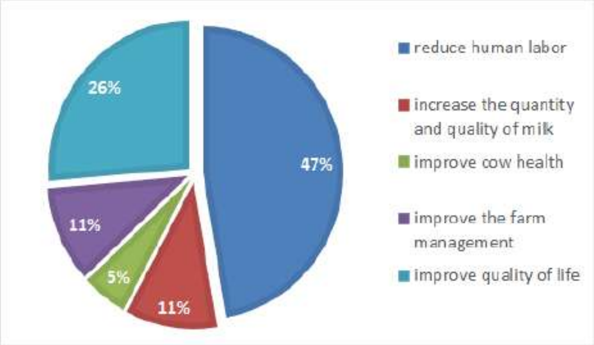

Figure 5. Reasons for the purchase of a milking robots

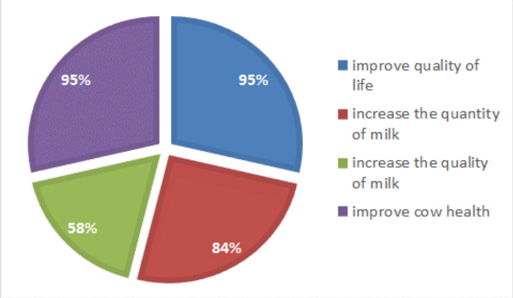

Figure 6. Achievements after purchasing the milking robots

After few years of using the milking robots farmers have gathered some experience and have received some first results and impressions. The vast majority of the respondents had positive experiences and fulfilled expectations in transition from conventional to robotic milking which agrees with the experiences of farmers in Canada who were also satisfied after transition from the conventional to the robotic milking *(Tse et al., 2017).* For example, 95% managed to improve quality of life and improve health of cows (reproduction, mastitis, hooves, etc.), which comply with statement of the *Koning (2010) and Hansen (2015).* In report of the *Tse et al. (2017)* 42% of the farmers reported to have decreased lameness, 49 % of them have decreased mastitis incidence, and 63% of the farmers have improved the conception rate, after they transfer to milking robots. *Hovinen et al. (2009)* points out that frequency of mastitis treatments decreased from 4.8/10,000 cow-days with conventional milking to 4.0/10,000 cow-days in AMS farms.

About 84 % of the farmers in this research increased the amount of milk and 58% of them managed to improve the quality of milk (Figure 6.), which agrees with previous research from *Koning (2010).* About 90 % of the examined farmers doesn’t have any negative experiences with milking robots. However, respondents emphasized the need for additional education in using milking robots. For example in the selection of bulls suitable for robotic milking and for the interpretation of data from the robotic software.

Conclusion

Based on the conducted research, it can be concluded that farmers mostly had positive experiences and fulfilled expectations in transition from conventional to robotic milking. Most of the examined farmers managed to improve quality of life and improve health of cows. Furthermore, they increased the amount of milk and the quality of milk. About 90 % of them doesn’t have any negative experiences with milking robots. However, farmers pointed out that certain education could be helpful for them for better using milking robots. Of the educations, they emphasized the greatest need for the selection of bulls suitable for robotic milking and the use and interpretation of data from the robotic software.

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BROMOTYMOL BLUE TEST SCORES FOR DETECTING RAW MILK QUALITY IN BUCKET MILK SAMPLES OF JERSEY COWS

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Abstract: Raw milk quality is keeping its popularity in dairy sector. Indirect analysis methods have constantly been investigated for this aim. The objective of the present study was to reveal the reliability of bromotymol test scores (BTS) for detecting bovine milk quality. A total of sixty bucket milk samples collected from small-scale Jersey farms located in Samsun province of Turkey was used as the material. To obtain BTS data, change of color of milk after the bromotymol reaction was assessed by a scale (1=green/normal; 2=blue/alkali and 3=yellow/acidic) and obtained data were evaluated as two subgroups (BTS1: normal and BTS2: acidic or alkali). To evaluate the reliability of BTS, the samples were compared with somatic cell count (SCC). In SCC analysis, direct microscopy was used. To evaluate raw values, two SCC subgroups (*SCC1:* $\leq 400x10^3$ *cells/ml and SCC2:* $>400x10^3$ *cells/ml*) were divided. To ensure homogeneity of variance, the SCC values were transferred to log10 base in the statistical processes. Pearson correlation coefficient of BTS and SCC was estimated to be weak (r=0.284). The regression model was calculated to be $\hat{Y}=-0.534+0.363X$ and the coefficient was found to be insignificant. Obtained findings clearly pointed out that using BTS values is not a favorable method to decide raw milk quality level in bucket milk samples of Jersey cows.

Key words: Bucket milk; milk acidity; raw milk quality; somatic cell count

Introduction

When the “milk production” term has been expressed, cattle raising principally comes to mind. However, in addition to obtaining the high amount of milk, achieving more quality has importance for market and customer demands.

Many study results have clearly shown that the weak degree of quality is negatively associated with milk yield (*Hadrich et al., 2018; Atasever et al., 2020; Franzoi et al., 2020*). The presence of intramammary infection or any abnormality of raw milk may only be detected by laboratory tests (*Kandeel et al., 2019*). *Nagasawa et al. (2019)* reported the correlations of bacterial load in milk and clinical severity in cases of coliform mastitis in cows. In spite of microbial analysis is the gold standard method to detect raw milk quality, some indirect techniques have also been used due to their faster and cheaper characteristics.

Today, somatic cell counting is the most reliable and common method to raw determine milk quality and any abnormality in the udder glands of the milking animals. Normally, somatic cells present in the milk, however, the cell amount speedily increases when any intramammary infection occurs. According to EU regulations, the upper limit for somatic cell count (SCC) in bovine milk that can be consumed by human has been declared to be 400x10^3 cells/ml (*Atasever, 2012*). Field studies *(Hortet and Seegers, 1998; Koldeweij et al., 1999; Chen et al., 2021)* revealed negative relations between SCC and milk production in the cattle farms. In addition, analyzing milk electrical conductivity, enzyme activity and acidity levels are other common indirect methods for detecting milk quality or mastitis in cows. As it appears, more and different analysis methods on this item are still needed. Bromotymol blue test scores (BTS) has rarely been as one of the alternative analysis methods. *Marschke and Kitchen (1985)* compared the California Mastitis Test (CMT) with BTS and CMT was revealed as more sensitive. Although, no detailed study has been noted on the reliability of BTS by SCC in the literature. Determining the sensitivity of BTS in the decision about raw milk quality may lead to new insights in the cattle farms.

The aim of the present study was to reveal the reliability of BTS for detecting milk quality in Jersey herds.

Materials and Methods

Bucket milk samples taken from small-scale Jersey farms located in Samsun province of Turkey were used as the material. For this aim, twenty samples were collected from each location (Atakum, Gebi and Istasyon) and in total, sixty samples were transferred to the laboratory of Animal Science Department of Ondokuz Mayis University within the sampling days. Bromotymol test scores (BTS) were obtained by the observing the change of color of milk after bromotymol reaction. In this process, a scale with 1 to 3 points (1=green/normal; 2=blue/alkali and 3=yellow/acidic) was used *(Kirdar, 2001)*. The samples were also analyzed by somatic cell count (SCC). For this aim, direct microscopic method *(Atasever, 2012)*.The strain was composed of 0.6 g of certified methylene blue chloride to 52 mL of 95% ethyl alcohol, 44 mL of tetrachlorethane and 4 mL glacial acetic acid. Total number of fields counted per slide was 40 and noted SCC values were transferred to log10 base for statistical analysis. To evaluate raw values, two SCC subgroups (*SCC1: ≤400x10*^{3} *cells/ml and SCC2: >400x10*^{3} *cells/ml*) were divided according to EU directives *(Atasever, 2012)*. Similarly, BTS values were evaluated as two subgroups (BTS1: normal and BTS2: acidic or alkali). To calculate the relations of BTS with SCC, Pearson correlation method was applied and a regression model was calculated. All statistical work was performed using SPSS 17.0 statistical program for windows.

Results and Discussion

The change of BTS and logSCC values by three milk collection points is presented in Table 1. Accordingly, Atakum was the most suitable region for SCC threshold which reflects the normal or abnormal status of the milk. Really, a high percentage (65%) of milk samples were noted as milk with lower SCC (≤400x10^{3} cells/ml). To support to this case, BTS percentage of milk collected from Atakum was found as harmonic with SCC results. This parallelism was assumed to be attractive and might be pointed out to associations of BTS with SCC. The milk samples those collected from Gebi, it could be commented a lower raw milk quality level by both markers. Additionally, the lowest raw milk quality was obtained from Istasyon location. Indeed, both BTS and logSCC levels were highest in this location.

Table 1. Change of BTS and logSCC by locations

Location	SCC1		SCC2		BTS1		BTS2		logSCC	BTS
	n	%	n	%	n	%	n	%	Means (±SE)	Means (±SE)
Atakum	13	65.0	7	35.0	14	70.0	6	30.0	5.40 ± 0.073^{A}	1.30 ± 0.105^{a}
Gebi	7	35.0	13	5.0	8	40.0	12	60.0	5.74 ± 0.08^{B}	1.60 ± 0.112^{ab}
Istasyon	2	10.0	18	90.0	6	30.0	14	70.0	5.94 ± 0.05^{B}	1.70 ± 0.105^{b}

Different superscript letters in the same column indicate statistically significance (a,b: P<0.05; A,B: P<0.01)
SCC1: somatic cell count ≤400x10^{3} cells/ml; SCC2: somatic cell count >400x10^{3} cells/ml; BTS1: green/normal; BTS2:blue/alkali or yellow/acidic

In the present study, the overall mean of SCC, logSCC and BTS were calculated to be 725x10^{3} cells/ml, 5.70±0.051 and 1.53±0.064, respectively. As seen, the mean of SCC higher than the threshold declared by the EU for the limit of SCC in raw milk for consumption by human *(Atasever, 2012).* Obtained SCC mean here was also higher than some study results *(Atasever et al, 2010; Koç, 2011; Sahin, 2023)* those obtained in Türkiye conditions. The difference between the findings of the present study and the other might be caused by the different location, breed, farm and management conditions. Besides, calculated BTS of all milk samples was found as relatively acidic or alkali. Therefore, taking marked precautions on boosting the quality level of the investigated milk marketing locations may crucially be suggested.

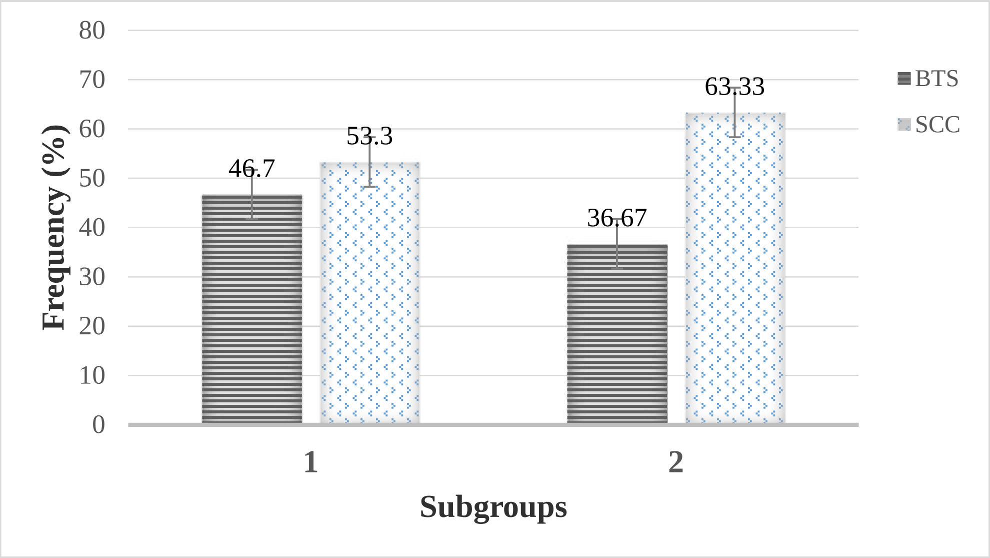

Figure 1. Distribution of BTS and SCC subgroups
(SCC1: somatic cell count ≤400x10^3 *cells/ml; SCC2: somatic cell count >400x10*^{3} *cells/ml; BTS1: green/normal; BTS2: blue/alkali or yellow/acidic)*

Normal and abnormal thresholds of both parameters are given in Figure 1. As seen, the percentage of milk samples by abnormal BTS and SCC is higher than another subgroup. As mentioned in the initial section, subgroup of BTS 2 points out to acidic or alkali structure. To support to this case, higher percentage of SCC was attractive by the groups. These findings were harmonic with the results given in Table 1 and therefore revising milk storage conditions in these locations could be advised to milk marketers here.

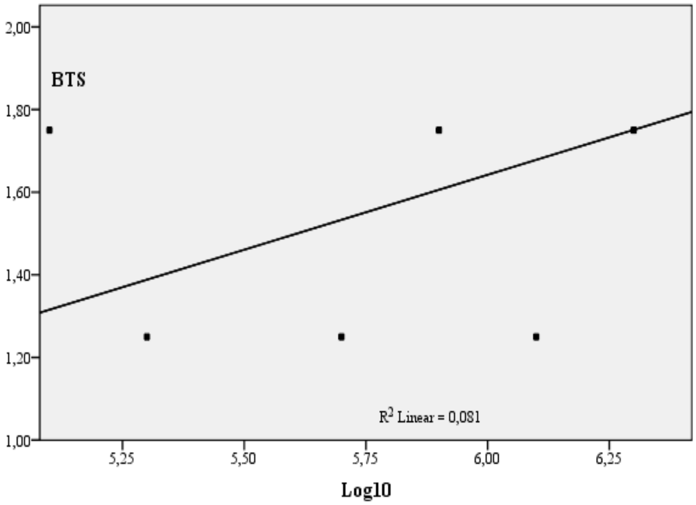

Figure 2. Linear regression curve of logSCC with BTS

The linear regression curve between logCC and BTS was presented (Figure 2). Accordingly, a positive bur weak relationship (r=0.284) was estimated between two parameters. Moreover, a regression model was calculated on these parameters (Table 2). As seen, the regression model was obtained to be $\hat{Y}=-0.534+0.363X$ and the coefficient was found to be insignificant. According to the regression model, an increase of one unit in logSCC induced to 0.363 unit increase in BTS. *Marcschke and Kitchen (1985)* emphasized that elevating the thresholds of BTS test results markedly decreased the test sensitivity, and therefore, this method might only be used on the herds with relatively high SCC in milk. *Kumar et al. (2018)* also reported that bromothymol blue strip test (BTB) values have low reliability to diagnose subclinical mastitis in buffaloes. In the view of the obtained finding here, investigating the revealed associations with more data and different locations by subsequent researchers may be suggested as a beneficial process.

Table 2. Model summary and coefficients on the relations of BTS with logSCC

Model Summary[b]					
Model	R	R Square	SE of the Estimate	SE of the Estimate	
1	0.284[a]	0.081	0.065	0.48647	
Coefficients[a]					
Model	Unstandardized		Standardized		
	B	SE	Beta	t	Sig.
1 (Constant)	-0.534	0.918		-0.582	0.563
logSCC	0.363	0.161	0.284	2.259	0.028

Conclusions

The association of BTS with SCC was examined in this study. Accordingly, analyzed milk samples dramatically tended to reflect an acidic structure. Similarly, abnormal samples were found within the high thresholds for both parameters.

Calculated weak correlation coefficient (r=0.284) and estimated regression model clearly show that BTS values of bucket milk samples collected from Jersey cows are not suitable to decide the raw milk quality.

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PRODUCTION AND QUALITY ASPECTS OF PROBIOTIC FERMENTED MILK WITH ADDITION OF HONEY

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Abstract: Probiotic fermented milks are known as high-value functional and sensory food. There is a limited range of fruit-flavoured probiotic fermented milks on the market. However, probiotics with added honey are not commonly found. In addition to sensory attributes, honey, as a natural ingredient, contributes to improving the functional properties of probiotic fermented milks. Therefore, the aim of this study was to investigate the possibilities of producing probiotic fermented milk with added honey and to analyze its chemical, physical and sensory properties. Milk with different fat content was used for probiotic fermented milk production. Probiotic beverage was produced using yogurt starter culture with probiotic strains. Two indigenous types of honey produced in Bosnia and Herzegovina were used, meadow and mountain honey. The raw materials used in the production of honey-flavoured probiotic fermented milk were analyzed. Chemical, physical (dry matter, fat, protein and acids content, pH, viscosity, water activity) and sensory properties of produced beverages were done. Chemical composition of honey-flavoured probiotic fermented milk was (%): dry matter 16.23-18.38; fat 0.90-2.80; protein 2.61-2.77 and acidity 0.750-0.789. pH and water activity ranged from 4.37 and 0.941 to 4.60 and 0.945 resp. Viscosity of samples was between 147 and 370 mPas. A good quality of honey-flavoured probiotic fermented milk was obtained, which was sensory highly rated. The best rated was the one with 2.80% of milk fat and addition of meadow honey.

Key words: probiotic fermented milk, honey, physical-chemical properties, sensory characteristics

Introduction

Probiotic fermented milk is fermented milk that contains live probiotic bacteria. It is considered a functional food due to its favorable influence on human health (*Samaržija, 2015*). The chemical properties of probiotics play an important role in the quality, taste and aroma of the product. Non-fat dry matter is usually enhanced, which results in an increase in dry matter and protein content and consequently an increase in viscosity, firmer consistency and better properties of probiotics (*Tratnik and Božanić, 2012*). Proteins are of particular importance for replacing fat or improving the structure of fermented milks with reduced fat content due to textural properties (*Lesme et al., 2020*). Among physical properties of fermented milk, viscosity, pH value and titration acidity have perhaps the most dominant influence on their sensory properties and acceptability. On the other hand, from the point of view of the development of microorganisms, water activity is an important factor (*Zamberlin et al., 2008).* The offer of fruit flavoured probiotics on the market is limited, while probiotics with the addition of honey are absent. In addition to sensory attributes, honey as a natural ingredient contributes to improving the functional properties of probiotics (*Coskun i Karabulut - Dirican, 2019).* Water content is the most important parameter of honey quality because it affects the storage and quality of honey since it determines the stability and resistance to microbiological spoilage during storage (*Petričko, 2015*). Total acidity is an important indicator of quality due to its connection with fermentation processes, taste and smell, and bactericidal properties of honey (*Gjuračić, 2016*). Electrical conductivity is a physical property that depends on the proportion of mineral substances and acids in honey; the higher it is, the higher the electrical conductivity of the honey (*Biber, 2017*). The occurrence and proportion of hydroxymethylfurfural (HMF) in honey depends on the type of honey, its pH value, the proportion of acids and moisture, and exposure to light (*Herceg, 2017; Batinić, 2014).* The goal of the work was to examine the possibility of producing probiotic fermented milk with the addition of honey, as well as to establish the best recipe. For this purpose, it was necessary to find the appropriate ratio of probiotics and honey, and to produce a functional product while retaining the desired physico-chemical and sensory properties of the product. Also, it was necessary to establish which milk fat content of this type of probiotic gives the best sensory properties. In addition, the use of indigenous types of honey produced in Bosnia and Herzegovina is also one of the important segments of this work, which enhances the traditional character of the product and promotes domestic production.

Material and Methods

The experiment was divided into two phases. Since experimental starter culture has in its composition yogurt starter bacteria besides probiotic strains, in the first phase, the ratio of honey and probiotic was determined sensory using yogurt available on the Sarajevo market (producer "Mliječna industrija 99" B&H). In the second phase, the production of probiotics was carried out by adding honey in concentrations that gave the best honey-flavoured fermented milk in the first phase. Two types of honey (meadow – flower and mountain – forest) from the producer "Eko pčelinjak Čolić", B&H and UHT milk ("Meggle" B&H) with different content of fat (0.90, 1.50 and 2.80%) were used for production. To achieve a standardized inoculum in the form of a batch (liquid) culture, a freeze-dried lyophilized starter culture, FLYABC (Bekar, Russia), was inoculated into milk. The composition of this culture, in addition to *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, was consisted of probiotic strains *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium animalis* subsp. *lactis* Bi1, *Bifidobacterium breve* Bbr8, *Bifidobacterium breve* Bl10 and *Lactobacillus plantarum*. After inoculation and thorough mixing to distribute the culture evenly, the probiotic was left to incubate at a temperature of 42°C for 6-7 hours, until the pH reached a value of 4.23. Before production, milk and honey, were analyzed. After the production of probiotics and the addition of previously determined concentrations of two types of honey, the physical, chemical and sensory properties of the produced honey-flavoured probiotic fermented milks were analyzed. The experimental production of honey-added probiotic fermented milks was done in three repetitions.

Analyzes of the basic chemical composition of UHT milk were done. Determination the of milk fat, protein, lactose, dry matter and fat-free dry matter content was made by *BAS ISO 9622 (2015)* (CombiFoss 5200-MilkoScan TM FT 6000). Laboratory tests of honey included the following physico-chemical analyses: determination of water content in honey by measuring the refractive index at 20^0C in an Abb'e type refractometer and using the Wedmor table for conversion to water percentage (AOAC Official Method 920.182) (*Rulebook on Control Methods for Honey and other Bee Products, 2009*) and determination of acidity (volumetric method); determination of electrical conductivity on the conductometer CYBERSCAN CON 510, EUTECH INSTRUMENTS, measuring range 0-20 mS/cm *(Rulebook on Control Methods for Honey and other Bee Products, 2009)*; determination of hydroxymethylfurfural (HMF) by the photometric method according to White using a spectrophotometer PERKIN ELMER LAMBDA 25 UV/VIS, measuring range 190-1,100 nm *(Rulebook on Control Methods for Honey and other Bee Products, 2009).*

The analyzed physical and chemical properties of honey-flavoured probiotic fermented milks were: content of dry matter, fat, protein, pH value, titration acidity expressed as acid content, viscosity and water activity. The dry matter content of probiotic fermented milks was determined using the method for milk by drying at a temperature of 102±2°C, while the fat content was determined using the Gerber method *(Rulebook on sampling methods and methods of chemical and physical analysis of milk and milk products, 1983).* Protein content was determined using the Kjeldahl method for milk (ISO 8968-1). The pH value was measured with a pH meter (METROHM 632) using a WTW SenTix 41 combined electrode. Titration acidity was determined by titration according to the Soxhlet-Henkel method, after which it was converted into acid content (*Sabadoš, 1996; Božanić et al., 2010; Bajt et al., 1995*). Viscosity was determined on a viscometer Viscotech Hispania, S.L (*Vranac and Muhović, 2011*) and determination of water activity by RotronicHydroLab 3 device (*Dervišević, 2019*). In the first phase, the sensory analysis was performed by hedonic scale. The evaluation was performed by a consumer group consisting of 20 evaluators. In the second phase, the sensory evaluation was carried out by an expert commission of 7 members, which evaluated the appearance, color, consistency, smell and taste with a total of 20 points. The research results were processed using the statistical program IBM SPSS Statistics 23. A comparison was made based on the results of the descriptive analysis, two-ways ANOVA and Tuckey test ($p<0.05$).

Results and Discussion

In the first phase, the share of honey in the probiotic drink was determined by evaluating the consumer group. According to the assessment, the best rated share of honey for probiotic fermented milk of high sensory quality with the addition of meadow honey was 13%, while for the one with the addition of mountain honey the best rated share was 12% honey. The evaluators noted that the best-rated probiotics with the addition of honey have a pronounced aroma of honey, which is complemented by the slightly sour taste of the fermented milk. After the assessment, the samples were produced in three replicates and coded as presented in Table 1.

Table 1. Coded samples

Sample	**Code**
0.90% milk fat probiotic + 13% meadow honey	L1
1.50% milk fat probiotic + 13% meadow honey	L2
2.80% milk fat probiotic + 13% meadow honey	L3
0.90% milk fat probiotic + 12% mountain honey	P1
1.50% milk fat probiotic + 12% mountain honey	P2
2.80% milk fat probiotic + 12% mountain honey	P3

The results obtained for chemical composition of the milk corresponded to the usual values and the declared fat content (content of fat 0.93-2.77%; dry matter 9.57-11.45%; non-fat dry matter 8.63-8.69%; protein 3.31-3.36% and lactose 4.46-4.48%). The physical and chemical parameters of honey for meadow and mountain honey were as follows: water content (%) 19.79 and 16.60; acidity (meq/kg) 32.00 and 16.50; electrical conductivity (mS/cm) 1.02 and 0.21 and HMF content (mg/kg) 18.50 and 16.30 (resp.). All obtained results, except the results for electrical conductivity, were in accordance with the current legislation of BiH (Rulebook on control methods for honey and other bee products, 2009) and correspond to the values indicated by other authors (*Grujić et al., 2009*; *Chakir et al., 2011; Esculedo et al., 2012*). The obtained values for acidity for mountain honey is slightly lower than those obtained by above mentioned authors. The reason for this can be the botanical origin, but also the way of storage. The values for the electrical conductivity of honey are not in accordance with the current legislation *(Rulebook on honey and other bee products, 2009)*. Differences in electrical conductivity were observed depending on the type of honey. A sample of meadow honey should have a conductivity of up to 0.80 mS/cm, while mountain honey should have a conductivity higher than 0.80 mS/cm. It is assumed that this low electrical conductivity for the sample of mountain honey is caused by the mixture of a large amount of acacia or flower honey. Meadow honey has a higher conductivity than allowed due to the mixture of forest honey in its composition. After the production of probiotic fermented milk with the addition of honey, the samples were analyzed (Table 2).

Table 2. Physico-chemical parameters of probiotic fermented milk with added honey

Parameters	L1	L2	L3	P1	P2	P3
Dry matter (%)	16.27^{a}	16.82^{b}	18.38^{c}	16.23^{ad}	16.50^{be}	18.09^{cf}
Fat (%)	0.90^{a}	1.50^{b}	2.80^{c}	0.93^{ad}	1.50^{be}	2.80^{cf}
Protein (%)	2.61	2.67	2.68	2.77	2.69	2.64
pH value	4.40^{a}	4.44^{a}	4.47^{a}	4.37^{b}	4.39^{b}	4.60^{c}
Acidity (%)	0.756^{a}	0.789^{b}	0.750^{c}	0.768^{d}	0.759^{a}	0.768^{d}
Viscosity (mPas)	177^{a}	160^{b}	370^{c}	147^{d}	157^{e}	277^{f}
a_w value	0.941	0.942	0.941	0.945	0.943	0.941

* values marked with different letters within the same row are statistically significantly different at the level $p<0.05$

Milk fat content has a statistically significant influence while the type of honey has no influence on the on the proportion of dry matter and fat since honey doesn't contain fat (*Vahčić i Matković, 2009*). Protein content in the samples varies slightly. The pH values were within the normal range for honey and probiotics. The pH value of honey varies from 3.20 to 6.50 (*Suárez-Luque et al., 2002*). pH value is affected by the type of honey, since honey has different acidity and sugar composition. According to the Codex standard for fermented milks (CODEX STAN 243-2003), the minimum acidity content expressed as % of lactic acid is a minimum of 0.600%. Different fat percentage and type of honey affected acid content of probiotics. The viscosity of the samples depends on the viscosity of milk, honey and the content of dry matter. Because of this, there is a link between the content of dry matter and viscosity, i.e. the higher the content of dry matter, the higher the viscosity and vice versa. Percentage of milk fat and the type of honey have an influence on the viscosity of honey-flavoured probiotics while water activity has no significant variations between the samples.

Based on the total sum of points, probiotic drinks with 2.80% milk fat (L3 and P3) were sensory rated the best and they can be classified in the extra quality class (18.01-20.00). There is a statistically significant influence of the different milk fat percentage on the sensory evaluation, as well as the type of honey (Table 3). Based on the comments given by the evaluators for the best-rated samples, they had a pleasant taste, and the taste of honey and its sweetness did not prevail.

Table 3. Sensory evaluation of probiotic fermented milk with added honey

Sensory attribute	MAX	L1	L2	L3	P1	P2	P3
Appearance	1	0.91	0.92	0.93	0.92	0.92	0.92
Color	1	0.97	0.96	0.97	0.96	0.96	0.96
Consistency	4	2.76	3.04	3.47	2.51	3.00	3.52
Smell	2	1.78	1.67	1.72	1.59	1.61	1.54
Taste	12	11.08	11.08	11.38	10.68	10.89	11.11
TOTAL	20	17.25^{a}	17.67^{b}	18.45^{c}	16.66^{d}	17.37^{e}	18.05^{f}

* values marked with different letters within the same row are statistically significantly different at the level $p<0.05$

Conclusions

It can be concluded that the ratio of 13% meadow honey and 12% mountain honey give the highest sensory quality of honey-flavoured probiotic fermented milk. The physico-chemical characteristics of products mainly depends on fat content of milk and type of honey. The resulting honey-flavoured probiotic fermented milks were of good quality, and the one with 2.80% fat and the addition of meadow honey was rated the best. Finally, it can be concluded that there is a possibility of producing probiotic fermented milk with the addition of honey of satisfactory quality.

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EFFECT OF SYSTEMATIC FACTORS ON MILK PRODUCTION PER MILKING, PRODUCTIVE AND LIFETIME DAY IN SIMMENTAL COWS

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Abstract: One of the very important functional characteristics of dairy cows is milk production per milking, productive and lifetime day. Due to the pronounced action of both genetic and paragenetic factors, the phenotypic expression of this trait is characterized by high variability.The effect of fixed (breeding area - farm, season and year of birth, total number of lactations) and continuous (age of cows at first insemination) paragenetic factors was carried out on a sample of 2 548 Simmental cows distributed in three breeding areas: Zlatiborski Suvati farms with free-stall housing system (n=502), farms in Dobrichevo with a tie-stall housing (n=956) and individual tie-stall in the area of the Agricultural Cooperative "Voćar" from Kotraža (n=1090).Based on the statistical analysis using the GLM procedure, it was determined that the general average for milk production per milking, productive and lifetime day was 12.79 kg, 9.31 kg and 5.47 kg, respectively.Breeding area, year of birth and total number of lactations significantly influenced ($P<0.01$) the phenotypic expression of all the traits. Season of birth did not significantly affect any of the observed functional traits ($P>0.05$), while age at first insemination as a continuous factor significantly affected milk production per milking day ($P<0.05$) and especially milk production per productive and lifetime day ($P<0.01$).

Key words: Simmental breed, milk production, milking day, productive day, lifetime day

Introduction

The life length and the duration of the period of exploitation of cows in the production of milk and calves, as well as the level of this production, greatly affect the overall results in cattle breeding. Although the natural life limit of cows, kept in optimal conditions, exceeds twenty or more years, certain limiting bio-economic factors determine the shortening of life and the period of their exploitation, which significantly increases the cost of this production and often makes it unprofitable.

Even though better conditions are continuously being created for the realization of desirable hereditary traits, it can be stated that the longevity and lifetime production of cows is decreasing. Numerous researchers think that the intensive, almost industrial conditions of production determine the shorter productive life of even averagely productive cows. This is also contributed to by the fact that unilateral selection of cows for high productivity regularly affects the weakening of the constitution and biological resistance, which often in intensive farming conditions ends with the premature removal of the cow from breeding, i.e. production, which affects the reduced lifetime production of milk and milk fat. This results in a high overhaul percentage, which affects the value of the selection differential, and this, in turn, affects the selection's success.

Several systematic non-genetic factors have a significant effect on total milk production during life, regardless of whether they are discontinuous-fixed in nature (breeding area, season and year of birth and calving, total number of lactations as well as their interactions) or continuous-non-categorical factors (age at first insemination or calving). In many herds, the main causes of cow exclusion are low production (30-35%), impaired physiological function of the organism (30-40%), and udder diseases (10-15%). Due to low production, cows after the first or second lactation are mostly excluded, while older cows are usually excluded due to infertility. A good indicator of adequate breeding of dairy cows is, among other things, the phenotypic expression and variability of life production characteristics, among which the most important are milk production per milking, productive and life day.

Ivanov (1990) and *Petrović et al. (2008; 2012; 2015)*, which studied the phenotypic manifestation and the effect of genetic and some paragenetic factors on lifetime milk production, state that milk production per milking day ranged from 10.86 to 14.07 kg, per productive day from 8.89 to 13.10 kg and from 5.46 to 8.01 kg per life day.

This paper aims to examine the effect of breeding area, season and year of birth, lactation in order and age of cows at first insemination in production conditions, using the appropriate methodology, on the phenotypic expression and variability of milk production per milking, productive and lifetime day in Simmental cows.

Material and Methods

The effect of systematic paragenetic factors on the phenotypic manifestation and variability of milk production per milking, productive and lifetime day was analyzed in 2 548 Simmental cows born in the period from 1995 to 2008, which were housed in three farms: 1) Zlatiborski Suvati dairy farm, Mt. Zlatibor which provides a free-stall housing system 2) Dobričevo dairy farm, Ćuprija provides tie-stall housing, and 3) Individual tie-stall farms of Kotraža region.

Based on the data from the registers of cows located in three breeding areas, milk production per milking day (kg) (MPMD), milk production per productive day (kg) (MPPD) and milk production per lifetime day (kg) (MPLD) were analyzed.

Values for milk production per milking, productive and lifetime day were calculated based on data (dates of birth, calving and exclusion) from cow registers:

- **Milk production per milking day** was obtained by dividing the lifetime milk production in kg by the number of milking days.
- **Milk production per productive day** is calculated by dividing lifetime milk production in kg by the length of productive life in days.
- **Milk production per lifetime day** was obtained by dividing the lifetime milk production in kg by the age of the cows expressed in days.

The effect of the following paragenetic factors was observed on the expression of milk production per milking, productive and lifetime day:

- **Breeding area (farm).** The research included three localities, two of which are in the hilly and mountainous area (dairy farm on Zlatibor and individual farms in the Kotraža area) and the Dobrichevo farm in the plain area.
- **Season of birth:** 1-Spring season (March, April, May), 2-Summer season (June, July, August), 3-Autumn season (September, October, November) and 4-Winter season (December, January, February).
- **Year of birth** (cows born from 1995 to 2008).
- **Total number of lactations** (1-10).
- **Age at first fertilization**.

The analysis of the effect of systematic environmental factors on the observed traits of life production (least square means (LSM), standard errors (SE_{LSM}), significance of the influence of paragenetic factors and coefficients of determination (R^2)) was performed using the general linear model of statistical software Statistica 6.0. The following models were used to analyze the effect of individual non-genetic factors on life production characteristics:

$$\mathbf{y}_{ijkl} = \boldsymbol{\mu} + \mathbf{BA}_i + \mathbf{SB}_j + \mathbf{YB}_k + \mathbf{L}_l + \mathbf{b_1}(\mathbf{x_1} - \bar{x}_1) + \mathbf{e}_{ijkl}\,,\ \text{where is}$$

y_{ijkl} - individual of the ith breeding area, the jth season of birth, the kth year of birth and the lth total number of lactations; **μ** - general population average with equal representation of all classes of effect (OP, SR, GR, L); **BAi** - fixed effect of the ith breeding area (1-3); **SBj** - fixed effect of the jth season of birth (1-4); **YBk** - fixed effect of the kth year of birth (1-14); **L_l** - fixed effect of the lth total number of lactations (1-10); **b_1** - linear regression coefficient of the effect of age at first fertilization and **e_{ijkl}** - another undetermined effect.

In the further analysis of the observed traits, the coefficients of determination (R^2) for each observed trait are shown, which represent the rest of the residual variance, i.e. the variance of the model divided by 100.

Results and Discussion

Table 1. Least squares means (LSM), standard errors of least squares means (SE_{LSM}), the significance of the influence of paragenetic factors and coefficients of determination (R^2) for milk production per milking, productive and lifetime day

Paragenetic influences	n	MPMD, kg LSM±SE_{LSM}	MPPD, kg LSM±SE_{LSM}	MPLD, kg LSM±SE_{LSM}
General average- μ	2548	12.79±0.041	9.31±0.05	5.47±0.04
Rearing area (A)				
Zlatibor	502	13.32±0.10^{a}	10.44±0.11^{a}	6.35±0.04^{a}
Dobričevo	956	13.31±0.07^{a}	9.08±0.08^{b}	5.35±0.10^{b}
Kotraža	1090	12.10±0.05^{b}	8.99±0.05^{b}	5.16±0.06^{c}
Season of Birth (B)				
Spring (1)	589	12.78±0.08	9.37±0.09	5.51±0,09
Summer (2)	727	12.84±0.08	9.34±0.08	5.54±0.07
Autumn (3)	570	12.80±0.09	9.30±0.10	5.33±0.09
Winter (4)	662	12.74±0.08	9.23±0.09	5.47±0.08
Year of Birth (C)				
1995	164	13.85±0.15^{a}	10.11±0.18^{a}	7.06±0.15^{a}
1996	129	13.69±0.17^{a}	10.18±0,19^{a}	6.61±0.15^{b}
1997	184	12.61±0.15^{ef}	9.42±0.17^{bc}	5.48±0.16^{c}
1998	169	13.22±0.19^{bc}	10.24±0.17^{a}	5.57±0.17^{c}
1999	246	12.44±0.15^{fg}	9.45±0.14^{b}	5.47±0.14^{c}
2000	250	11.79±0.13^{h}	9.08±0.12^{bcd}	4.94±0.13^{d}
2001	192	12.73±0.15^{def}	9.05±0.14^{cd}	5.67±0.15^{c}
2002	213	12.88±0.14^{cde}	9.37±0.13^{bc}	5.58±0.14^{c}
2003	187	13.42±0.13^{ab}	8.75±0.16^{de}	5.65±0.13^{c}
2004	167	13,00±0.15^{cd}	9.12±0.17^{bcd}	5.64±0.15^{c}
2005	172	13.10±0.13^{bc}	9.34±0.19^{bc}	5.56±0.16^{c}
2006	106	12.59±0.20^{ef}	9.03±0.29^{cd}	4.77±0.17^{d}
2007	168	12.45±0.14^{fg}	8.45±0.21^{e}	4.47±0.12^{e}
2008	201	12.17±0,11^{g}	9.01±0.16^{cd}	4.46±0.14^{e}

Table 1. (continue)

Total number of lactations (D)				
1	396	11.27±0.11^{e}	9.14±0.13^{c}	2.68±0.04^{h}
2	512	12.00±0.09^{d}	8.89±0,11^{c}	4.27±0.05^{g}
3	466	12.72±0.09^{c}	8.94±0.10^{c}	5.32±0.06^{f}
4	385	13.16±0.09^{b}	9.07±0.10^{c}	6.03±0.07^{e}
5	314	13.72±0.09^{a}	9.76±0.12^{c}	6.92±0.08^{d}
6	217	14.01±0.11^{a}	10.06±0.14^{bc}	7.52±0.10^{c}
7	143	14.07±0.13^{a}	10.05±0.15^{bc}	7.79±0.11^{b}
8	81	14.19±0.14^{a}	10.30±0.18^{ab}	8.15±0.14^{b}
9	23	14.37±0.35^{a}	11.06±0.43^{a}	8.98±0.34^{a}
10	11	13.64±0.21^{ab}	10.15±0.37^{abc}	8.36±0.28^{ab}
Age at fertilization (E)				
b_{xy}±S_b		0.247±0.172 *	-0.183±0.257 **	-0.100±0.598 **
Anova	**df**			
A	2	**	**	**
B	3	ns	ns	ns
C	13	**	**	**
D	9	**	**	**
E	1	*	**	**
Koef. deter. – R^2		0.330**	0.150**	0.725**

Mean values in columns marked with the same letters do not differ (P>0.05) based on the LSD test
F-test (Anova) and t-test (coeff. lin. regression-bxy): N.S. - P>0.05; * - P<0.05; ** - P<0.01;

The general average, corrected for the effect of observed paragenetic factors, for milk production per milking, productive and lifetime day was 12.79, 9.31 and 5.47 kg.

The ***breeding area*** or farm most often significantly affects the characteristics of life production due to different ways of rearing, nutrition, care, climatic conditions, age structure and size of the herd, as well as several other effects related to the way of work and management on the farm itself. In the conducted research, the breeding area had a very significant effect (P<0.01) on milk production, both by milking and by productive and lifetime days. The greatest manifestation of these traits was on the farm in Zlatibor due to the free-stall housing system and using grazing in the summer season. During the analysis of the effect of the breeding area on the performance of milk production by milking, productive and lifetime days, *Petrović et al. (2012; 2019)*, state its predominantly highly significant (P<0.01) effect on milk production per milking, productive and lifetime day.

The systematic effect of the ***season of birth*** of cows on longevity traits is due to changes in climatic conditions, primarily temperature and precipitation, which also causes changes in the way of housing (free-stall and tie-stall) and the health status of cows as the most important factors that determine the expression of these traits. The season of birth of cows in our research did not significantly affect (P>0.05) any of the observed characteristics of life production.

The effect of the ***year of birth*** on the expression of longevity traits is manifested through different climatic conditions by year, in which the throat had production, and through them the quality and quantity of available food. In addition, breeding technology and the level of health care have been improving over the years, and during each year more or less annual selection success has been achieved. In the conducted research, the year of birth had a very significant (P<0.01) effect on the manifestation of the observed characteristics of life production (milk production per milking, productive and lifetime day). Despite the improvement of breeding technology and the level of health care in our research, the characteristics of life production by year of birth had a slightly decreasing trend. Increased selection criteria and intensification of production contributed to this, which directly affected the relationship between planned and unplanned exclusion, and thus the manifestation of life production characteristics and the profitability of milk production. The largest part of cow exclusion from production refers to unwanted exclusion, which can have a 2.5 times larger share compared to planned (*Pinedo et al., 2010*). According to several authors (*Nienartowicz-Zdrojewska et al., 2009; Chiumia, 2011; Ansari-Lari et al., 2012; Stojić et al., 2012*) the main reasons that lead to unplanned removal of cows from production reproductive disorders, mastitis, leg and hoof diseases and injuries.

The effect of the ***total number of lactations*** on longevity traits was very significant (P<0.01). The increase in the number of lactations, directly and indirectly, influenced the increase in the observed characteristics of life production. When considering at the research results of the effect of lactation on the reasons for exclusion in cows, it is observed that the dominant reasons for exclusion change according to lactation. Thus, the dominant reasons for the exclusion of first heifers are selection reasons (most often low production) and problems in reproduction (*Seegers et al., 1998; Stojić et al., 2012*). Of the total number of cows excluded during one year, first-calves accounted for 20 to 35% (*Maher et al., 2008; Pinedo et al., 2010; Chiumia, 2011*). Also when it comes to heifers, it was established that heifers that later became pregnant for the first time are excluded earlier, due to a higher risk of low production, as well as those with less pronounced typical characteristics (*Dürr, 1997*).

The ***regression effect of age at first insemination*** was highly significant (P<0.01) in milk production per productive and lifetime day, while its effect on milk production per milking day was significant (P<0.05). The linear regression coefficient for milk production per milking day was b_{xy}= 0.247, while for milk production per productive and lifetime day, it was negative and amounted to b_{xy}=-0.183 and b_{xy}=-0.100.

The ***coefficients of determination***, which indicate the level of explanation of variation in the observed characteristics of life production by the applied model, were highly significant, which indicates an adequate selection of paragenetic factors in the applied model. Their value was very different and ranged from only 0.150 (15%) for milk production per productive day to 0.725 (72.5%) for milk production per lifetime day.

Conclusion

Based on the applied model for analyzing the effect of systematic environmental factors and the age of cows at first insemination on the expression and variability of milk production per milking, productive and lifetime day, it can be concluded:

- The general average for milk production per milking productive and lifetime day was 12.79, 9.31 and 5.47 kg.
- The effect of breeding area, year of birth and lactation group on milk production per milking, productive and lifetime day was very significant ($P<0.01$), while the effect of the season of birth of cows was not significant ($P>0.05$).
- Age at first insemination, as a continuous factor, had a highly significant ($P<0.01$) effect on milk production per productive and lifetime day and a significant ($P<0.05$) on milk production per milking day.
- The calculated coefficients of determination (R^2) were highly significant based on the applied model, for all longevity traits they were very significant ($P<0.01$) and ranged from 0.150 (15%) for milk production per productive day to 0.725 (72.5%) in milk production per day of life.

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THE EFFECT OF MASTITIS PREVALENCE RISK ON THE DAILY PRODUCTION OF DAIRY COWS CONCERNING THE MILK RECORDING YEAR

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Abstract: Aiming determination of the mastitis prevalence risk in the dairy cattle population as well as the effect of mastitis prevalence risk on milk production (daily milk, fat and protein yield) at successive milk recordings concerning the year of milk recording test-day records of dairy Simmental and Holsteins collected from 2005 to 2022 were analysed. The obtained results indicate the variability in mastitis prevalence risk as well as the differences in the effect of mastitis risk due to the milk recording year. Furthermore, higher prevalence risk was determined in the Simmental breed as well as the lower increase of daily milk yield at the first successive milk recording. Furthermore, in both breeds there is a visible trend of increasing percentage of healthy animals in the past 6 - 7 years, most likely caused by improved management and preventive measures at dairy cow farms. The determined results indicate that the daily lactose content can operate as an excellent indicator of a possible mastitis problem on dairy farms and prevent a set of various costs without any additional investments on the farm except for employee education enabling more efficient and environmentally sustainable dairy cattle farms.

Key words: milk production, daily lactose content, mastitis prevalence risk, dairy cattle

Introduction

Mastitis represents one of the most frequent and expensive disorders in dairy cattle implying the inflammation of mammary glands. It causes physical changes in the udder, such as swelling, warmness, redness, pain, and necrosis, and results in a decrease in milk production and deterioration of animals' health status. Mastitis could be caused by bacterial infections (*Staphylococcus aureus, Streptococcus species, Escherichia coli*, and others); non-infectious factors (mechanical injury to the udder, irritation, or hormonal imbalances); and environmental factors (insufficient hygiene, dirty or wet bedding, unsanitary milking, and others).

Furthermore, the inflammation of the udder parenchyma of dairy animals could appear in clinical or subclinical form and is correlated with increased use of antibiotics leading to possible resistance of the causative agent *(Ebrahimi et al., 2019)*. Regardless of the form, mastitis causes significant economic losses due to quality deterioration and reduced milk production *(Halasa et al., 2007).* Furthermore, *Özkan Gülzari et al. (2018)* pointed out the environmental impact of mastitis prevalence reporting that prevention and management of subclinical mastitis results in the lowering of the greenhouse gas emissions per kg of produced milk on a dairy farm consequently enabling improved profits for the farmers due to declines in milk losses, optimization of culling rate and decreased feed and other variable costs.

Therefore, in order to enable economically and environmentally efficient dairy farms it is necessary to develop efficient ways of monitoring dairy herd and prevention of mastitis prevalence. *Pyorala (2003)* emphasized that except for somatic cell count, daily lactose content could be used as an adequate indicator of mastitis prevalence. *Silanikove et al. (2014)* stated that the inflammation in the mammary gland causes cell damage and decreased synthesis of lactose. *Babnik et al. (2004)* defined that daily lactose content lower than 4.5% indicates the risk of mastitis prevalence.

Since milk production is highly variable due to various genetic and environmental effects, this research aimed to determine the mastitis prevalence risk (defined accordingly to the daily lactose content) in the dairy cattle population as well as the effect of mastitis prevalence risk on milk production (daily milk, fat and protein yield) at successive milk recordings concerning the year of milk recording.

Materials and Methods

Test-day records of dairy Simmental and Holstein cows bred in Croatia gathered from January / 2005 to December / 2022 were statistically analysed. Data were collected during regular milk recording that is performed accordingly to the alternative milk recording method (AT4 / BT4; measuring of milk yield and milk sampling occur during evening or morning milking every four weeks). Milk samples were analysed in the Central Laboratory for Milk Quality Control

(Croatian Agency for Agriculture and Food) on the Milcoscan FT6000 applying infrared spectrophotometry for determination of lactose content in milk. Logical control of test-day records included correction of the following variables: stage of lactation (5 - 300 days), parity in (1 - 10), and age at first calving in (21 - 36 months). Test-day records with missing data regarding stage of lactation, parity, age, breed, and missing or meaningless values of daily milk yield in line with ICAR standards *(ICAR, 2017)* were deleted from the dataset. After logical control, dataset contained 4,922,751 test-day records of Simmental and 3,953,637 test-day records of Holstein cows.

The mastitis prevalence risk was defined accordingly to the daily lactose content (DLC), whereby DLC ≥ 4.5% indicates healthy animals, while DLC < 4.5% indicates the mastitis prevalence risk. The mastitis prevalence risk in the population of dairy cows (Holstein and Simmental breeds) was defined as the percentage (%) of cows at risk from the total number of animals. Furthermore, the mastitis prevalence risk was calculated separately by the milk recording year. The analysis of the effect of mastitis prevalence risk on daily milk production during successive milk recordings included only cows with a determined risk of mastitis (DLC < 4.5%). The daily milk yield measured on the day of the milk recording when the mastitis prevalence risk was determined was defined as a reference. Furthermore, the mastitis index was defined concerning the number of days after the confirmed risk as follows: D-0 = record on the milk control when the risk of mastitis is determined, A-1 = within 35 days, A-2 = between 36 and 70 days, A-3 = between 71 and 105 days, and A-4 = more than 105 days. The effect of the mastitis prevalence risk on the daily milk yields was analysed separately by the recording year and breed using the MIXED procedure of SAS (SAS Institute Inc., 2019) and following statistical model:

$$y_{ijklmno} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3\, ln(305/d_i) + b_4\, ln^2(305/d_i) + A_j +$$
$$+ R_m + T_n + e_{ijklmno}$$

Where:

y_{ijklm} = estimated daily milk yield;

μ = intercept;

b_1, b_2, b_3, b_4 = regression coefficient;

d_i = stage of lactation (i = 6 to 300 dan);

A_j = fixed effect of age at first calving j (j = 21 to 36 month) *only for first parity,

P_k = fixed effect of parity k (k = 1., 2., 3., ≥ 4);

S_l = fixed effect of milk recording season l (l = spring, summer, autumn, winter);

R_m = fixed effect of herd size m (m = 1, … 6),

T_n = fixed effect of mastitis index n (n = D–0, A–1, A–2, A–3, A–4);
e_{ijklm} = residual.

The differences in milk production (milk, fat, and protein) during successive milk recordings after detection of the mastitis risk are equal to the product of the difference between the estimated daily yields (LSMeans) during the next and previous recording and the interval (in days) between these milk recordings. Estimated differences in daily milk yields (milk, fat and protein, in kg) at successive milk recordings after the detection of mastitis prevalence risk was presented separately by each breed and year of milk recording.

Results and Discussion

The prevalence of healthy and cows at mastitis risk concerning the year of milk recording for the Simmental breed in the period from 2005 to 2022 is presented in Figure 1. Based on the conducted analysis, a noticeable variability of prevalence is perceptible depending on the year of milk recording. The highest percentage of cows at mastitis risk was determined in 2013 (55.86%), while the lowest prevalence risk was observed in the year 2022 (32.81%) when there were more than 67% of healthy cows.

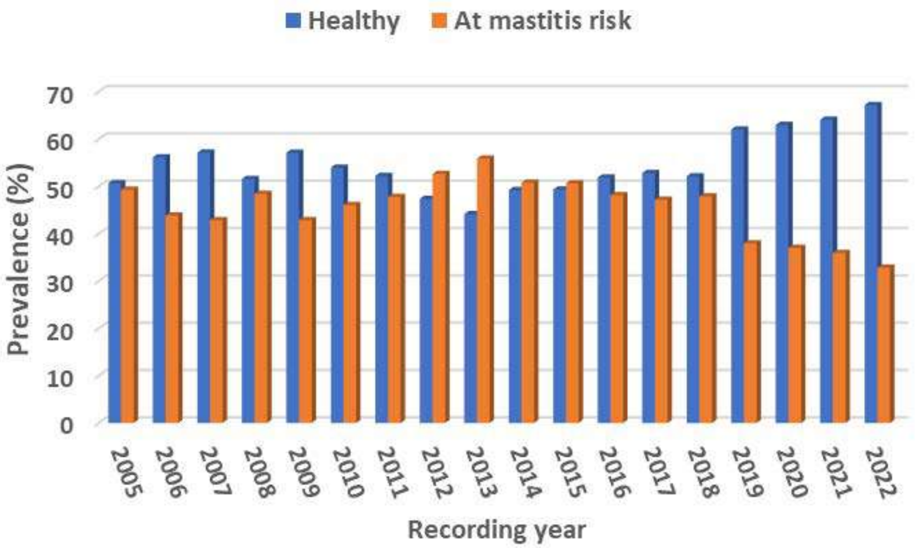

Figure 1. Prevalence of healthy and cows at mastitis risk in regard to year of milk recording for Simmental breed

The prevalence of healthy and cows at mastitis risk accordingly to the year of milk recording for Holstein breed in the period from 2005 to 2022 is presented in Figure 2. The highest percentage of healthy animals was determined in 2022 (69.65%), while the highest prevalence risk was observed in the year 2013 (55.25%).

Comparing the risk prevalence results for both breeds, the highest risk is visible in 2013 in both breeds and the highest proportion of healthy animals in 2022. Furthermore, in both breeds there is a visible trend of decreasing the risk of mastitis in the past 6-7 years, most likely caused by improved management and preventive measures at dairy cow farms. In addition, a lower prevalence of risk is visible in the Holstein breed, which, although it is significantly more demanding and sensitive compared to the Simmental, is mostly bred on farms with better technologies and far greater investments in the knowledge and training of employees.

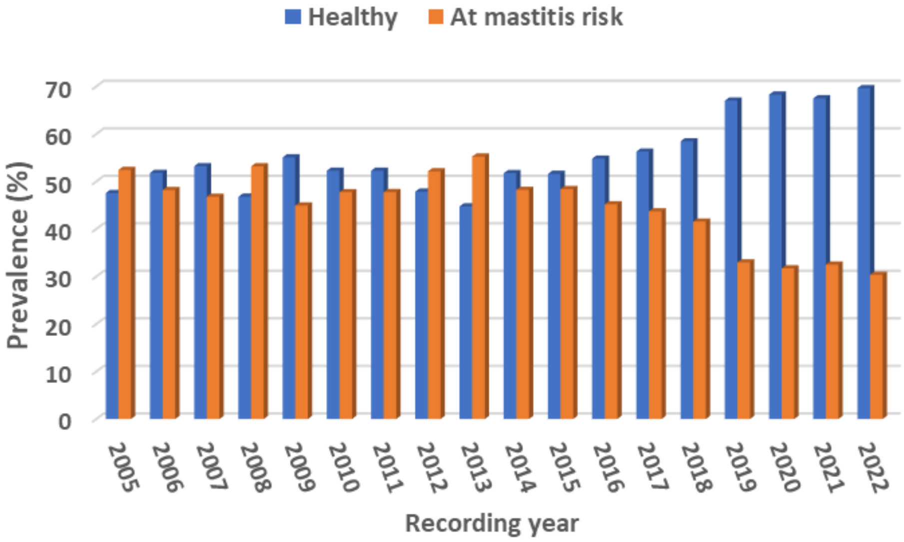

Figure 2. Prevalence of healthy and cows at mastitis risk in regard to year of milk recording for Holstein breed

Estimated differences in daily milk production: milk, fat, and protein yield (kg) at successive milk recordings after the detection of mastitis prevalence risk for the Simmental breed is presented in Table 1. Analysis of the differences at subsequent production showed high variability due to the recording year and the time after the first detection. At the first successive milk recording, after the prevalence risk was detected, an increase in daily milk yield in the amount from 1.49 kg/day (2005) to 20.66 kg/day (2010) was determined. Daily fat yield decreased in all analysed years, except for 2010 (0.03). The determined difference in milk fat yield amounted from -0.28 kg/day in 2011 to -0.85 kg/day in 2009. Daily protein yield, at the first successive milk recording, varied from -0.16 kg/day (2009) to 0.52 kg/day (2010). During the following successive milk yield recordings (second, third, and fourth), a decrease in daily milk production was mostly recorded. The total estimated difference in daily milk production amounted from -4.15 kg/day (2019) to 32.20 kg/day (2005) for milk yield, from -1.28 kg/day (2019) to 0.02 kg/day (2005) from daily fat yield, and from -0.67 kg/day (2019) to 0.94 kg/day (2005) for daily protein yield.

Table 1. Estimated differences in daily milk production: milk, fat, and protein yield (kg) at successive milk recordings after the detection of mastitis prevalence risk for Simmental breed

Year	1. milk recording			2. milk recording			3. milk recording			4. milk recording			Total		
Trait	DMY	DFY	DPY	DMY	DFY	DPY	DMY	DFY	DPY	DMY	DFY	DPY	DMY	DFY	DPY
2005	1.49	-0.57	-0.11	-6.03	-0.27	-0.20	8.51	0.21	0.29	28.24	0.65	0.95	32.20	0.02	0.94
2006	4.43	-0.76	-0.07	-1.58	-0.05	-0.06	-1.04	-0.07	-0.08	1.18	0.11	0.04	2.99	-0.77	-0.17
2007	3.80	-0.80	-0.09	-2.03	0.02	-0.11	-2.01	-0.18	-0.07	-0.94	-0.06	-0.10	-1.18	-1.02	-0.37
2008	3.13	-0.76	-0.11	-0.89	0.01	-0.03	-1.51	-0.16	-0.08	-2.31	-0.11	-0.17	-1.58	-1.02	-0.39
2009	3.05	-0.85	-0.16	-2.16	0.02	-0.09	-1.28	-0.12	-0.07	-2.83	-0.10	-0.18	-3.22	-1.06	-0.50
2010	20.66	0.03	0.52	-20.88	-0.78	-0.75	-0.52	-0.02	-0.04	-1.47	-0.14	-0.14	-2.21	-0.90	-0.41
2011	11.91	-0.28	0.25	-9.97	-0.40	-0.39	-1.16	-0.07	-0.04	-3.12	-0.18	-0.17	-2.33	-0.94	-0.35
2012	4.68	-0.56	-0.07	-0.91	-0.06	-0.05	-0.56	-0.06	0.00	-4.69	-0.18	-0.28	-1.48	-0.87	-0.40
2013	5.69	-0.49	-0.05	-0.86	0.04	-0.04	-2.69	-0.13	-0.11	-4.47	-0.25	-0.26	-2.33	-0.84	-0.46
2014	7.43	-0.60	0.01	-2.57	-0.01	-0.08	-2.71	-0.14	-0.11	-3.93	-0.16	-0.21	-1.80	-0.91	-0.39
2015	7.58	-0.56	0.00	0.05	0.06	0.00	-3.60	-0.31	-0.16	-4.57	-0.15	-0.27	-0.55	-0.96	-0.43
2016	10.50	-0.36	0.10	-0.97	-0.11	-0.07	-4.05	-0.22	-0.17	-2.69	-0.16	-0.21	2.79	-0.84	-0.35
2017	11.28	-0.46	0.10	-3.31	-0.01	-0.11	-3.01	-0.18	-0.13	-4.43	-0.18	-0.27	0.54	-0.82	-0.41
2018	11.44	-0.51	0.10	2.51	0.22	0.05	-4.75	-0.28	-0.17	-4.23	-0.20	-0.26	4.96	-0.76	-0.28
2019	8.61	-0.62	-0.02	-2.40	-0.13	-0.12	-3.86	-0.22	-0.19	-6.50	-0.31	-0.34	-4.15	-1.28	-0.67
2020	8.05	-0.64	-0.01	-3.43	-0.08	-0.16	-1.71	-0.12	-0.05	-4.15	-0.29	-0.27	-1.24	-1.13	-0.49
2021	14.49	-0.46	0.14	-1.15	-0.02	-0.06	-4.51	-0.13	-0.17	-2.97	-0.21	-0.24	5.86	-0.81	-0.33
2022	13.74	-0.48	0.18	0.76	0.31	0.01	-4.87	-0.33	-0.14	-3.76	-0.21	-0.30	5.87	-0.72	-0.24

*Year – year of milk recording; DMY – daily milk yield (kg); DFY – daily fat yield (kg); DPY – daily protein yield (kg)

Estimated differences in daily milk production: milk, fat, and protein yield (kg) at successive milk recordings after the detection of mastitis prevalence risk for the Holstein breed is presented in Table 2.

Table 2. Estimated differences in daily milk production: milk, fat, and protein yield (kg) at successive milk recordings after the detection of mastitis prevalence risk for Holstein breed

Year	1. milk recording			2. milk recording			3. milk recording			4. milk recording			Total		
Trait	DMY	DFY	DPY	DMY	DFY	DPY	DMY	DFY	DPY	DMY	DFY	DPY	DMY	DFY	DPY
2005	8.39	-0.48	0.09	-15.18	-0.54	-0.54	-2.61	-0.52	-0.23	-19.16	-0.27	-0.48	-28.56	-1.80	-1.17
2006	7.04	-0.91	-0.06	2.23	0.32	-0.04	-5.49	-0.15	-0.18	10.64	0.40	0.16	14.42	-0.34	-0.11
2007	14.35	-0.70	0.14	-1.17	-0.06	-0.05	0.02	0.13	-0.01	5.35	0.05	0.01	18.54	-0.59	0.09
2008	12.55	-0.79	0.03	5.09	0.36	0.14	-2.61	-0.14	-0.07	2.27	0.07	-0.10	17.31	-0.50	0.00
2009	12.34	-1.02	0.06	0.88	0.04	-0.05	0.96	-0.02	0.03	5.90	0.17	0.05	20.08	-0.82	0.09
2010	18.65	-0.69	0.26	-7.65	-0.03	-0.25	-3.48	-0.11	-0.14	0.98	-0.14	-0.17	8.49	-0.97	-0.30
2011	17.99	-0.71	0.33	-3.08	0.02	-0.12	0.11	-0.04	0.00	3.96	0.00	0.01	18.98	-0.73	0.20
2012	18.40	-0.74	0.28	0.47	0.10	0.08	0.33	-0.08	-0.06	1.98	-0.04	-0.12	21.18	-0.76	0.17
2013	20.80	-0.65	0.26	3.58	0.23	0.07	1.81	0.06	0.05	-0.22	-0.17	-0.21	25.98	-0.53	0.16
2014	20.26	-0.78	0.35	6.58	0.54	0.23	0.29	-0.06	0.00	6.50	0.12	0.01	33.63	-0.18	0.58
2015	25.82	-0.41	0.55	2.86	0.18	0.02	1.25	0.09	0.01	2.63	0.01	-0.15	32.56	-0.14	0.43
2016	21.70	-0.75	0.41	3.13	0.10	0.11	-0.38	0.10	-0.02	10.21	0.16	0.17	34.66	-0.39	0.66
2017	27.71	-1.10	0.67	-0.65	0.08	-0.11	-3.83	-0.09	-0.12	1.75	0.00	-0.09	24.97	-1.11	0.34
2018	24.01	-1.26	0.54	9.07	0.35	0.25	-6.39	-0.22	-0.23	6.87	0.20	0.02	33.56	-0.93	0.57
2019	28.88	-0.64	0.74	0.94	0.10	0.00	0.34	0.00	-0.01	11.17	0.41	0.20	41.33	-0.13	0.93
2020	27.72	-0.73	0.67	3.94	0.31	0.10	5.21	0.17	0.21	6.51	0.17	0.03	43.38	-0.09	1.01
2021	24.57	-0.99	0.63	4.75	0.28	0.27	-1.42	-0.11	-0.10	7.41	0.32	0.06	35.32	-0.50	0.86
2022	36.79	-0.61	1.11	-0.82	-0.01	0.04	-1.76	0.19	-0.14	14.14	0.54	0.36	48.34	0.12	-1.37

*Year – year of milk recording; DMY – daily milk yield (kg); DFY – daily fat yield (kg); DPY – daily protein yield (kg)

Similarly, to the Simmental breed, an increase in daily milk yield at the first successive milk recording was found in Holstein cows. The determined increase in daily milk yield amounted from 7.04 kg/day (2006) to 36.79 kg/day (2022). Daily fat yield, at the first subsequent milk recording, decreased in amount from -1.26 kg/day (2018) to -0.48 kg/day (2005). On the other hand, daily protein yield mainly increased in amount from 0.03 kg/day (2008) to 1.11 kg/day (2022), with the exception in the year 2006 when the decline was determined (-0.06 kg/day). In the following subsequent milk yield recordings (second, third, and fourth), an increase in daily milk production parameters was mainly recorded. The total estimated difference in daily milk production amounted from -28.56 kg/day (2005) to 48.34 kg/day (2022) for milk yield, from -1.80 kg/day (2005) to 0.12 kg/day (2022) from daily fat yield, and from -1.37 kg/day (2022) to 1.01 kg/day (2020) for daily protein yield.

The determined increase in daily milk yield at the first successive milk recording was higher in Holstein than in Simmental cows, in all analysed recording years. Also, the determined total difference (predominantly increase, except for 2005) was higher in Holsteins than in Simmentals. Furthermore, in both breeds, the total decrease in daily fat yield was mainly determined, while the total daily protein yield mainly decreased in Simmental and increased in Holstein cows.

Factors such as season, herd size, husbandry system, average milk yield per cow, and somatic cell counts, may be related to pathogens that cause mastitis, and to the prevalence rate of clinical mastitis cases in dairy herds *(Tomazi et al., 2018)*. The milk lactose content could be an indicator of the health of the mammary gland, since it is more sensitive to increases in SCC than other components, which is a consequence of the leakage of the blood-milk barrier, as well as the reduced ability to synthesize secretory cells *(Nóbrega and Langoni, 2011).* So, the presence of intramammary inflammatory processes leads to a decrease in lactose production in the epithelial cells of the mammary gland, as well as an increase in the permeability of tight junctions between alveolar epithelial cells, which results in the loss of lactose and its passage into the bloodstream. *Costa et al. (2019)* reported that cows with a lactose content of ≤4.553% had a higher rate of health damage compared to cows with a lactose content of ≥5.045%. They also stated that subclinical mastitis is genetically related to lactose in milk and that more productive cows are genetically more susceptible to mastitis than less productive cows. The obtained results indicate the variability in mastitis prevalence risk as well as the difference in the effect of mastitis risk due to the year of milk recording. The effect of season on the incidence of cows with intramammary infections was confirmed by *Nóbrega and Langoni (2011).* While *Gantner et al. (2011)* and *Weber et al. (2020)* confirm changes in milk composition, somatic cell counts, and mastitis prevalence due to environmental changes.

Conclusion

The obtained results indicate the variability in mastitis prevalence risk as well as the differences in the effect of mastitis risk due to the milk recording year. Also, the differences regarding the breed were observed. Observed could indicate a faster recovery of Holstein compared to Simmental cows. Further, in both breeds there was a visible trend of increasing percentage of healthy animals in the past 6 – 7 years, most likely caused by improved management and preventive measures at dairy cow farms. The determined results indicate that the daily lactose content could operate as an indicator of a possible mastitis problem on dairy farms and prevent a set of various costs without any additional investments on the farm except for employee education enabling more efficient and environmentally sustainable dairy cattle farms.

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MEDICINAL AND AROMATIC PLANTS IN LIVESTOCK FARMING: A PROMISING APPROACH FOR BOOSTING HEALTH AND PERFORMANCE

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Abstract: In recent times, there has been a revived interest in utilizing medicinal and aromatic plants (MAPs) for the treatment of diseases and enhancing the productivity of farm animals. Thanks to their bioactive compounds, MAPs can enhance the immune system, reduce the risk of disease and improve overall health and welfare. This is especially important in the post-antibiotic era, where the search for alternative options is becoming increasingly urgent. Although the mode of their action is still unclear, today it is known that MAPs can modulate the microbiota and promote gut health, leading to improved digestion and nutrient absorption. Some components of MAPs act against viruses, bacteria and parasites. Additionaly, the use of MAPs in livestock farming can also have environmental benefits. Livestock methane emissions are a significant contributor to greenhouse gas levels. Recent studies have shown that certain bioactive compounds found in MAPs could inhibit methanogenesis and reduce the number of methanogenic microbes in animal gastrointestinal tracts. As a result, researchers are exploring the potential of MAPs in methane reduction strategies. Plants could also play a prominent role in obtaining functional and enriched foods to contribute to animal product quality and human health in general. This review highlights the importance and potential of using medicinal plants in farm animal breeding, particularly in the context of the One Health concept, which acknowledges the interconnectedness of human, animal, and environmental health.

Key words: medicinal plants, livestock, health, performance, phyto additives, ethnoveterinary medicine

Introduction

Throughout history, herbs have been utilized for their medicinal properties and have played a significant role in promoting health and well-being. Furtheremore, they have always been a primary source of drugs and treatment strategies in various traditional medicinal systems. Determining the exact time when herbs were first used for medicinal purposes is challenging, but carbon dating from ancient Babylon (Iraq) suggests that they were being cultivated for medicinal purposes as far back as 60,000 years ago *(Qazi and Molvi, 2016).* A Sumerian clay tablet discovered in Nippur, dating back nearly 5000 years, holds the earliest recorded evidence of using medicinal plants for creating medicines and for treatment purposes. This tablet includes 12 recipes for making medicines and mentions over 250 different plants, including notable alkaloid-containing plants such as poppy, and mandrake *(Wong, 1971).* This finding provides valuable insight into the rich tradition and utilization of medicinal plants in ancient civilizations, shedding light on how people utilized plants for improving health and treating ailments thousands of years ago. Today, the term Medicinal and Aromatic plants (MAPs) is most often used for all plants used for this purpose.

The objective of this review is to present the latest knowledge about the importance and potential of using medicinal plants in the breeding of farm animals with a particular focus on preserving their health, suppressing diseases, improving production performance, and reducing methane emissions. In addition, the potential and effects of their use in supporting the One Health concept and the enrichment of foods of animal origin were discussed.

Translating tradition into modern livestock farming

For many medicines of plant origin, which were known by the oldest human civilizations and which were used throughout the millennia until today, modern science has confirmed their active action and included them in pharmacotherapy. Recognizing the importance of traditional medicine, the World Health Organization (WHO) acknowledges the need to explore and utilize ancient medicinal practices to meet the primary healthcare requirements of animals. The WHO also acknowledges that traditional medicine can play a crucial role in the advancement of livestock in third-world countries *(WHO, 2008).*

The use of medicinal plants in animal husbandry has so far been most widely used in organic production. Although, the EC Regulation for Organic Farming states that organic livestock should be treated preferably with phytotherapeutic products, only few registered herbal veterinary medicinal products currently exist on the European market *(Mayer et al., 2014).* In addition to the growing need for natural products, there are other indications that encourage the use of medicinal plants. First of all, these are national and international efforts to combat antimicrobial resistance and promote the One Health approach. Nowadays promoting "clean, green, and ethical" animal production that limits the use of drugs, chemicals, and hormones while considering animal welfare and reducing the environmental impact of food production presents challenges to livestock production systems *(Ostojić Andrić et al., 2018).*

Antibiotic growth promoters have been banned in livestock production in the European Union since 2006. due to concerns about the transmission and spread of resistant bacteria in the food chain. Therefore, alternative feed strategies using botanicals, such as aromatic plant extracts, have been investigated. These plant products have advantages over commercial antibiotics because they are residue-free and generally considered safe. Furthermore, they can be used as alternatives to synthetic drugs, because of their potent properties and more complex bioactivity *(Kuralkar and Kuralkar, 2021).* Bioactive plants and botanicals have also gained attention in recent years as potential growth promoters for animals. They may be helpful in some aspects of the proposed approach because they are often inexpensive and considered environmentally safe *(Hashemi and Davoodi, 2011).* Although the use of medicinal plants has a long tradition in Serbia, the works of the 19th-century folk educator and pedagogue Vasa Pelagić (1839-1899) are considered the first important reading in which traditional and scientific knowledge was collected and presented on the appearance, purpose and method of use in the treatment of people as well as in the breeding of farm animals. Recently, with the increased studies on this topic around the world, the interest of researchers in Serbia has also increased.

Summarized data from different studies have shown that about 700 wild growing species have been used as medicinal plants in Serbia *(Zlatković et al., 2014).* Research on the use of medicinal plants in farm animals is not so common in Serbia and is predominantly reliant on information gathered from surveyed respondents who possess knowledge of traditional phytotherapy practices. From the recent literature, we can single out studies that investigated the knowledge of the practice and tradition of healing with medicinal plants in the area of Pirot *(Matejić et al., 2020; Marković et al., 2021),* Negotin *(Janaćković et al., 2019),* Suva mountain *(Jarić et al., 2015),* Kopaonik *(Jarić et al., 2007),* Rtanj *(Zlatković et al., 2014).* Until recently, the use of medicinal plants in veterinary medicine was mostly based on results obtained in human medicine. However, it is encouraging that the number of clinical studies on the effects of using medicinal plants in veterinary medicine is gradually increasing. Some of the studies investigated the effects of application of plants and their preparations on health and production performance of farm animals *(Runjaic-Antic et al., 2010; Davidović et al., 2011;*

Kostadinović and Lević, 2018; Pajić et al., 2019; Drašković et al., 2020; Petričević et al. , 2021; Kurčubić et al., 2019; 2023).

MAPs and their bioactive compounds

According to the State of the World's Plants report *(Royal Botanic Gardens, 2016)*, there are approximately 391,000 plant species on Earth. Out of this vast number, it is believed that 35,000 to 70,000 plant species have been utilized for medicinal purposes, as reported by the World Health Organization (WHO) in 1998. The use of herbal products has grown significantly worldwide.

Herbs have an inherent ability to generate a diverse range of chemical substances through their regular metabolic activities and each herb has its own distinct combination and characteristics. These substances can be categorized into two main groups: primary metabolites, including sugars and fats, which are present in all plants, and secondary metabolites (PSMs), also referred to as phytobiotics or phytochemicals, which are specific to certain plant species *(Hashemi and Davoodi, 2011).* PSMs are bioactive compounds that may have beneficial or adverse effects but in comparison to inorganic chemicals, products derived from plants exhibit natural characteristics, lower toxicity levels, absence of residues, and have found extensive applications in traditional as well as alternative veterinary medicine. Furthermore, investigation of medicinal plants has led to the isolation of many PSMs which have become well-known pharmaceuticals (acetylsalicylic acid, morphine, digitoxin, quinine etc.).

The most important bioactive compounds present in herbs include alkaloids, glycosides, flavonoids, phenolic acids, saponin, tannins, terpenes, anthraquinones, essential oils and steroids. They act as antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, anticoccidial and growth promoters *(Kostadinović and Lević, 2018).*

Utilization of MAPs-most frequent form of use and mode of action

The MAPs can be utilize in various forms. Sometimes the plants are used fresh or made into poultices. Far more common is the use of their extracts combined with other substances and administration in the form of drinks, through feed, or applied to the skin. The most important forms in the narrower sense include the use of whole plants or parts of plants (botanicals), essential oils, and phytoadditives.

Botanicals refer to the different parts of plants that are processed or used in their entirety. When herbs are used in their entirety, including the leaves and other parts, and combined with other herbs, their active ingredients work together in harmony, resulting in the desired effects being achieved naturally *(Kuralkar and Kuralkar, 2021).* Botanicals work by disrupting the cell membrane of microbes, interfering with their virulence properties, and stimulating the growth of beneficial bacteria in the gut. They also act as immunostimulants, protect the intestine from microbial attack, and enhance the production and activity of digestive enzymes. Additionally, botanicals stimulate the growth of absorptive cells in the gastrointestinal tract *(Jamroz et al., 2003; Vidanarachchi et al., 2010).*

Essential oils are liquids that have a strong scent and are extracted from different parts of plants such as leaves, flowers, and seeds. They possess various properties such as antimicrobial, anti-inflammatory, antioxidative, and coccidiostatic that can benefit health. Essential oils can improve digestion and immunity, prevent the growth of harmful bacteria in the gut, and reduce odour and ammonia *(Omonijo et al., 2018; Diniz do Nascimento et al., 2020).* They work by increasing the release of digestive enzymes and decreasing the availability of nutrients for bacteria in the gut *(Omonijo et al., 2018; Reyer et al., 2017).*

The ban on the use of antibiotic growth promoters (AGP), applied in the European Union since 2006, has stimulated interest in the use of plant extracts, known as *phytogenic additives - PFA* (phyto additives, phytobiotics). PFAs is used to improve production outcomes and health in livestock farming. These additives act as antioxidants, metabolism enhancers, and regulators of the growth of pathogenic microorganisms such as bacteria and fungi *(Stevanović et al., 2018).* Moreover, their use supports the concept of sustainable agriculture and the production of healthier food. In order to be a viable alternative additive to antibiotic growth promoters, PFAs must provide similar advantages which means they should act like them: increase growth, improve feed efficiency, and reduce the occurrence of certain diseases.

MAPs for boosting livestock performance

The use of herbal and plant-based remedies has become more prevalent in livestock production. This shift can be attributed to various reasons, such as the negative effects of conventional synthetic drugs, the increasing expenses of traditional treatments, worries about harmful residues in food, the growth of microbial resistance, and the establishment of organic livestock production systems.

In terms of boosting livestock performance phytogenic additives -PFAs are the most common form of MAPs utilization. Research on the effect of phytoadditives in the nutrition of farm animals is most numerous in poultry. PFAs are shown to enhance feed efficiency and increase weight gain *(Zhang et al., 2009;*

Khattak et al., 2014), reduce pathogenic load *(Engida et al., 2023),* booster immune system and enhance meat quality characteristics in poultry *(Mnisi et al., 2023).*

There is an extensive collection of literature available on the impact of herbal feed supplements on the growth and meat quality of broiler chickens and pigs *(Jamroz et al., 2003; Vidanarachchi et al., 2010; Pajić et al., 2019; Petričević et al., 2021).* On the other hand, research on the effects of natural feed supplements on growth, immunity, and antioxidant status of ruminants is limited, possibly due to their complex digestive system.

Livestock production heavily relies on nutrient digestibility for optimal results. The addition of PFAs to livestock feed may improve digestibility through various mechanisms *(Reyer et al., 2017; Stevanović et al., 2018).* These mechanisms include increasing appetite, promoting saliva and intestinal mucus production, enhancing bile acid secretion, and boosting the activity of digestive enzymes like trypsin and amylase *(Oso et al., 2019).* PFAs may also have positive effects on intestinal morphology and exert local effects at the intestinal border, as well as induce systemic changes in macronutrient metabolism. The combination of these different actions leads to the observed improvement in nutrient digestibility when PFAs are added to feed.

The performance of farm animals is commonly understood to be closely associated with the health and function of their gut, which is influenced by various factors including diet, intestinal integrity, gut microbiome, and immune system of animals. In order to promote the growth of beneficial bacteria, and stimulate digestion and absorption, numerous feed additives have been developed *(Ghasemi et al., 2014; Abdelli et al., 2021).*

As mentioned previously, many experimental findings suggest that using traditional herbal medicines as supplements can improve feed to gain ratio and benefit animal health. However, conflicting research findings highlight the necessity for further investigation to ascertain optimal dosage and mechanisms of action. The biological effects of phytogenic feed additives can vary significantly depending on the experimental methods used to evaluate their suitability as growth-promoting feed additives for animals. To test the effectiveness of herbal feed additives on animal production, it is crucial to carefully select the appropriate traditional medicinal plants with bioactive compounds and determine the appropriate dietary doses *(Bostami et al., 2021).*

MAPs for curing, health promotion, disease prevention and management

The utilization of medicinal plants extends beyond human disease treatment and is also widely employed for addressing animal ailments. In fact, as revealed by *Marković et al. (2021)*, most of the herbal species are used to treat both veterinary and human diseases. Extracts and other natural substances in MAPs (plant derived bioactive compounds including lycopene, carotenoids, L-theanine, fucoidan, humic acid etc.), are used to treat various health conditions such as anxiety, depression, insomnia, pain, and inflammation *(Arain et al., 2018; Arif et al., 2019).* This type of complementary and alternative medicine is known as phytotherapy (also herbal medicine or botanical medicine) *(Nabi et al., 2023).* Nowadays, knowledge of phytotherapy is incorporated into ethnoveterinary medicine which encompasses the understanding, techniques, practices, and beliefs surrounding traditional health treatments. According to *Viegi et al. (2003)*, approximately 70% of animals treated with herbal remedies are cattle, horses, sheep, goats, and pigs, followed by poultry (9.1%), dogs (5.3%), and rabbits (4.3%). Although not the subject of this study, it is worth mentioning that animals possess a natural ability to self-medicate using herbs, which is referred to as zoo pharmacognosy.

Although the repertoire mechanisms of action of bioactive compounds in MAPs is not fully elucidated, one of their primary mode of action is related to their antimicrobial effects which allow controlling potential pathogens *(Mohammadi et al., 2018).*

Some studies have indicated that specific phytotherapeutic compounds may have antiviral properties, and could potentially be used as alternative or complementary treatments for viral infections in animals. For instance, certain compounds such as flavonoids, terpenes, and alkaloids found in different plants have demonstrated antiviral activity against various viruses, including influenza, herpes simplex virus, HIV, and coronavirus *(Setayesh et al., 2022).*

In the study conducted by *Pajic et al. (2019)*, the herbal preparation, which included essential oils derived from *Thymus vulgaris, Origanum vulgare, and Coriandrum sativum*, exhibited a potent anticoccidial effect and had a significant impact on oxidative stress parameters. Aromatic plants and their essential oils contain phenolic compounds that act as powerful natural antioxidants. These compounds, which include flavonoids, have the ability to efficiently eliminate free radicals and stop oxidative reactions, making them highly effective antioxidants.

Medicinal plants can serve as both, health promoters and remedies for various diseases. They are utilized in addressing a range of conditions, including infections like anthelmintic and acaricidal treatments, as well as in surgical, gynecological interventions, and bovine mastitis management *(Chakraborty and Pal, 2012).*

A study by *Schmid et al. (2012)* showed that breeders from 21 organic farms were in general satisfied with the outcome of MAPs usage in their animals (mostly cattle). Skin alterations and sores, gastrointestinal and metabolic diseases as well as infertility and diseases of the female genitalia were treated with homemade mono-species herbal remedies. The single most applied species were *Matricaria recutita L., Calendula officinalis L., Symphytum officinale L., and Coffea arabica L.*

Herbal products have the potential to improve the overall health and well-being of livestock, in addition to treating illnesses. They can enhance the immune system, aid in digestion, and supply necessary nutrients, which can reduce the likelihood of disease and promote optimal growth and performance. For instance, certain studies have demonstrated that compounds present in plants like echinacea, elderberry, and garlic possess antiviral properties and can enhance the immune response *(Nabi et al., 2023).*

More and more research has demonstrated that phytochemicals can enhance the body's ability to fight off microbial infections, leading to better health. Through interactions with immune system, PFAs can modulate immune responses through various mechanisms. One way is through dietary immunomodulation. This involves modulating the expression of cytokines, which are essential for both the adaptive and innate immune systems. Another way is through effects of some substances that can help combat antigenic challenges. The active components in black cumin for example, including thymol, nigellidine, nigellimine, thymoquinone, dithymoquinone, and thymohydroquinone have such pharmacological effects *(Ghasemi et al., 2014).*

Another type of potentially beneficial action of MAPs is the positive influence on the microbiota of the gastrointestinal tract (GIT). Many studies showed that the composition and metabolic activity of the GIT microbiota are key factors in enhancing immune response, reducing pathogen load in the GIT and promoting the growth of beneficial bacteria *(Ghasemi et al., 2014; Abdelli et al., 2021).* In this way, by including MAPs in the diet of farm animals, long-term benefits can be achieved in ensuring their health and well-being in general.

As a rational approach to livestock management, preventing diseases should be the primary focus, and maintaining animal health is crucial. Medicinal plants can also play a role in achieving this goal. Numerous herbal products have antimicrobial, antiparasitic, and antiviral properties, which can aid in preventing and managing various diseases in livestock. By incorporating herbal remedies into healthcare practices, farmers can decrease their reliance on antibiotics and other pharmaceutical interventions, minimizing the risks associated with drug resistance.

Some of the most important MAPs in Serbian ethnoveterinary medicine

The Serbia and Balkans region in general is renowned for its abundant biodiversity and traditional ecological knowledge within the broader context of Europe *(Shabih et al., 2022).* As mentioned earlier, studies on the use of MAPs in farm animals is not so common in Serbia and is predominantly reliant on information gathered from questionaries. The literature highlights studies on the use of medicinal plants for healing in various areas of Serbia, including Pirot, Negotin, Suva mountain, Kopaonik, and Rtanj *(Marković et al., 2021; Matejić et al., 2020; Janaćković et al., 2019; Jarić et al., 2015; 2007; Zlatković et al., 2014).*

Until recently, the use of medicinal plants in veterinary medicine was mostly based on results obtained in human medicine. However, it is encouraging that the number of clinical studies on the effects of using medicinal plants in veterinary medicine is gradually increasing *(Runjaic-Antic et al., 2010; Davidović et al., 2011; Kostadinović and Lević, 2018; Pajić et al., 2019; Drašković et al., 2020; Petričević et al., 2021; Kurčubić et al., 2019; 2023).*

According to the reviewed literature, one of the most commonly used plants for livestock in Serbia is *Hypericum perforatum L.* It has a very wide range of indications and is used for infectious diseases, digestive intoxications, diarrhea, skin diseases (wounds and burns) and many other animal diseases. Depending on the disease, this plant can be used fresh, but also in the form of water extract or oil.

The water extract of *Helleborus odorus* has antiseptic properties, so it is used as a disinfectant, while its specific effect is also reflected in lowering the temperature when applied externally in the ear.

Some herbs have shown their usefulness in relieving udder edema in cows: *Alium sativum L., Cichorium intybus L., Agrimonia eupatoria Ledeb. and, Potentila reptans L.* are mainly applied topically in the form of lining. *Calendula officinalis L. and Celosia argentea* are used in the treatment of dermatological changes in pigs.

MAPs for reducing methane emissions

Global warming is a major environmental problem and methane produced by ruminants contributes significantly to it. A feeding strategy that involves adding plant secondary metabolites is one way to reduce methane emissions in ruminants. They have the potential to affect ruminal fermentation and reduce the population of methanogenic bacteria and protozoa which are the main CH_4 –producing microbes.

Protozoa in the rumen are directly proportional to methane production, and reducing their population can decrease methane emissions.

Considering saponins can act as protozoan defaunators, while tannins can inhibit methanogen growth by reducing H_2 production during fibre digestion, some research has addressed the effects of these plant compounds on methanogenesis in ruminants. Study by *Antonius et al. (2023)* showed that supplementation of *M. oleifera, C. longa Linn., and P. timoriana* in ruminant diet effectively increased total gas production, dry matter and organic matter digestibility, and reduced CH_4 gas emissions and protozoa populations during rumen fermentation.

Some other bioactive compounds from plants, such as polyphenols and flavonoids also could be useful in strategy against ruminal methanogenesis. In vitro study by *Jo et al. (2022)* showed that the addition of extracts (containing polyphenols and flavonoids) from oriental MAPs *(Vitis vinifera L. and Rhus succedanea L.)* to ruminant diets can decrease methane production and the presence of methanogenic archaea.

While many studies have shown the potential benefits of using MAPs in methane emission reduction strategies, further research is needed to validate and implement their use in ruminant diets, as the current studies are primarily based on in vitro analysis. Additionally, a more precise selection of MAPs potentially inhibiting methanogenesis is necessary.

MAPs for functional and enriched foods

Functional food is a type of food that has been changed or created to offer extra health benefits beyond its basic nutritional value. These foods are often intended to improve specific functions in the body or enhance overall health. They may contain bioactive compounds like antioxidants, prebiotics, phytochemicals, or probiotics that can potentially benefit health. Enriched food, on the other hand, refers to products that have had certain nutrients added to increase their nutrient content beyond what is naturally present *(Castillo et al., 2018).*

Despite the new trends that reject the consumption of meat, milk and their derivatives, it is clear that their consumption provides multiple benefits in terms of nutrition and health. The benefits provided by foods of animal origin could be further enhanced by changes in the design of farm animal rations, including supplementation with natural plant extracts. Manipulation of the process of ruminal biohydrogenation through natural supplements could in the future be the basis of nutritional enrichment of milk and meat of ruminant origin. In this way, the antioxidant effect on the final products would be ensured, and the modern demands of consumers related to the quality and safety of foodstuffs would additionally be met.

Conclusion

The use of MAP in improving the health and performance of farm animals could have an increasing application. It fits into the modern one health concept and supports a holistic approach for the overall well-being of humanity. By embracing this approach, we can move towards a more sustainable model of livestock farming that benefits animals, humans, and the environment.

The advantages of using MAPs compared to conventional preparations in animal husbandry can be briefly described in the following words: availability, cost-effectiveness, harmlessness and efficiency. However, despite the legacy of tradition and new knowledge, this promising field of research has only just begun. The number of potentially medicinal plant species around the world is large, folk knowledge about the medicinal properties of plants is mostly preserved in less developed parts of the world or those that have traditionally relied on ethnomedicine such as India and China. However, in some regions, traditional plant-based treatments have been overlooked due to the rise of modern medicine and pharmacology. Despite this, there is an opportunity to revitalize and optimize the use of MAPs in animal husbandry worldwide, combining traditional principles with modern scientific knowledge and trends. Collecting traditional knowledge about medicinal plants from around the world is crucial to prevent it from being lost. Subsequent research should prioritize analyzing selected plants, detecting their active ingredients, and determining their effectiveness and dosage based on scientific principles.

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IMPROVING THE DEVELOPMENT OF THE COMPETITIVENESS OF PIG AND CATTLE PRODUCTION IN THE REPUBLIC OF CROATIA BY APPLYING GENERIC STRATEGIES

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Abstract: Livestock production should be considered from an existential point of view, because meat is an input that is used daily for human consumption. Statistical data show that the total livestock production does not meet the total demand for meat. Scientific research has shown that it is necessary to improve the competitiveness of production by implementing generic strategies that reduce costs, differentiate production and focus on a specific product. For this reason, it is necessary to maintain the competitiveness of pig and cattle production, as well as strive for continuous production growth. The aim of this paper was to show how the competitiveness of pig and cattle production in the Republic of Croatia can be increased by implementing business strategies. The emphasis was to show how tax relief contributes to the competitiveness of production. In the research results, a supply and demand curve for pigs and cattle was formed based on current prices and production volume, and quantitatively was shown how much are the differences when the tax burden is reduced. Raising the level of competitiveness of production, in addition to being significant for the company, is also very significant for the state, as it also increases the total GDP.

Key words: generic strategies, competitiveness, pig production, cattle production, tax relief

Introduction

Scientific research on the competitiveness of the economy is important for economic theory and economic policy, and accordingly, they should completely analyze the state of competitiveness of agricultural production in the Republic of Croatia. Research should cover the relationship between costs and prices, and put that relationship in relation to competitiveness. According to *Golubović and*

Džunić (2012), the international competitiveness of the national economy represents the ability of that economy to prevail and maintain part of the world market and to prevent excessive penetration of imports, all without the help of non-economic measures, with a satisfactory net effect in foreign exchange and ensuring a good standard of living of the population. Furthermore, strengthening economic factors of competitiveness (costs, input prices, national exchange rate, subsidies, export credits, tax credits) and strengthening technological factors of competitiveness (quality, design, delivery time, service) are of the greatest importance for agricultural production. The aforementioned factors should be focused on strengthening international competitiveness, given that the economy of the Republic of Croatia is open and there are various models to attract investments and develop the production and processing industry.

In order to improve competitiveness, it is necessary *(Burda and Viplos, 2012):*

- establish a diagnosis of current non-competitiveness and
- practically implement a valid strategy for achieving future competitiveness.

The ability of the national economy, that is, of a country, to successfully compete depends on the quantity and quality of resources with which it is supplied and on the efficiency with which these resources are used. The environment and a good economic policy can compensate for a certain shortage of domestic market resources through investment attraction models *(Porter and Stern, 2003).*

In this sense, it is possible to use three generic strategies *(Porter, 1990)*:

- Strategy of current institutional arrangement.
- The strategy of innovation of the institutional arrangement emphatically focused on the growth of competitiveness.
- Strategy for the development of the competitive capabilities of producers, the non-economic sector, and the entire social-economic fabric.

The ***strategy of the current institutional arrangement*** implies that the domestic market is open to the outside world, market mechanisms mainly regulate economic life, private ownership dominates the economic system, monetary and macroeconomic balance is maintained, investment is encouraged - primarily foreign direct investments, the economic and legal regime is improving, non-economic institutions are more and more directed to market arrangements with the economy, development, and other policies are not in the intended and strong interaction with explicit or implicit competitiveness policy because it is not considered necessary, the state (government and other state institutions) completely leaves the problem of competitiveness producers and their autonomous organization, competition, cooperation and the like *(Schuller and Lidbom, 2009).* The effectiveness of this strategy depends on the current state of competitiveness, the size of the domestic production system and market, the length of experience in a well-organized production environment, and the overall competitive national fabric - both economic and otherwise. This strategy can be applied by developed countries that have developed market mechanisms *(Cvetanović, 1999)*, which is not the case with Croatia.

The ***strategy of innovation of the institutional arrangement*** emphatically aimed at the growth of competitiveness is significantly larger, more diverse, more valid, and more modern than the content of the previous strategy. It can be well applied in those countries where a complete institutional system is arranged. The effectiveness of this strategy has not been confirmed to the expected extent, because competitiveness is an achievement that requires the following conditions to be maintained, constantly and predictably, simultaneously *(Stanković et al., 2007)*: a competitively oriented and powerful institutional arrangement (this strategy has this), the willingness to become and remain competitive, the ability to choose - create opportunities in which competitiveness can be achieved, the availability of the necessary resources (from material to managerial) to achieve competitiveness and a permanent commitment to achieving, maintaining and raising competitiveness. Given that Croatia is not a large market, that it does not have a well-developed domestic market, and that the opinion of experts is frequently neglected, the effectiveness of this strategy could not be used in the right way.

The ***strategy for the development of the competitive abilities*** of producers, the non-economic sector and the entire socio-economic fabric is obtained by upgrading the content of the previous strategy so that each, or most producers can, with acceptably low risk, recognize opportunities by using which they can achieve the necessary competitiveness, and harmonize these opportunities with their resource and other potentials (and vice versa) and that it possesses the ability of permanent behavior that ensures the competitiveness of products/services. The essence of this strategy is to find together a valid arrangement and environment and an overall capable producer *(Porter and Stern, 2003)*, i.e. to qualify both macro and micro level actors equally well. An overall capable producer, in addition to other characteristics, also possesses mental models that see reality undistorted and are capable of making and implementing good decisions in that reality *(Nijkamp and Siedschlag, 2011)*. In the present, with regard to global and additionally local radical changes, the largest number of decisions faced by businessmen concern changes to the existing state of affairs. The profit motivation of the producer is a necessary condition for the producer to be rational and willing to become competitive *(Krugman, 2009)*. Therefore, not all, not even the majority of producers motivated by profit, are internationally competitive. This strategy can be applied for the development of livestock production in Croatia for the reason that it implies systemic measures that interfere with the functioning of the political, social, economic and social sectors. Namely, it is necessary to define which products of livestock production have export capabilities, how the state can support this through its development role, which markets are interesting for export, how to develop the national brand and in what way, what are the interactions between this strategy, export policy, fiscal policy and monetary policy.

The aforementioned theoretical analysis represents the basis for conducting research with the aim of raising the level of competitiveness in the production of pigs and cattle in the Republic of Croatia by implementing generic strategies in the business process.

Materials and Methods

To conduct this research, the data of the Ministry of Agriculture of the Republic of Croatia, and the State Statistical Office for the year 2021 related to pigs and cattle were processed, and the key concepts were production volume, price movements, trade and taxation. The data are presented in the Table 1.

Table 1. Presentation of pig and cattle production in 2021 in the Republic of Croatia

Components	**Pig**	**Cattle**
Total pigs produced	971,000	427,600
Increase, or decrease in production compared to the previous year +/-	-6.0%	+1.1
Share of the Republic of Croatia in the EU in %	0.69	0.57
Share in total agricultural production in %	9.4	11.5
Self-sufficiency in %	59.0	47.0
Import	620,336	161,953
Export	365,604	72,256
Trade balance +/-	-254,732	-89,697
Price of live livestock per kg in EUR	1.45	1.96
Increase decrease in price compared to the previous year +/-	+2.4	+2.8
Average value added tax in %	13	13
Average product tax in %	18	18
Average income tax in %	5	5

The data in the table show that the production of pigs and cattle occupies a small share of the total European production, i.e. the values are below 1%, although the territory of the Republic of Croatia is abundant with fertile land suitable for the production of animal feed. Furthermore, observing the share of pig and cattle production in the total production, it is evident that it is on average 10%, and that the current volume of production is self-sufficient on average at 53%. The difference is settled from imports, which is why the balance of the trade balance is negative. One of the main factors contributing to these figures is fiscal policy and the level of tax rates. To determine economic policy measures, it is necessary to look at the demand and supply of pigs and cattle. Given that every sector faces limitations in terms of production volume, resource spending, and profit maximization, it is necessary to take a detailed look at all types of limitations that are imposed. The most common are technological, economic and market restrictions. The factors that affect the supply are output price, production costs, production volume, and income. Due to all the above, in the continuation of the research, a demand curve and a supply curve for pigs and cattle will be formed, and based on these data, the correlation calculation will show the intensity of the connection between production and taxation, and finally, the regression model will show how much the change of one variable affects the change of another variable. After summarizing the results, a business strategy will be proposed that could contribute to raising the level of competitiveness in the production of pigs and cattle.

Results and Discussion

According to the data from Table 1, a curve of demand and curve of supply for pigs and cattle was formed. The quantity was measured in thousands of tons.

Table 2. Calculation of the selling price of pig and cattle

Demand curve		**Supply curve**	
Pig	D = 980 – 15p	Pig	S = 870 + 20p
Cattle	D = 940 – 15p	Cattle	S = 935 + 20p

*D – demand, S – supply, P – price

Based on the above functions, the price of pigs was formed, which is 1.39 euros/kg, while the average price of cattle is 1.75 euros/kg. According to current prices, the need for pigs is 1,225,732 animals, and for cattle 517,600. The data refer to live animals.

However, on the one hand, the mentioned prices are not competitive in the European market, and on the other hand, they have a destructive effect on agricultural production. One of the ways to make the unit price more competitive is to lower the fiscal levies that burden it. If we start from the assumption that VAT on agricultural products will be reduced successively to 5% on agricultural products, the regression model will show in which direction the volume of production will move (Figure 1).

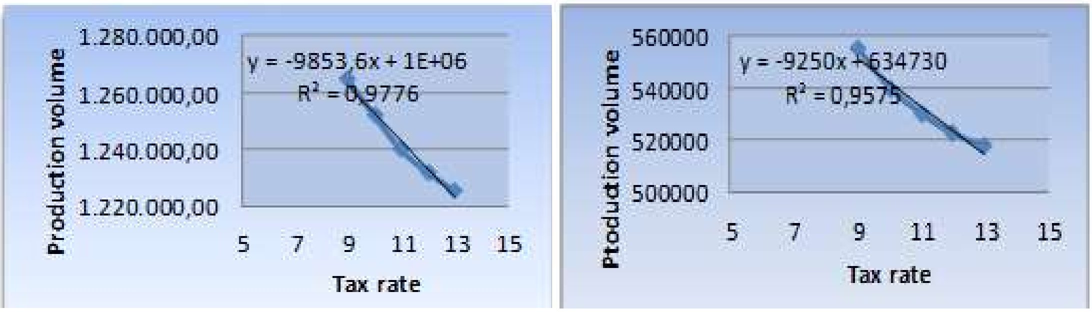

Figure 1. Regression model of the relationship between the tax rate and the volume of pig and cattle production

The regression model Y=bx+a show that if the tax rate were to increase by 1%, we can expect a decrease in the total volume of production by 9,853 pigs, or a decrease in the production of 9,250 cattle. The downward trajectory of the curve shows that each percentage reduction in the tax rate causes an average increase in the volume of production by 9.6%. Furthermore, it is also important to show how fiscal relief has a positive impact on production costs (Figure 2).

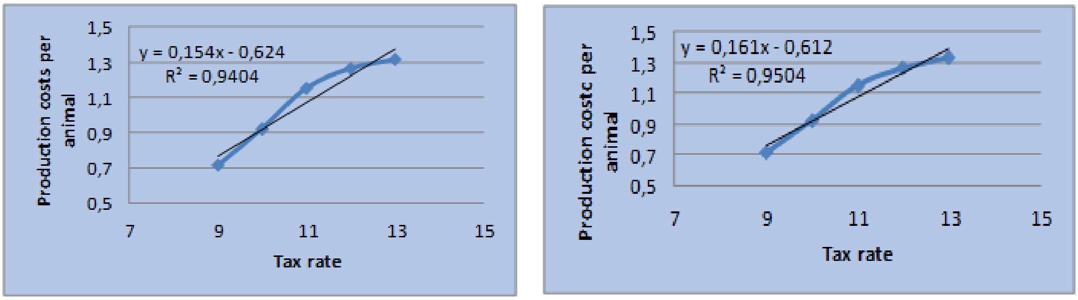

Figure 2. Regression model of the relationship between tax rate and production costs in pig and cattle production

The regression model Y=bx+a show that if the tax rate were to increase by 1%, it would cause an increase in production costs by 0.15 euros per piece of pigs, or 0.16 euros per piece of cattle. The upward trajectory of the curve shows that each percentage increase in the tax rate causes an average increase in unit costs of production by 9.5%. By calculating the correlation, it was determined that there is a strong negative relationship between taxation and the volume of production, amounting to -1, and a strong positive relationship between taxation and unit costs.

Conclusion

The results of the research showed that the equilibrium price of pigs should be 4.2% lower than the market price, that is, the equilibrium price of cattle should be 11% lower than the market price. A decrease in the market price would stimulate the demand for pigs and cattle on the domestic market, and only an increase in demand would have a positive effect on the increase in production, which would reduce the negative balance of the trade balance. Furthermore, each further increase in the tax rate by 1% causes a reduction in the volume of production of pigs by more than 1%, or cattle by more than 2%, but causes an increase in unit costs by an average of 9.5%. The opposite happens with tax reductions. Therefore, for the Republic of Croatia, the strategy of the current institutional arrangement would be the most suitable, given that it is a country that has an open market to the outside world, and in which private ownership dominates, and it is necessary to continuously encourage the process of investing in the modernization of production, new technologies and the development of cooperatives, and maintain continuous interaction between economic policy and producers, that is, the profit sector in order to strengthen the domestic industry and strengthen the competitiveness of domestic products.

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THERMOREGULATION OF SPORTS HORSES

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Abstract: Horses are very well adapted to various weather/climate conditions due to their thermoregulation mechanisms. Thermoregulation is the result of complex and sophisticated biological processes that are affected by many environmental factors, not just the ambient temperature. In addition to the weather/climate, there are other factors that reduce or even limit the function of natural thermoregulation. Metabolic processes naturally generate a large amount of heat and thus make an emphasized contribution as a component of regulatory mechanisms. The challenges of sports horses are numerous, but through care and proper work, the consequences of equestrian sports can certainly be mitigated. Due to different climatic conditions and extremes, the horse through millions of years of evolution has adapted to the extremes through different mechanisms of thermoregulation. In order to ensure proper management and increase the various well-being of horses, further research is necessary in order to gain a deeper understanding of the mechanisms of thermoregulation in horses.

Key words: horse, thermoregulation, care

Introduction

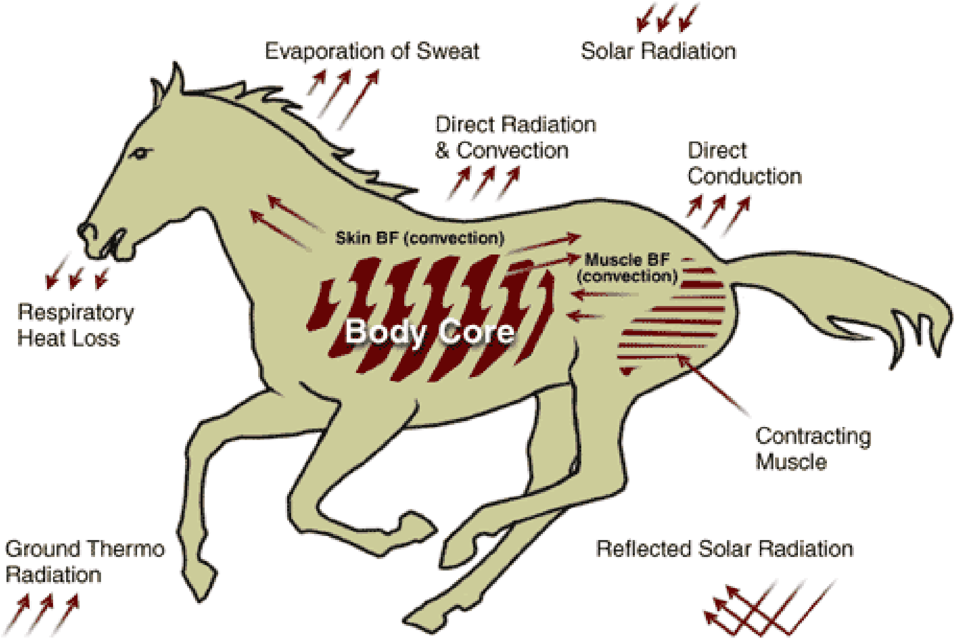
From an evolutionary point of view, horses are very well adapted to various climatic weather conditions due to their thermoregulation mechanisms. The original habitat of the wild ancestors of our domestic horses was different steppe landscapes such as desert, steppe and grasses, bushes and trees, as well as areas of savannah and tundra. The common characteristic of such landscapes is that they are very warm during the day and much cooler at night, which results in large fluctuations in temperature within 24 hours. On the other hand, wind and intense sun are typical for these landscapes. The horse has been adapted to such climate conditions for at least 25 million years. Horses are therefore born with excellent mechanisms for adaptation to heat and cold, as well as temperature oscillations. There are various studies on thermoregulation in young horses during training *(McConaghy, 1994; Geor et al., 1995; 2000; McConaghy et al., 1995;*

McCutcheon et al., 1999; McCutcheon and Geor, 2000; McKeever et al., 2010), but there is not much information on what happens during the specific care of a sports horse due to covering and shaving. Therefore, more research and knowledge are required on how best to manage sport horses, especially when they are sweaty after exercise in winter in order to ensure good welfare *(Mejdell et al., 2020)*.

Considering the extreme importance of the property of thermoregulation, the aim of this paper was to show the natural thermoregulation of sports horses and the influence of horse care on thermoregulation.

Horse thermoregulation

Thermoregulation mechanisms in horses are very important during work in different weather/climate conditions. Heat production occurs through the metabolism of feed necessary for work. Purebred racehorses produce enough heat to raise their body temperature by 3.25º C to 5.42º C. Similarly, like athletes, the equine athlete's thermoregulatory system uses convection, radiation, respiratory losses, and evaporation to release heat from the body (Picture 1).



Picture 1. Equine Thermoregulation (https://horses.extension.org/equine-thermoregulation)

Furthermore, *Mrowka and Reuter (2016)* explained that as homeothermic mammals, horses regulate and maintain their core temperature through thermoregulation, which involves physiological and behavioral processes *(Holcomb et al., 2013). Hodgson et al. (2014)* emphasised that many mechanisms are used to remove heat and reduce core temperature, including evaporation, conduction, radiation, and convection.

Evaporation is the primary process in which body tissues conduct heat to the skin, via blood flow and skin capillaries, transferring it to the external environment *(Kohn and Hinchcliff, 1995)*. Evaporation involves the removal of heat through sweat. The cardiovascular system plays an important role in transferring heat from areas of production, such as muscle, to areas where dissipation may occur, such as skin *(Hodgson et al., 1994; Sjaastad et al., 2016; EFSA, 2022)*. During physical labour, blood flow to the skin increases to allow sweat to evaporate. The amount of heat dispersed by 1 litter of sweat is equivalent to one to two minutes of high-intensity exercise or five to six minutes of submaximal endurance exercise. Although horses do not normally pant, the respiratory tract can dissipate heat through air exchange. Furthermore, a horse's high body mass to surface area ratio reduces the individual's ability to efficiently achieve rapid heat transfer. *Morgan (1998)* reported a basal rate of total heat loss in horses of 142 W m2, which remains stable at ambient temperatures ranging from 5°C to 25°C. Further, convection and radiation represent the direct transfer of heat from the skin to the environment. According to *Mazan (2022)*, horses are extraordinary athletes, whose respiratory systems are exceptionally capable of delivering the high flow of air and oxygen that is required for top sports performance. *Hodgson et al. (1994)* emphasised that a basic understanding of thermoregulatory responses in the exercising horse is imperative to avoid heat-induced illness.

Cutting and covering of the sport horse

Winter cutting of horses is performed to improve heat dissipation during training and to facilitate care. It is often combined with a blanket (picture 2) to keep the horse warm *(Wallsten et al., 2012)*. According to *Steinhoff-Wagner (2019)*, coat clipping/cutting is a common practice in sport horses; but the timing, purpose, technique, and clips, similarly like horse's management and feeding differ significantly.

According to *Mejdell et al. (2020)* cutting and blanket use interrupts natural thermoregulatory mechanisms in horses in the Nordic countries. According to the study by *Wallsten et al. (2012)*, thermoregulatory mechanisms for heat dissipation are mobilized in uncut horses, and defense mechanisms against the cold in cut horses at the beginning of training. Blankets increased heat dissipation in uncut horses and moderated reactions in cut horses. The mechanisms are physiologically and energetically demanding, and if horses are losing water and electrolytes through sweating and increased evaporation from the lungs and skin, fluid balance should be considered. *Mejdell et al. (2020)* pointed out that in Nordic countries there is a learning method in which horses could learn how to communicate to the handler whether they wanted to have a blanket on or not. The need to use a blanket depends on a number of thermoregulatory factors, such as ambient temperature, wind, and precipitation. *O'Brien et al. (2020)* stated that the photoperiod is considered the most dominant environmental sign enabling animals to predict and adjust to seasonal changes.

Accordingly to *O'Brien et al. (2020),* four studies on horses and ponies were performed to detect the effect of extended photoperiod and warmth, and the provision of horse blankets on the growth of winter coats and maintenance of the summer coat. They concluded that, in order to enable the successfully manage of equine coat growth and preserve optimal thermoregulation in both competition and breeding stock, the correct timing of light application is crucial which requires careful monitoring of environmental temperature.

Picture 2. Horses blanket (https://mystable.ca/canadian-sporthorse-association)

Effects on thermoregulation

Mechanisms of thermoregulation primarily involve structural components consisting of skin thickness, hair/coat, and sweat glands. The coat adapts to environmental and habitat conditions. It also develops differently in foals, old and adult animals, and in certain metabolic diseases such as Cushing's disease. By raising the coat, the insulating function at low temperatures is enhanced by an additional layer of air, and the structure of the coat works together with the sebaceous glands of the skin to drain away water. The skin (thickness) also takes on a special function.

According to *Padalino et al. (2019)* when the ambient temperature exceeds 25°C, horses are potentially exposed to heat stress. Therefore, it is recommended to provide shade for horses during hot days. Furthermore, it can be observed that horses eat more in the annual cycle towards winter in order to increase the thickness of the skin or layer of fat. Self-motivated behavior also protects the horse from unpleasant weather conditions *(Zeitler-Feicht, 2008)*. Horses instinctively seek shelter, especially when temperatures are low, wind and precipitation are persistent, or when there is direct sunlight *(Zeitler-Feicht, 2008).* Breed- and type-specific differences can also be identified with regard to structural components: it can be roughly divided into northern and southern types, which differ, for example, in terms of subcutaneous fat and hair/coat structure.

Thermoregulation represents the result of complex and sophisticated biological processes that are influenced by many environmental factors, not only environmental temperature *(Sjaastad et al., 2016).* The lower critical temperature (LCT) is not a fixed value but depends on animal-related factors such as age, breed, physiological status, acclimatization, coat quality, diet, and body condition *(Autio, 2008).* For example, during cold adaptation, the metabolic rate increases and can become 2-3 times higher than the maintenance level *(Sjaastad et al., 2016).* For these reasons, the LCT is valid only for the studied population. For example, *Morgan (1998)* found the LCT to be +5°C in trotters acclimated to 15−20 °C. *McBride et al. (1985)* estimate that the thermoneutral zone is between +10 and -15 °C in adult castrate horses acclimatized to winter. *Cymbaluk and Christison (1989)* evaluated the LCT of Quarter crossbred and Quarter horses fed ad libitum and cold-acclimated to −11 °C, while *Autio et al. (2007)* suggested the LCT is between −9 and −16 °C in cooling weaned foals of different breeds. In neonatal foals (thoroughbred, Arabian, ponies) the mean LCT is between +10 and +24 °C (summarized according to *Autio, 2008*). Nevertheless, studies on LCT are valuable for demonstrating the expected cold resistance.

The term zone of least thermoregulatory effort has been proposed to define the range of ambient temperature below which an animal is not heat stressed and does not need to mobilize energy reserves, nor pant or sweat, to maintain a stable body temperature *(Hillman, 2009)*. In humans, the zone of least thermoregulatory effort roughly corresponds to the comfort zone, where people feel thermal comfort *(Hillman, 2009).*

According to *McKeever et al. (2010)*, older horses had a reduced ability to thermoregulate during training and these differences may be the result of different absolute plasma volumes (PV) at the beginning of the work. Training regimens and athletic events should be designed accordingly to prevent heat stress in older equine athletes. If the mechanisms of heat loss are weakened by aging, then the only way a horse could lower its body temperature is to reduce the rate of heat gain by reducing the intensity of exertion.

In addition to the weather, there are other factors that reduce or even limit the function of natural thermoregulation. Metabolic processes naturally generate a large amount of heat and thus make a decisive contribution as a component of regulatory mechanisms. In old horses, however, these processes are greatly slowed down, meaning that horses must expend more energy to keep their body temperature stable *(Hodgson et al., 1994)*. Even chronically or seriously ill horses cannot always keep up with the seasonal adjustment. Partial cutting can help if the coat does not fall out in the spring and if the horse sweats excessively. A horse has sweat glands all over its body. In this way, the body temperature can be effectively and quickly lowered by the evaporation of sweat. Cutting in the winter interferes with the horse's natural thermoregulation mechanisms and forces the horse to be adequately covered to prevent the horse from freezing.

The consequences of permanent blanket covering are as follows:

- Increase in body temperature (after only a few hours of sun exposure; also proven with light, breathable fly mats in summer). Clinical symptoms are exhaustion, reduced ability up to circulation problems, and/or colic.
- Increased risk of infection: Horses gradually lose the ability to thermoregulate as a result of the constant, unnatural supply of heat from the blanket. This makes the immune system more susceptible to disease.
- Ischemic damage due to improper fit of the carpet or permanent pressure (pain due to reduced blood flow to the skin, especially in the withers. Consequence: back problems). Therefore, only blankets with a good fit, light materials, and good breathability should be used.
- Increased risk of skin infections: if the skin's ability to breathe and the natural exchange of moisture on the body's surface are disturbed, the ideal ground for fungal skin diseases is created.

Conclusion

Due to different climatic conditions and extremes, the horse through millions of years of evolution has adapted to the extremes through different mechanisms of thermoregulation. In order to ensure proper management and increase the various well-being of horses, further research is necessary in order to gain a deeper understanding of the mechanisms of thermoregulation in horses.

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POPULATION TRENDS OF GOATS IN SERBIA AND CROATIA FROM 2012 TO 2021

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Abstract: This paper aims to examine the state of goat production and the population trends of goats in Croatia and Serbia from 2012 to 2021. Goat keeping in both countries suffered immensely in the second half of the 20th century as a result of the infamous Law on Prohibition of goat keeping, which was unique of its kind and which led to the almost complete annihilation of the goat sector in countries which were at the time part of Yugoslavia. With the abolition of this law, both countries have made an effort to revive goat production by importing high-producing breeds, crossbreeding domestic breeds, herd book keeping and government funding. In the past ten years, Serbia has experienced a significant rise in the number of herd book goats and a decline in the total number of heads of goats. The Republic of Croatia had a positive trend in the total number of goats which increased by about 10.6%, and a higher percentage of herd book goats compared to the total number of goats compared to Serbia. However, Serbia has about 2.7 times bigger total goat population. Total production of goat milk and meat in both countries is considered low when seen from the perspective of Europe, as only 1.3% and 0.5% of European goats are raised in Serbia and Croatia, respectively. In Serbia, production systems are still predominantly extensive to semi-intensive, and therefore production potential of animals is not exploited to the maximum. Also, Serbia has weak and unstable markets for goat milk and meat, making this production unpredictable and varying. Even though the goat sector has come a long way since its downfall in 1954, it still has a long way to go to become sustainable. On the other hand, in Croatia, goat milk has been a sought-after product in recent years, and farmers have achieved fair prices, establishing the goat dairy industry. After Croatia joined the European Union, goat farmers gained access to several significant sources of financing, making it the most important event for the goat sector in Croatia.

Keywords: goats, Croatia, Serbia, breeds, production, population and trends

Introduction

In Serbia and Croatia, goats have been bred for centuries as an important source of animal products: milk, meat, hair and skin. *Vondraček (1998/1999)* claims that goats have been present even before the Roman times in the Croatian region Istria. Their importance has not diminished over the centuries, and in the 19th century, around 750 000 goats were bred in Dalmatia alone *(Ožanić, 1955)*. Just before World War II, there were approximately 1,800,000 goats in the territory of the State of ex-Yugoslavia, which included both Serbia and Croatia, along with the remaining four republics. These were mainly domestic Balkan goats. The number of goats significantly declined during the war, but it increased again after the war to around 1,300,000 heads, as reported by *Memiši and Žujović (2012)*. Unfortunately, a rapid decline in the number of goats in Serbia and Croatia occurred in the second half of the 20th century, precisely from 1954, when the Law on the Prohibition of goat keeping was adopted *(Maksimović et al., 2017)*. The consequences of the Law mentioned above are still present. However, in the 1980s, the Law was tacitly abolished, and several highly productive breeds of goats (Alpine, Saanen, German Improved Fawn) were imported into Serbia and Croatia, which laid the foundations for more intensive goat breeding and milk production *(Mioč, 2022).*

Due to decades of neglect and ostracism following the prohibition law, the goat sector in Serbia has suffered, resulting in it being the least favoured livestock sector. For many years, there has been a need for clear strategy or direction for this production, and this continues to be the case. Breeders start with tiny herds avoiding all kinds of risks *(Memiši and Žujović, 2012)*. Through the decades of absence of goats and goat products on the markets and rapid industrialisation favouring cow milk, consumers' tastes and habits changed, putting bovine species ahead in dairy. In recent years, the government of the Republic of Serbia has been trying to revitalise and stop further deterioration of goat production, primarily by funding breeders to produce quality breeding animals *(Maksimović et al., 2021)*. Most effort has been made to increase the number of quality breeding animals in the past decade. Funding revived the goat sector, and genetics was improved by importing high-yielding breeds such as Alpine and Saanen. However, this is only the first step, as the goat sector needs much more attention in all its aspects.

In Croatia, goat breeding has made significant progress in the last three decades. All breeds were standardised and genotyped (especially the autochthonous breeds), and breeding programs for most breeds were rewritten and published. The procedure for protecting the authenticity of certain goat products like dried goat meat was started (*Mioč and Držaić, 2022*). Protecting originality and achieving geographical authenticity marks helped the farmers make their products more recognisable, protect them from unfair competitors and increase the product's price. Croatia has been a member of the European Union since 2015. Thus, most of the legislation had to be adapted according to European regulations and new Laws on animal husbandry and veterinary medicine were adopted. Although joining the European market did not bring significant changes in terms of increasing the number of goats or the amount of goat products in the Croatian sector, it did enable farmers to access funding from various European sources. Additionally, progressive farmers received considerable support as a result. Nonetheless, Croatian goat production still falls short of being self-sufficient.

Goat breeds in Croatia and Serbia

In both countries, there are several autochthonous and imported breeds of goats. Of the imported breeds, Alpine, Saanen and Boer are bred in Serbia and Croatia. Croatian autochthonous breeds are Croatian spotted goat (Figure 1), Croatian white goat (Figure 2) and Istrian goat (Figure 3), while in Serbia, there are two breeds, Balkan goat (Figure 4) and Serbian white goat (Figure 5).

Croatian spotted goat

The Croatian spotted goat is a typical Mediterranean-type goat breed known for its resilience, adaptability, longevity and modest production. It is the most numerous Croatian bred, mainly raised in Dalmatia's hill and mountain areas. Formerly, it was known as the Balkan goat. These goats have strong bodies covered with white, black, grey and brown hair. Horns are present in both goats and bucks. The average body weight of adult goats and bucks is 30 to 50 kg and 40 to 60 kg, respectively. The milk production is low, between 150 and 350 litres in the lactation (150 – 220 days) *(Mioč, 2022).*

Figure 1. Croatian spotted goat
(Source of the picture: Hrvatski savez uzgajivača ovaca i koza, 2023)

Croatian white goat

The origin of the Croatian white goat is unknown, but it is supposed that domestic goats were bred with Saanen goats, thus creating this breed. The estimated number of Goats is around 5000. It was mainly bred in small herds, along with sheep, for milk production. The average body weight of adult goats and bucks is 35 to 50 kg and 45 to 70 kg, respectively. The milk production is low, averaging between 250 and 400 litres of milk during the lactation period of 230-260 days *(Mioč, 2022).*

Figure 2. Croatian white goat
(Source of the picture: Hrvatski savez uzgajivača ovaca i koza, 2023)

Istrian goat

Istrian goats have a big physical frame, strong constitution, and strong bones. The base coat colour is white, but grey or cream-coloured hues are allowed. Large, rough, and coarse horns bent backwards in bucks are desirable. The body weight of adult goats is between 57 and 66 kg and adult bucks' is between 70 and 120 kg. Goats produce between 500 and 800 kg of milk during the 220-280 days of lactation *(Antunović et al., 2019; Mioč, 2022).* This breed was officially recognised in 2012, and it is critically endangered, with 100 individuals remaining.

Figure 3. Istrian goat
(Photo: Ivan Vlahek)

Balkan goat

As mentioned in the description of the Croatian spotted goat, the Balkan goat is the same breed, i.e. genetically the same. Balkan goat refers to all autochthonous goats originating from Balkan Peninsula. It is a small goat with long hair that comes in every possible colour or combination of colours. Balkan goats of both sexes have horns, but occasionally some individuals can be without horns. This goat is resilient, healthy, agile, and capable of surviving in harsh environments. Body mass in adult does can vary from 35-45 kg, and in bucks, 45-55 kg. Milk production ranges from 150-350 kg, depending on the duration of lactation, and fertility is around 130% (*GOP*, *2020*). It is bred extensively for combined production, i.e. milk and meat. It can be found all over Serbia, but mainly in the south and southeast regions.

Figure 4. Balkan goat
(Photo: Bogdan Cekić and Ivan Ćosić)

Serbian white goat

The Serbian white goat, also called the Domestic white goat, is an improved version of the Balkan goat, created through the crossbreeding of Balkan does and Saanen bucks to enhance milk production and body conformation. Although the crossbreeding process was unintentional, the Serbian white goat has since been recognised as a distinct breed. Serbian white goat has white to white-beige hair, which is short with patches of longer hair along the back line, on the flanks and on the belly. Its format makes it smaller than Saanen and bigger than Balkan goat. Mature goats weigh about 45 kg, and bucks are 60 kg of body weight on average. Milk yield can vary from 150 to 450 kg in lactation, which also varies in length. Fertility is 130% on average but can be as much as 180% in good rearing practices (*GOP*, *2020*). This goat is mainly found in southern and southeast Serbia and is bred for milk and meat.

Figure 5. Serbian white goat
(Photo: Aneta Lilić)

Goat production systems in Serbia and Croatia

Goat keeping in Serbia is predominantly extensive to semi-intensive. Autochthonous breeds, such as Balkan and Serbian white goats, are kept exclusively in extensive pastoral systems. In contrast, higher productive breeds like Alpine and Saanen are kept in semi-intensive production systems. Fully intensive production systems are rare in Serbia and can be only found sporadically, usually in the northern part of the country, i.e. AP Vojvodina region. Goat production in Serbia is directed towards milk-meat, but predominantly milk (*Žujović et al., 2011*). Milk is considered the main goat product, but the dairy market is unstable, and prices fluctuate annually. Therefore, producers can turn to kids' production for meat in those years when the milk prices are too low. This means that the milk is then exclusively used to nourish kids. That way, farmers cope with loss from low milk prices, but such practices of discontinuing milk production from year to year lead to lower milk yield in subsequent lactations, and kids suckling for a prolonged period can cause udder problems *(Maksimović et al., 2021)*.

In Croatia, two main types of goat production systems exist. There are two primary types of goat production systems in Croatia. The first type is extensive, mainly located in Dalmatia, where Croatian native breeds of goats (Croatian spotted goat, Croatian white goat) are raised for meat production. The second type is an intensive dairy production system in which the Alpine breed dominates. Most dairy producers are located in central and northern Croatia, gathered by the „Vindija“ company as its cooperators or have their small dairy processing plants. In recent years goat milk has been a sought-after product, and farmers have achieved fair prices. This has, in return, stabilised the goat dairy industry.

Neither Croatia nor Serbia is among the high-producing European countries regarding the goat sector. According to Faostat data, in 2021, total goat milk and meat production in Croatia was 7,003 tonnes and 500 tonnes, respectively, while Serbia produced 35,400 tonnes of milk and 3,256 tonnes of goat meat in total (Table 1.). But, since official statistics do not include home consumption or informal market sales, precise measuring of actual production is impossible. In addition, goat milk is predominantly consumed locally. In contrast, cow milk is usually channelled into formal markets for processing *(Miller and Lu, 2019)*, and the same goes for goat meat, even more so.

Table 1. Goat milk and meat production in 2021 in Serbia, Croatia, Europe and the world

Country/Product	Milk (in tonnes)	Meat (in tonnes)
Serbia	35,400	3,256
Croatia	7,003	500
Europe	3,109,796	89,173
World	20,725,282	6,397,747

Source: Faostat (2023)

Total number of goats in Serbia and Croatia

During the observed period, Serbia's total number of goats varied between 231,837 in 2012 and 182,558 in 2017. In Croatia, this number ranged between 65,000 (from 2012 to 2017) and 82,290 (2019) individuals. The general trend in the number of goats in the two countries is opposite; in Croatia, it is ascending, and in Serbia, it was descending from 2012 to 2017 (Table 2; Table 3). *Sredojević et al. (2020)* reported that in Serbia, the number of goats has been declining since 2008, with an average decline of 8.7 thousand per year. According to the recent Faostat data, 15,156,962 goats were raised in 2021 in Europe. Of these, 1.3% and 0.5% were raised in Serbia and Croatia, respectively *(Faostat, 2023)*.

In Serbia in 2015, there were 62,930 goat herds, of which 54.21% contained one or two goats. Only 0.09% of herds had 100 or more goats *(Nastić and Potrebić, 2015)*. The average herd size was nine heads of goats *(Sredojević et al., 2020)*. *Maksimović et al. (2021)* stated that this structure might be changed recently, but it is clear that most holdings keep less than ten heads of goats which is under any limit that allows sustainable production. Most goats in Serbia are reared in West, East, and South Serbia, located south of Belgrade *(Maksimović et al., 2021)*.

In Croatia, 49% of goats are raised in three Dalmatian counties: Split Dalmatia County (20%), Zadar County (19%) and Šibenik Knin County (10%).

Most herds are medium in size, and 72.7% contain below 100 individuals *(Mioč and Držaić, 2022)*. More than 200 individuals per herd are present in 7.3% of herds. The most numerous goat breed was the Croatian spotted goat, followed by the Alpine breed *(HAPIH, 2022)*.

Table 2. The total number of goats and the number of quality breeding goats in Serbia and Croatia from 2012 to 2016

Year		2012	2013	2014	2015	2016
Country	Parameter					
Serbia	Total number	231,837	225,073	218,603	202,828	200,150
	Quality breeding animals	3,415	3,453	5,072	7,026	8,212
Croatia	Total number[*]	65,000	65,000	65,000	65,000	65,000
	Quality breeding animals	8,196	6,792	6,480	6,277	6,519

[*] Total number was estimated

Table 3. Total number and number of quality breeding goats in Serbia and Croatia from 2017 to 2021

Year		2017	2018	2019	2020	2021
Country	Parameter					
Serbia	Total number	182,558	195,932	191,280	202,325	195,037
	Quality breeding animals	12,926	16,512	18,554	19,009	18,571
Croatia	Total number[*]	65,000	68,000	82,290	71,140	71,872
	Quality breeding animals	6,837	7,395	7,459	7,548	8,735

[*] Total number in 2017 and 2018 was estimated

Quality breeding animals in Serbia and Croatia

Quality breeding goats have known pedigree and production data. Their production and reproduction are monitored, and the records are used as breeding efficiency indicators. In both Serbia and Croatia, the Alpine is the breed with the most quality breeding animals (Figure 6). From 2012 to 2021, the number of quality breeding Alpine goats in Serbia increased from 3,000 to 16,000. Croatia's number did not change significantly in the same period and varied by around 5,000 goats or 52% of the quality breeding goat population. This confirms that the Alpine is the primary breed in the two countries' intensive and semi-intensive goat production systems (Figure 9 and Figure 10), where dairy production dominates. The number of quality breeding Saanen goats in Serbia and Croatia was around 700 individuals, significantly lower than that of Alpine goats. In both countries, farmers prefer the Alpine goats because they are considered more resilient and adaptive than the Saanen goats, and their production is similar. Among other non-native breeds, the Boer goat had a minor presence in Croatia, which was recently introduced into Serbia in 2021. From 2012 to 2015, Croatia had a small breeding population of German improved fawn goats. In Europe, the dairy sector based on the Alpine, Saanen, Murciano-Granadina and several other highly productive breeds also dominates. Even though Europe holds only 1.9% of the world's goat population, it produces 15.1% of goat milk and 35.1% of cheese *(Morales et al., 2019)*. Leading European goat milk producers are the Netherlands and Mediterranean countries.

During the observed period, the Croatian spotted goat and Balkan goat were the most numerous native quality breeding goats (Figure 7). Moreover, both breeds had a significant increase in number. The number of quality breeding Croatian spotted goats increased from around 600 in 2012 to 2,300 in 2021, while the number of Balkan goats increased from 200 to about 700 individuals in the same period. Both breeds of goats are reared in hilly and mountainous regions with harsh environmental conditions, primarily for meat production *(Maksimović et al., 2019; Mioč, 2022)*. Croatian white goat and Serbian white goat breeds are less frequent, accounting for 4% and 0.8% of the total quality breeding populations (Figure 4; Figure 5). The Istrian goat is critically endangered, with only 100 individuals left *(HAPIH, 2022)*. However, in 2022 a plan was set to revive, protect and promote Istrian goats, thus preventing their extinction *(Vlahek et al., 2023)*.

The percentage of quality breeding goats from the total number of goats in Serbia and Croatia from 2012 to 2021 is presented in Figure 8. In Croatia, it varied between 9.06% and 12.61%. In Serbia, it was 1.47% in 2012 and had an increasing trend until 2019 (9.7%). However, both percentages are relatively low, and the number of quality breeding animals should be increased in the years to come.

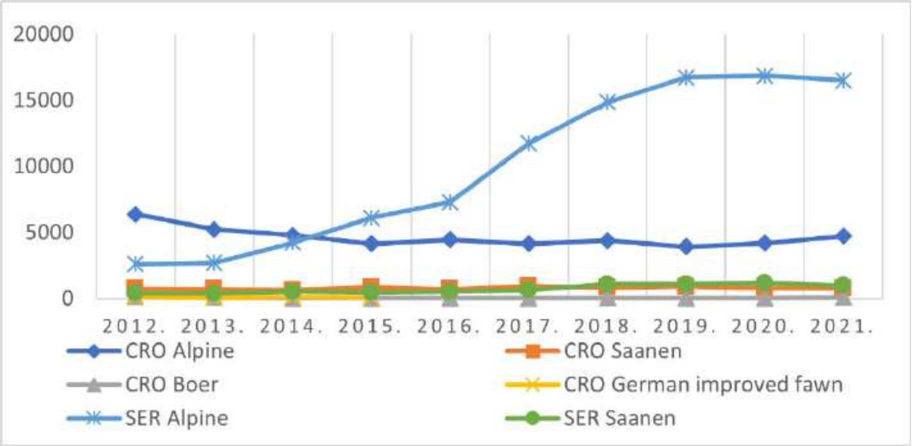

Figure 6. The number of quality breeding allochthonous goats in Serbia and Croatia from 2012 to 2021

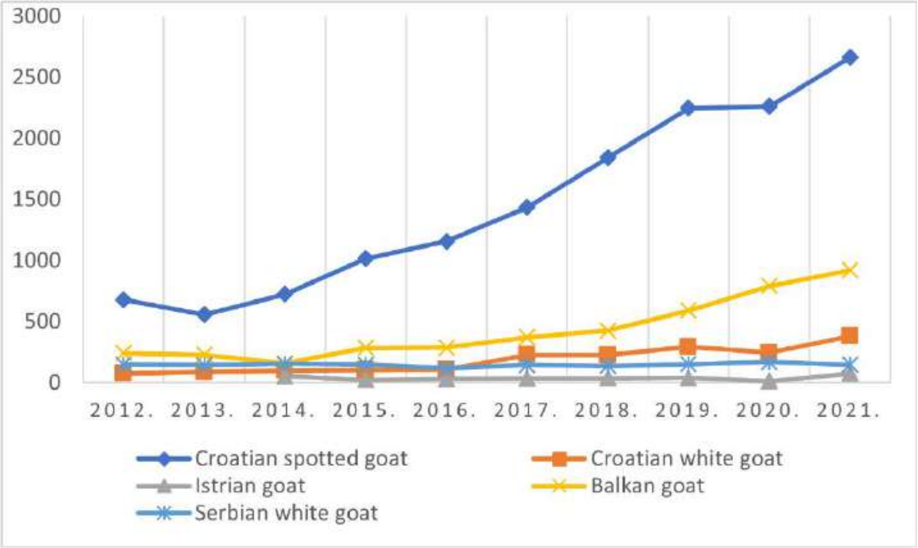

Figure 7. The number of quality breeding autochthonous goats in Serbia and Croatia from 2012 to 2021

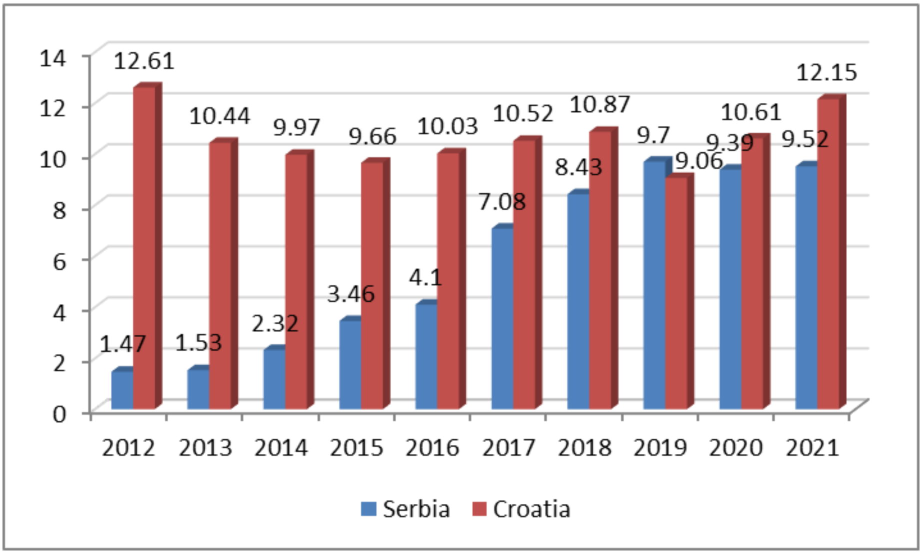

Figure 8. Percentage (%) of quality breeding goats in Serbia and Croatia

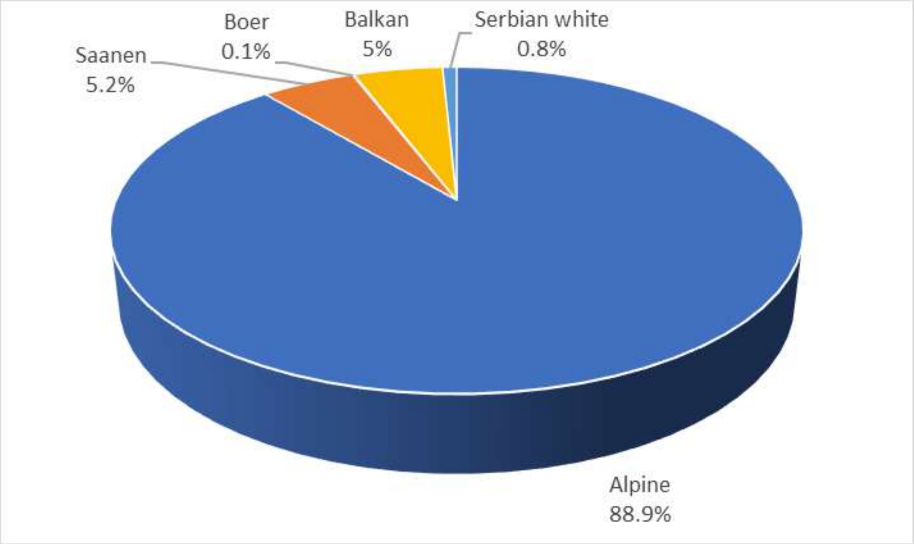

Figure 9. Structure of quality breeding goats in Serbia by breed in 2021

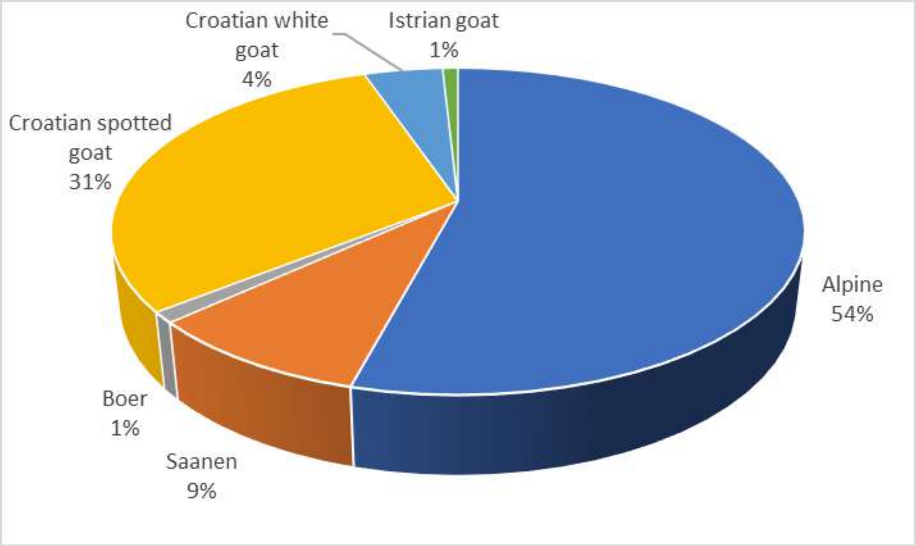

Figure 10. Structure of quality breeding goats in Croatia by breed in 2021

Government funding for the goat sector in Serbia and Croatia

As seen from Tables 2 and 3 and Figure 8, the number and percentage of animals under selection control in Serbia have increased significantly during the past ten years. This period brought an increase of roughly 5.4 times the number of controlled animals. In 2012, the number of registered goats accounted for 1.47% of the total goat population in Serbia, while in 2021, a significant increase to roughly 9.52% was recorded. The main reason behind this increase is the government's founding of goat breeders, currently the leading motivation for farmers to raise goats. So far, all goat breeds of national interest for breeding in Serbia have been included in funding through breeding-selection programs. Farmers receive 59 Euros per year per head for animals registered in the Central herd book and 38 Euros for goats of autochthonous breeds (genetic resources) under performance control (*Maksimović et al., 2021*). This funding proposes a lower limit of 10 breeding heads of goats that are under productivity control. In addition, in 2017, the funding for producing male breeding animals (bucks) was implemented as a new stimulating measure (*Maksimović et al., 2017*). Funding was initially established to help revitalise goat production, which was almost on the brink of extinction and has since become a necessity in goat breeding.

Compared to Serbia, Croatian governmental funds for goat keeping are significantly lower. Financial support for the quality breeding of female animals is 17,26 Euros *(APPRRR, 2021)*. However, other sources of financing became available after joining the European Union. For example, farmers can apply for support from the European Fund for Agricultural Development. In addition, several measures include the goat sector, like Measure 14 (support for animal welfare), Measure 10 (support for agriculture, environment and climate changes) or Measure 11 (support for organic production) *(APPRRR, 2023)*. Many farmers benefited from these measures since some funding was bountiful and allowed them to improve their farms significantly.

Conclusion

Goat production in both countries was revived with the import of highly productive goat breeds. In addition, productivity was improved through planned selection, intensification of production, and better rearing conditions. Despite financial stimulative measures leading to an increase in the number of quality breeding animals, the total number of goats in Serbia has been decreasing over the years, with goat breeders becoming almost entirely dependent on government funding. The unstable market for goat products needs to be addressed to ensure more sustainable production. Keeping autochthonous breeds in Serbia has been almost completely abandoned. However, the genetic potential of imported high-producing breeds still needs to be fully exploited because most production systems are extensive to semi-intensive. Even though the goat sector has come a long way since the 1954 introduction of that notorious Law of Prohibition on goat keeping, it still has a long way to go to become sustainable.

Contrary to Serbia, goat milk has been a sought-after product in Croatia in recent years, and farmers have achieved fair prices, establishing the goat dairy industry. Croatia also showed a positive trend in the total number of goats in the last ten years, which increased by about 10.6% and a higher percentage of quality breeding animals, so-called ‘’herd book goats’’, in relation to the total number of goats compared to Serbia. Furthermore, after Croatia joined the European Union, goat farmers gained access to several significant sources of financing. Progressive farmers who capitalised on these resources increased their competitiveness in the market.

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COMPARISON OF BODY WEIGHT, FAMACHA © BCS AND HAIR SCORES İN SAANEN GOATS DURING PREGNANCY AND BIRTH PERIOD

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Abstract: The aim of this study was to compare FAMACHA © card scores, BCS scores, body weight (BW) and hair score values at the beginning of gestation, advanced gestational and parturition periods in 22 Saanen goats whose oestrus was synchronized. Every 15 days from the mating period to the postpartum period, eye score scoring, BCS scoring, hair scoring and FAMACHA © card scoring of 22 had Saanen goats were determined and live weights were weighted. In terms of the properties measured in the study, the averages of the early period of pregnancy and the advanced period of pregnancy and the birth periods were compared. As a result of statistical analysis, the effect of BCS on body weight was found to be statistically significant. Correlation between BCS and FAMACHA © Graphic score was found to be negative (-0.392) and moderate significant. In the initial period of pregnancy, the effect of BCS on Famacha and body weight wasn't statistically significant, but its effect on hair scoring was found to be significant ($P < 0.05$). The effect of BCS on BW, FAMACHA © and hair score in late pregnancy period and delivery period was found to be statistically significant ($P < 0.05$). As a result of good care and feeding practices during the pregnancy period, BCS of Saanen goats increased during the birth period and body weight and FAMACHA © were positively affected.

Key words: Saanen goat, pregnancy, FAMACHA © card, BCS, hair Score, body weight

Introduction

Productivity, profitable production and minimum animal loss in sheep and goat breeding may be possible with some practical and simple applications throughout the year. Recently, many studies have been conducted on practical, easy and economical applications in herd management in sheep and goat breeding.

Some methods have been developed for this topic, and some of the scoring methods developed specifically for animal fields, animal welfare and health have been highlighted to facilitate herd management (*Russel et al., 1969; Cross and Parker, 1981; Malan et al., 2001; Ware , 2005).*

Internal parasites are a major threat to sheep and goat breeding. Especially the problems caused by sheep and goats in the digestive system can cause irreversible damage and even death, causing a decrease in the performance of the breeder and economic loss. In animals overloaded with parasites, a decline in reproductive performance, a slowdown in growth rate and a decrease in all types of productivity occur. The prevention and control of parasites transmitted to sheep and goats is becoming increasingly difficult as overuse of existing anthelmintic drugs results in increased resistance to anthropathic drugs. The fact that the breeders have limited resources makes this situation even more important. Although sheep and goats are a sustainable production activity that can be adapted to many different production systems and with relatively few inputs, it should be kept in mind that they are faced with serious production constraints. The control of internal parasites, especially intestinal nematodes such as Haemonchus contortus, is a primary concern for breeders *(Koyuncu et al., 2019).*

In animals that have lost a lot of blood due to bloodsucking parasites, the mucosa in some parts of the body, such as the gums and perineum, is pale. In small ruminants, anemia can be easily detected, especially by the color of the lower eyelid mucosa of the eye. While a normal animal will have healthy, red mucous membranes, the animal with a heavy Haemonchus load will show a light pink or nearly white mucosa *(Kaplan et al., 2004; Koyuncu et al., 2019).* Using the FAMACHA® system, developed cards can be used to categorize the inner color of the eyelids *(Koyuncu et al., 2019).*

Body condition score (BCS) is an easily applied tool to assess the fat reserves of animals. This tool can be applied when feed is scarce and feeding strategies need to be implemented, or to select animals for continuous assessment of the animals' nutritional, health, and reproductive status *(Kenyon et al., 2014).* BCS is considered the most suitable parameter for evaluating animals in field conditions due to its easy detection and no equipment required for BCS *(Mendizabal et al., 2011).*

During some physiological periods that require high food consumption (such as breastfeeding and pregnancy), ruminant animals may become more vulnerable to metabolic disorders reflected in biochemical and hematological parameters *(Antunović et al., 2011)*. Biochemical and hematological tests are useful diagnostic tools for identifying nutritional diseases and detecting associated changes in nutrition and health status before animals show visible physical changes

(Carta et al., 2020). However, these tests involve high costs that negatively affect the income of the growers.

To solve this problem, a subjective method based on the Faffa Malan Map (FAMACHA ©) has been proposed to determine abnormal hematological values in sheep *(Wyk and Bath 2002).* This method was created to control hemoncosis, a serious parasitic disease characterized by the development of severe anemia in sheep and goats. The method is based on coloration of the conjunctiva of sheep ranging from dark red to pink in healthy sheep, or nearly white in sheep with progressively worsening anemia. These changes have been associated with HCT (hematocrit) values and indirectly with anemia *(Morales et al., 2010; Amaducci et al., 2016)*. Recently, in hair sheep, *Soto-Barrientos et al. (2018)* reported that the FAMACHA © method was unable to detect animals in need of anthelmintic treatment (reported 750 EPG (50-55% false negatives)). They also reported that fewer than 2 BCSs were a condition for sheep with 750 EPG eggs in the stool (1.1% false negatives).

Because FAMACHA © and BCS are widely used in sheep breeding systems to indicate animals' degrees of anemia and body energy reserves, both tools could possibly help identify animals with hematological changes associated with anemia (regardless of worms or other causes of anemia). Therefore, the purpose of this study is to determine the relationships between FAMACHA ©, BCS score and hematological values in sheep without gastrointestinal parasites (Torres-Chable et al., 2020).

It has been suggested that the FAMACHA score is a reliable indicator for identifying sheep in need of treatment for Haemonchus contortus infections *(Wyk and Bath 2002)*. This has been demonstrated in several studies where significant correlations exist between FAMACHA and packed cell volume (PCV) *(Kaplan et al., 2004; Papadopoulos et al., 2013; Chylinski et al., 2015)*. As an indicator of TST (Targeted Selective Treatment), the FAMACHA score should also reflect the EPG (the nematode egg concentration per gram faeces) for H. contortus, but the findings are not consistent across studies. *Kaplan et al. (2004); Burke et al. (2007) and Notter et al. (2017)* found significant correlations between FAMACHA and EPG ranging from 0.21 to 0.44, and no relationship was found in other studies *(Chilinsky et al., 2015; Moors and Gauly, 2009)*. All of these studies, with the exception of *Chylinski et al. (2015),* investigated mixed GIN (gastrointestinal nematodes) infections where sheep were artificially infected with H. contortus. The results of using the FAMACHA score in dairy sheep breeds are limited, as most studies have been conducted on non-dairy sheep. The aim of this study was to compare FAMACHA © card scores, BCS scores, body weight (BW) and hair score values at the beginning of gestation, advanced gestational and parturition periods in 22 Saanen goats whose oestrus was synchronized.

Material and Methods

This study was carried out between October 2020-April 2021 in the Goat Breeding Unit of Aydın Adnan Menderes University Faculty of Agriculture Animal Husbandry Research Application Center. The coordinates of the enterprise are 37 ° 45′03.31 ′ ′ N and 27 ° 45′27.16 ′ ′ E, 52 m above sea level. Mediterranean climate prevails in the region.

The animal material of the study consists of 22 Turkish Saanen goats. Goats were obtained from the Goat Breeding Unit of Aydın Adnan Menderes University, Faculty of Agriculture, Animal Research and Application Center.

Measurements were taken from goats whose oestrus was synchronized in October 2020. Live weights (LW), hair scoring, FAMACHA scoring and body condition scores were taken from the brood goats every 15 days from the beginning of pregnancy until the time of birth.

Live Weight Measurement and Body Condition Scoring

Precision scales sensitive to 50 grams were used in the weighing. Body condition score (BCS) was obtained after weighing. Body condition scoring was calculated by taking into account the amount of fat on the spines (Processus spinosus) and the wing protrusions (Processus transversus) that make up the structure of the vertebra, the fullness of the angle between the two protrusions and the formation of the fat layer on this fullness, taking into account the average of the independent predicted values of two individuals who predicted independently by hand palpation.

Scoring values are given in 0.25 point intervals from 1 to 5. Animals with low scores were determined to be less fat, while animals with high scores were determined to have more *(Russel et al., 1969).*

Body Condition Score 1: Ribs are felt very easily and no fatty tissue is felt on the ribs. Paralumbal fossa is evident. With hand palpation, only bone comes to the hand. The animal is very weak.

Body Condition Score 2: Ribs are very easy to feel. There is very little fatty tissue on the ribs. Hunger cessation is again evident.

Body Condition Score 3: Ribs are easy to feel. There is some fatty tissue on the ribs. Hunger cupping is very slightly pronounced.

Body Condition Score 4: Ribs feel very light and a lot of fat tissue comes to the hand on palpation by hand. Hunger cupping is not clear.

Body Condition Score 5: Ribs can only be felt when pressed with the hand. Adipose tissue is quite high. The hunger pit has completely disappeared. The animal is quite fat.

FAMACHA © Score

The Faffa Malan Map (FAMACHA ©) was created to control hemonosis, a serious parasitic disease characterized by the development of severe anemia in sheep and goats. The method is based on coloration of the animal's conjunctiva color ranging from dark red to pink in healthy animals or almost white with progressively worsening anemia. These changes have been associated with HCT values and indirectly with anemia *(Morales et al., 2010; Amaducci et al., 2016).*

Hair Scoring

In this scoring, considering the external appearance of the coat of goats, scores between 1-3 were given considering the bristle shine, bristle structure and pattern (1: Good, 2: Medium, 3: Bad).

Statistical analysis

The data were analyzed by using Friedman two-way analysis of variance and Kruskal-Wallis analysis of variance and SPSS 22 for inter-group comparisons. Statistical error level was taken as $p<0.05$.

Results

According to BCS groups, average live weight was found between 47.34 kg and 56.49 kg. Overall live weight average was found to be 52.05 kg. The difference in live weight between BCS 2 ≤, 2.01-2.5 and ≥2.51 groups was found to be statistically significant. Hair scoring between BCS 2 ≤, 2.01-2.5 and ≥2.51 groups were found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS 2 ≤ group was found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS 2.01-2.5 group was found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS ≥2.51 group was found to be statistically significant (Table 1).

Table 1. LW, FAMACHA © and Hair average score and standard errors according to BCS groups in early period of pregnancy (Mean±SEM)

Bcs grups	**N**	**LW**	**Famacha**	**Hair score**
2 ≤	10	47.34±1.93^{aA}	2.22±0.20^{bA}	1.96±0.12^{cA}
2.01-2.5	7	52.32±3.21^{aB}	2.20±0.14^{bA}	1.46±0.15^{cB}
≥2.51	5	56.49±3.45^{aC}	2.16±0.22^{bA}	1.21±0.15^{cC}
	22	*	N.S	*

a, b, c,… The differences between averages with different letters on the same line are statistically significant.
A, B, ... The differences between averages with different letters in the same column are statistically significant.
* $P < 0.05$; N.S. = Non Significant.

Live weight averages according to BCS groups vary between 48.79 kg and 63.74 kg, and the overall live weight average is 57.04 kg. The difference in live weight between BCS 2 ≤, 2.01-2.5 and ≥2.51 groups was found to be statistically significant. FAMACHA © between BCS 2 ≤ and 2.01-2.5 groups was found to be statistically insignificant. The difference between the ≥2.51 group and the FAMACHA © other BCS groups was found to be significant. Hair scoring between BCS 2.01-2.5 and ≥2.51 groups wasn’t statistically significant. The difference between the hair scoring of BCS 2 ≤ group and the other BCS groups was found to be significant. The difference between live weight, FAMACHA © and hair scoring in BCS 2 ≤ group was found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS 2.01-2.5 group was found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS ≥2.51 group was found to be statistically significant (Table 2).

Table 2. The advanced period of pregnancy least squares means and standard errors for the effect of body condition score on live weight, FAMACHA © and hair score (Mean±SEM)

Bcs grups	**N**	**LW**	**Famacha**	**Hair score**
2 ≤	8	48.79±1.77^{aA}	2.77±0.17^{bA}	1.90±0.19^{cA}
2.01-2.5	8	58.60±2.41^{aB}	2.75±0.19^{bA}	1.30±0.30^{cB}
≥2.51	6	63.74±4.40^{aC}	2.00±0.02^{bB}	1.25±0.09^{cB}
	22	*	*	*

a, b, c,… The differences between averages with different letters on the same line are statistically significant.
A, B, ... The differences between averages with different letters in the same column are statistically significant.
* $P < 0.05$

Live weight averages according to BCS groups vary between 43.50 kg and 52.45 kg, and the overall live weight average is 48.68 kg. The difference in live weight between BCS 2 ≤, 2.01-2.5 and ≥2.51 groups was found to be statistically significant. The FAMACHA © difference between BCS 2 ≤, 2.01-2.5 and ≥2.51 groups was statistically significant. The difference in hair scoring between BCS 2 ≤, 2.01-2.5 and ≥2.51 groups was statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS 2 ≤ group was found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS 2.01-2.5 group was found to be statistically significant. The difference between live weight, FAMACHA © and hair scoring in BCS ≥2,51 group was found to be statistically significant (Table 3).

Table 3. The birth periods least squares means and standard errors for the effect of body condition score on live weight, FAMACHA © and hair score (Mean±SEM)

Bcs grups	**N**	**LW**	**Famacha**	**hair score**
2 ≤	12	43.50±4.36^{aA}	3.50±0.50^{bA}	2.50±0.50^{cA}
2.01-2.5	7	50.09±1.98^{aB}	2.00±0.85^{bB}	1.33±0.49^{cB}
≥2.51	3	52.45±3.86^{aC}	1.80±0.45^{bC}	1.20±0.45^{cC}
	22	*	*	*

a, b, c,… The differences between averages with different letters on the same line are statistically significant.
A, B, ... The differences between averages with different letters in the same column are statistically significant.
* P <0.05

Correlation between BCS and FAMACHA © Graphic score was found to be negative and moderate significant (-0.392) (Table 4).

Table 4. Correlations between BCS and the FAMACHA© Chart scores

	FAMACHA © Chart Score
BCS	-0.392*

Average BCSs according to FAMACHA © Chart scores are given in Table 5. BCS averages corresponding to the FAMACHA © Chart score are lower. According to the FAMACHA © Chart, the average BCS corresponding to the first and second points not considered anemic was found to be 2.28 (Table 5).

Table 5. BCS's according to the FAMACHA© Chart Scores (Mean±SEM)

Eyes scores	**BCS**
1,01-2	2.28±0.48[a]
2,01-3	2.02±0.31[b]
3,01-4	1.55±0.07[c]
P	*

a, b, c,… The differences between averages with different letters on the same line are statistically significant.
A, B, ... The differences between averages with different letters in the same column are statistically significant.
* P <0.05

Discussion

In this study, it was determined that the FAMACHA © and hair score values of 2.01-2.5, ≥2.51 BCS groups were the best. It was determined that as the BCS increased, the FAMACHA © chart scores decreased (Table 1,2,3). There was a high and negative correlation between BCS and FAMACHA © graphic scores (Table 4). *Yılmaz et al. (2014) and Yılmaz et al. (2016*)/ Similar results were found in their study. While BCS increased, FAMACHA © chart scores decreased. In other words, if the BCS was high, the FAMACHA © scores were low, which means the animal was not anemic. Otherwise, the animal is anemic. The 1.0 BCS is an extremely lean goat with no fat reserves, and the 5.0 BCS is a very overweight (obese) goat. In most cases, healthy goats should have a BCS of 2.5 to 4.0. 1.0, 1.5, or 2.0 BCS indicates that BCS with management or health issue is 4.5 or 5. BCS has been associated with the ability to accumulate body energy reserves (usually fat deposits) and reproductive efficiency, and more recently as an indicator of well-being in small ruminants *(Caldeira et al., 2007; Kenyon et al., 2014; Carlos et al., 2015).* In our study, it was determined that goats in 2.01-2.5, ≥2.51 BCS groups had a healthier pregnancy period. It was also observed that FAMACHA © and hair score values (Table 1, 2, 3) were better.

In a study in sheep, statistical differences were found in hematological values only in animals with BCS>4, while no hematological differences were found in other groups with lower BCS *(Torres-Chable et al., 2020).* Again in the same study, no significant difference was observed between the FAMACHA© score and BCS groups *(Torres-Chable et al., 2020).* In our study, there was a statistical difference between the FAMACHA© score in 2.01-2.5, ≥2.51 BCS groups.

Conclusion

As a result of statistical analysis, the effect of BCS on live weight was found to be statistically significant. Correlation between BCS and FAMACHA © Graphic score was found to be negative and moderate significant (-0.392). In the initial period of pregnancy, the effect of BCS on Famacha and Live weight wasn't statistically significant, but its effect on hair scoring was found to be significant (P <0.05). The effect of BCS on LW, FAMACHA © and hair score in late pregnancy period and delivery period was found to be statistically significant (P <0.05). It was determined that BCS and LW of goats before and during the mating period had a positive effect on the number of goats and live weight of goats at birth. It was seen again in this study that proper nutrition is very important in the last months of pregnancy. The decrease in BCS and LW of goats that gave birth to many is an indication of this. (Table 3) As a result of good care and feeding practices during the pregnancy period, BCS of Saanen goats increased during the birth period and body weight and FAMACHA © were positively affected.

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NEW ASPECTS IN RISK STATUS EVALUATION OF SMALL RUMINANT LOCAL BREEDS IN SERBIA

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Abstract: Several factors have led to a decline in autochthonous breeds, and the need for conservation programs based on risk assessment has increased. The aim of the study was to compare and validate two models for risk assessment of local small ruminant breeds in Serbia. The first model considered only the effective population size (Ne), while the alternative model included Ne, the number of females and sub-factors representing the influence of different elements. The results indicate an increasing trend in most breeds and populations over the last five years, with the exception of Tsigai and Chokan Tsigai, which showed a decrease. However, the increasing trend is insufficient, especially for Pirot, Karakachan, and Bardoka, as well as for the Domestic (Serbian) white goat, which is classified as critically endangered in both models. Vlashko vitoroga, Chokan tsigai, and the Balkan goat were at high risk. The models produced different results for the Krivovir, Sjenica, and Svrljig strains. In the first model, Krivovir was classified as potentially endangered, whereas Sjenica and Svrljig were not. In the second model, Krivovir was classified as highly and the other two strains as potentially endangered. These differences are due to the additional factors in the second model, which lead to a more comprehensive assessment for future risk assessments. Although the new model is appropriate for Serbia, it is important to test it on a variety of native species to increase confidence. Future conservation programs should also include genetic characterization and implement adaptive models to obtain more reliable conclusions.

Key words: sheep, goat, genetic resources, endangerment assessment, conservation

Introduction

The term animal genetic resources refers to the diverse pool of genetic material, such as genes and genetic traits, found in various domestic animal breeds and populations. Basically, these are local and autochthonous breeds that are specific to a particular area. These resources include inherent genetic variability that allows animals to adapt to different environmental conditions, exhibit different physical and physiological characteristics, and possess different production capabilities. Because of the unique genetic combinations, conservation and utilization of genetic resources is critical for several reasons, including biodiversity, adaptation and resilience, productivity, disease resistance, and adaptation to climate change.

Different specific environmental factors such as climate, geological and hydrological parameters, nutrition, animal migrations, and other factors have contributed to the formation of different ecotypes and breeds of sheep and goats that differ in morphological, reproductive, and productive characteristics. They represent a unique genetic combination and an important link in the agricultural biodiversity, tradition and cultural heritage of Serbia *(Ružić Muslić et al., 2015)*. On the territory of Serbia, the representatives of autochthonous sheep breeds are Pramenka (Zackel) with its differentiated strains *(Cekic et al., 2018)* and Tsigai (Tsigai and Chokan Tsigai), and the representatives of goats are Balkan goat and Domestic (Serbian) white goat *(Maksimović et al., 2023).* Due to various factors (political, demographic, economic, etc.), autochthonous breeds were displaced by imported, conditionally more productive breeds intented for intensive systems, which affected the decreasing number of animals of local breeds. *Reist-Marti et al. (2003)* claimed that the introduction of imported (exotic) genotypes threatened at least 1000 breeds with extinction worldwide, representing a major loss of genetic diversity. For sustainable management of animal genetic resources in a sustainable way, their monitoring and characterization must be introduced *(ERFP, 2021).* As the size of local breed populations decreases, the need for risk assessment increases.

Assessment of livestock risk status is a key component of early warning and response systems in countries *(Verrier et al., 2015)* and has been studied in many countries *(Polak et al., 2021)* using a variety of methods *(Urošević et al., 2022)*. Initially, assessments were based on total population size and the proportion of matings in the pure breed *(Scherf, 2000),* but later methods incorporated other factors, such as demographic, genetic, and socioeconomic factors *(FAO, 2007),* as well as the time required to reach a critical population size *(Gandini et al., 2005).* Furthermore, *Alderson (2009)* claimed that in addition to the above factors, the occurrence of the same breed in many countries, as well as anthropogenic, climatic, and epidemiological factors should be considered when assessing risk status. Also, *FAO (2013)* published guidelines for breed conservation based on total population size, breeding population size, and reproductive capacity of species, and established the Domestic Animal Diversity Information System (DAD-IS) and the European Farm Animal Biodiversity Information System (EFABIS) to assess risk status.

Nowadays, the methods for assessing risk status and their specific factors are country-dependent, and most of them focus on population size and the measures needed to reduce inbreeding rates and maintain heterozygosity *(Notter, 1999).* Although there is a lack of harmonization of procedures, most conservation programs classify breeds according to extinction risk based on their endangerment status *(Alderson, 2009).* Based on this fact, the aim of this study is to evaluate the endangerment status of local breeds of small ruminants in Serbia by using two models, comparing and validating their results.

Materials and Methods

Data on population size, and number of breeding animals (male and female) under control of productive traits were taken from databases and annual reports on activities and results of control of breeding programs implementation in 2022 of the two main breeding organizations for small ruminants in the Republic of Serbia - Institute for Animal Husbandry, Belgrade (for Central Serbia) and Department of Animal Science, Faculty of Agriculture, University of Novi Sad (for Vojvodina region), as well as DAD-IS database of *FAO (2021).*

Two approaches were used to assess the risk status of local breeds in Serbia. The first one corresponds to the official model of the Ministry of Agriculture, Forestry and Water Management described in the Decree on the List of Domestic Animal Genetic Reserves, the Method of Conservation of Domestic Animal Genetic Reserves and the List of Native Domestic Animal Breeds and Endangered Native Breeds *(Official Gazette 30/22),* while the second one represents the model described and proposed by *Polak et al. (2021).* In both, effective population size (Ne) was used as the main parameter for representing genetic diversity and inbreeding rates in animal populations

The official model focused on risk assessment using only effective population size, using the formula: **$Ne = 4Nm \times Nf/(Nm+Nf)$**, where Nm is the number of breeding males and Nf is the number of breeding females. In terms of the degree of endangerment, populations are divided into four groups: critically endangered ($Ne \leq 50$), highly endangered ($Ne > 50$ and $N \leq 200$), potentially endangered ($Ne > 200$ and $N \leq 1000$), and not endangered ($N > 1000$).

The alternative model used the correction of Ne by multiplying it by a factor of 0.7 (Ne_c), assuming random selection of males and females for breeding *(Santiago and Caballero, 1995).* In addition to Ne_c, the method considered additional factors and included the total number of breeding females (L) and a complex factor (SSF) composed of six elements: geographic concentration within a country (GC), demographic trend over the past five years (DT), cultural value of the breed (VC), control of ancestry (PC), presence of *ex situ* conservation (EX), and anthropogenic factors that include breeder age, implementation of existing conservation programs, and potential financial support for breed conservation (AF). To mitigate the potential effects of subjectivity and its potential influence on results, the SSF factor is divided by 1/2, reducing the likelihood of estimation error. If L is less than 150, breed is classified as critically endangered, regardless of the final outcome. Further descriptive details of the parameters and subfactors are published in *Polak et al. (2021).* Each of the listed parameters was defined by the thresholds and scoring values listed in Table 1, and the following formula was used to calculate the risk status (RS) of each strain/breed: **$RS = (L + Ne_c + 0.5SSF)/3$**. Based on the RS value, populations are sorted as follows: ≤ 1 - critical; > 1 ≤ 2 - highly endangered breed required action; > 2 < 3 - potentially endangered breed requiring monitoring; > 3 - not at risk.

Table 1. Scoring range regarding the factors and sub factors for risk evaluation

Parameter	Score value				
	0	0.5	1	2	3
Total number of females **L**	< 150	/	151 - 1000	1001 - 6000	6001 - 20000
Corrected Effective population size **Ne_c**	≤ 50	/	50 - 200	201 - 1000	> 1000
Geographical concentration	≤ 25%	25 - 75%	> 75%	/	/
Demographic trend	Down	Stable	Upward	/	/
Cultural and historical value	Present	Small value	No value	/	/
Parentage control	Lacking	Existing to a small extent	Existing	/	/
Ex situ conservation	Lacking	Existing to a small extent	Existing	/	/
Anthropogenic factors	Lacking	Existing to a small extent	Existing	/	/

Results and Discussion

Regarding the number of animals in the last five years, Table 2 shows that there is an upward trend in most populations, except for Chokan Tsigai and Tsigai, where the number of animals is lower in 2022 compared to 2021. Although for most observed populations this trend is promising, it is still not satisfactory. This is of particular concern for breeds that are not transboundary and are not regionally distributed, such as the Pirot strain of Pramenka and the Domestic (Serbian) white goat. In these populations, intervention by importing new, unrelated animals is not possible, and the risk of inbreeding is higher. In a small population, inbreeding increases because after one generation each individual is related to some degree to every other individual *(Meuwissen, 2009)*, which leads linearly to a decrease in genetic variance *(Falconer and Mackay, 1999).*

Table 2. Number of local breeds under control of productive parameters in the last five years

STRAIN / BREED	YEAR				
	2018	**2019**	**2020**	**2021**	**2022**
SHEEP					
Pirot strain	129	149	197	195	302
Karakachan strain	165	176	186	225	252
Krivovir strain	816	1,103	1,118	1,600	2,068
Bardoka strain	117	107	142	144	207
Sjenica strain	74,604	105,573	139,319	176,534	199,571
Svrljig strain	14,807	18,166	19,585	25,857	29,285
Lipa strain	941	1,280	1,636	2,102	2,866
Vlashko vitoroga (Racka)	331	492	1,060	1,131	1,150
Chokan tsigai	1,098	1,834	2,092	2,505	2,485
Tsigai	5,580	5,613	5,161	4,362	4,227
GOAT					
Balkan goat	427	590	791	921	1.153
Domestic (Serbian) white	138	152	168	147	201

The goal of comparing the two methods was to evaluate various factors that classify a particular breed as being in danger of extinction. The results presented in Table 3 include the risk assessment according to the official model, considering all four endangerment categories. Thus, in the group of critically endangered populations, Pirot, Karakachan and Bardoka strain, Vlashko vitoroga and Chokan tsigai are at high risk, while Krivovir and Lipa strain and Tsigai are at potential risk. On the other hand, the most numerous populations of local sheep breeds (Sjenica and Svrljig) are not threatened with extinction. In the case of goats, both breeds are threatened with extinction, while the Serbian (domestic) white is critical and the Balkan goat is highly endangered. As mentioned above, this model is based on Ne, one of the most important parameters in monitoring local breed populations *(Verrier et al., 2015)*, as it allows predicting changes in genetic variance *(Wright, 1931).*

Table 3. Risk status of small ruminants local breeds according to official model

STRAIN / BREEDS	**Number of females**	**Number of males**	**Ne**	**Risk status**
SHEEP				
Pirot strain	290	12	46.09	critically endangered
Karakachan strain	240	12	45.71	critically endangered
Krivovir strain	1.980	88	337.02	potentially endangered
Bardoka strain	198	9	34.43	critically endangered
Sjenica strain	194,342	5,229	20,367.98	non endangered
Svrljig strain	28,367	918	3,556.89	non endangered
Lipa strain	2,780	86	333.68	potentially endangered
Vlashko vitoroga (Racka)	1,102	48	183.99	highly endangered
Chokan tsigai	2,445	40	157.42	highly endangered
Tsigai	4,059	168	645.29	potentially endangered
GOAT				
Balkan goat	1,115	38	146.99	highly endangered
Domestic (Serbian) white	191	10	38.01	critically endangered

Using the model proposed by *Polak et al. (2021)*, the results regarding risk assessment of local strains and breeds are similar to the previous model, and the Pirot, Kakarachan, and Bardoka strains and the Domestic (Serbian) white goat are listed in a group of critically endangered populations, while Lipa, Vlashko Vitoroga, Chokan Tsigai and Tsigai, and the Balkan goat are highly endangered populations requiring action, as listed in Table 4. This similarity may indicate that the newly proposed model can be effectively used to assess the risk status of small ruminants under the conditions prevailing in the Republic of Serbia. The main difference between these two models is with respect to the strains Krivovir, Sjenica and Svrljig. While the results of the official model showed that the Krivovir strain is potentially endangered, and the other two strains are stable, non endangered, the results of the alternative model showed that Krivovir is highly endangered, while Sjenica and Svrljig are potentially endangered, and that surveillance is still needed. This discrepancy is the result of the use of additional subfactors detailed in terms of demographic, cultural, anthropogenic, and conservation aspects that enhance the risk assessment approach. The results suggest that these factors have a real and strong influence on the sustainability of a strain, breed or population.

Table 4. Risk status of small ruminants local breeds according to number of females, corrected effective population size and additional sub factors

STRAIN / BREEDS	**L[1]**	**Score L[2]**	**Ne$_c$[3]**	**Score Ne$_c$[4]**	**Sub factors**							**RS[12]**	**Risk status[13]**
					GC[5]	**DT[6]**	**VC[7]**	**PC[8]**	**EX[9]**	**AF[10]**	**SSF[11]**		
SHEEP													
Pirot strain	290	1.00	32.26	0.00	0.50	0.00	1.00	0.50	1.00	1.00	4.00	1.00	CE
Karakachan strain	240	1.00	32.00	0.00	0.50	0.00	1.00	0.50	1.00	1.00	4.00	1.00	CE
Krivovir strain	1.980	2.00	235.91	2.00	1.00	0.00	1.00	0.50	0.00	1.00	3.50	1.92	HE
Bardoka	198	1.00	24.10	0.00	0.50	0.00	1.00	0.50	1.00	1.00	4.00	1.00	CE
Sjenica strain	194,342	3.00	14,257.58	3.00	1.00	1.00	1.00	0.50	1.00	0.50	5.00	2.83	PE
Svrljig strain	28,367	3.00	2,489.83	3.00	1.00	0.00	1.00	0.50	0.50	0.50	3.50	2.58	PE
Lipa strain	2,780	2.00	233.57	2.00	1.00	0.50	1.00	0.50	0.50	1.00	4.50	2.08	PE
Vlashko vitoroga (Racka)	1,115	2.00	128.79	1.00	1.00	0.50	1.00	0.50	0.50	1.00	4.50	1.75	HE
Chokan tsigai	2,453	2.00	110.20	1.00	1.00	0.50	1.00	0.50	1.00	1.00	5.00	1.83	HE
Tsigai	4,097	2.00	451.70	2.00	1.00	0.50	1.00	0.50	1.00	1.00	5.00	2.17	PE
GOAT													
Balkan goat	1,115	2.00	102.89	1.00	0.50	0.00	1.00	0.00	1.00	1.00	3.50	1.58	HE
Domestic (Serbian) white	191	1.00	26.61	0.00	0.50	0.00	1.00	0.50	1.00	1.00	4.00	1.00	CE

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[1]L - Number of breeding females; [2]Score value regarding the L; [3]Ne$_c$ - Effective population size corrected for uncontrolled breeding; [4]Score value for Ne$_c$; [5]GC- sub factor of geographical

concentration; [6]DT - sub factor for trend of demography; [7]VC - sub factor representing the cultural value of breed/strain; [8]PC - sub factor representing the value of parentage control; [9]EX - value regarding to existence of *ex situ* conservation; [10]AF - value of anthropogenic factors; [11]SSF - Total value of sub factors; [12]RS - risk status; [13]CE - critically endangered, HE - highly endangered breed requiring action, PE - potentially endangered breed, requiring monitoring.

One of the most important factors contributing to the increase in the risk of extinction is anthropogenic factors. For conservation of local breeds, the socio-political context of humans must be understood as it must have some impact on the livelihood of breeders *(Gizaw et al., 2008)*. Conservation of local breeds should be cost-effective and based on a multi-criteria decision *(Wainwright et al., 2019). Meuwissen (2009)* claims that the best strategy to conserve an endangered breed is to make it profitable. In the Republic of Serbia, there are governmental measures in the form of subsidies whose main goal is to achieve profitability and economic efficiency of genetic resources *(Nastić et al., 2020).* However, they cannot always cover the increasing production costs. Another critical factor is the low awareness of breeders about the importance of local breeds.

In addition, demographic trends over the last five years are an important factor that can be used to objectively assess population trends. The main areas for breeding local sheep and goat populations are rural areas, and with depopulation, the number of livestock in general is decreasing, as well as the number of genetic resources.

The cultural value of the breed and its historical significance are important factors that should be considered in risk assessment. These breeds have great potential in the artisanal processing of milk, meat, and wool into traditional products that have a strong influence on folklore, gastronomy, tourism and religious tradition. In other words, the loss of local breeds has an impact on the loss of cultural value and the loss of historic landscapes *(Meuwissen, 2009).*

A model based on a multifactor analysis is certainly more comprehensive than one that uses only Ne as a risk assessing element. In addition, the use of a larger number of indicators leads to a more continuous view of the degree of risk. Obtaining the information, defining the indicators, and establishing the score values is also relatively straightforward, as the two main breeding organizations have information to track breeds in terms of population size and structure, as well as productive traits. In addition, combining several different indicators can minimize the possibility of undermining the purpose of the score. In other words, when the number of indicators in the model is increased, the reliability of the assessment at risk increases and the possibility of error is reduced.

However, it should be noted that while this model works for small ruminant breeds in Serbia, its suitability should be investigated for other domestic species. To ensure even greater reliability, future conservation programs should include, in addition to these factors, the aspect of genetic conservation, the main objective of which would be to maintain breed diversity. The models could be modified according to the availability of new information on novel indicators or subfactors so that the information is wholesome for their calculations.

As stated by *Ružić-Muslić et al. (2021)*, the concept of sustainable use of genetic resources consists in their identification, description, development and monitoring, leading to their conservation. To ensure efficient management of genetic resources, it is important to maintain breed registers, conduct genetic evaluations, promote controlled breeding initiatives, and support sustainable breeding practices. These measures are essential to prevent genetic erosion and improve the overall quality of small ruminant populations. The models used to assess vulnerability must be able to evolve as new information is obtained and as legislation and objectives change.

Conclusion

Sustainable use of genetic resources requires their identification and description, development and monitoring, and consequently methods for their conservation. One of the ways to perform monitoring and propose development measures is to assess the risk of extinction. In this study, two risk assessment models were used and their results are compared. In the majority of the observed populations, there is an increasing trend in the number of animals, with the exception of Tsigai and Chokan Tsigai, which are decreasing. However, in the populations of Pirot, Karakachan, Bardoka (sheep) and Domestic (Serbian) white goat, which are classified as critically endangered in both risk status assessment models, the number of animals is insufficient. Although the results regarding the risk status of both models are similar, the alternative model provides a broader perspective as it takes into account demographic, sociocultural, geographic, anthropogenic, and conservation factors. This model was proposed for Poland, but the results show that it can also be used to assess local breeds in Serbia. Further research is needed to assess the risk status for other species of livestock genetic resources. To ensure greater reliability of results, future work should also focus on aspects of genetic characterization, with the main goal of obtaining meaningful information that can be implemented in conservation programs.

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PRNP GENE POLYMORPHISMS IN HEALTHY GREEK SHEEP FROM 2017 TO 2022 - NATIONAL DATABASE FROM RESISTANT RAMS

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Abstract: Scrapie is a slowly progressive infectious disease of sheep and goats that causes central nervous system degeneration. It is one of several transmissible spongiform encephalopathies (TSEs). The main constituent of the infectious agent is the scrapie isoform (PrP^{Sc}) of the normal cellular (PrP^{C}) prion protein (PrP). In sheep, three polymorphisms of the host *PRNP* gene that encodes the PrP, at codons 136, 154, and 171 are closely linked to susceptibility or resistance to natural and experimental scrapie. This information is used to implement national breeding programs to reduce the susceptibility to scrapie. For many years, Greece was a country with a high percentage of positive cases, but after the implementation of the National Surveillance Programme, scrapie cases have decreased. In the present study, we present the PrP Types (1 to 5, according to the UK National Scrapie Plan) of 11569 blood samples collected from clinically healthy rams during 2017-2022, in comparison with blood samples analyzed at 2012-2016. Samples were analyzed by Real - Time PCR (SNP detection - TaqMan probes). Types 1 and 2 linked with resistance to the disease showed an exceptionally high percentage, especially in 2019 (53.76% and 46.24, respectively). In contrast, Type 3, associated with susceptibility to the disease, was relatively reduced in 2019 (15.88%). The private initiatives of farmers, in collaboration with the Veterinary Research Institute, have resulted in the establishment of a substantial number of farms with a significant population of resistant rams.

Key words: Scrapie, scrapie types, sheep, Real-Time PCR

Introduction

Scrapie is a slowly progressive but fatal infectious disease of sheep and goats that causes central nervous system degeneration. The main constituent of the infectious agent is an aberrant isoform (PrP^{Sc}) of the normal cellular (PrP^{C}) prion protein (PrP), which is a cell–surface glycoprotein *(O'Rourke et al., 1997)*. The cellular prion protein (PrP^{C}) suffers a conformational change, and its α-helix-rich folded structure turns into a pathogenic β-sheet-rich conformer PrP^{Sc} *(Baral et al., 2019)* with abnormal biological properties, such as insolubility and resistance to proteolytic degradation.

Since 1998, two types of scrapie have been identified: classical scrapie and atypical. Classical scrapie that occurs in young and old animals and exhibits vertical and horizontal transmission is documented in both sheep and goats, and atypical scrapie that was initially described in Norwegian sheep in 1998 *(Balch, 2022)*.

According to epidemiological studies, the transmission of classical scrapie occurs predominantly through oral exposure in a contaminated environment, principally from the shedding of PrP^{Sc} in the placenta and placental fluids *(Onodera et al., 1993; Andreoletti et al., 2002)*, also feces *(Terry et al., 2011)* and carcasses of infected animals *(Miller et al., 2004)*, by direct contact of animals, but also through the ingestion of milk and colostrum *(Konold et al., 2008)* or intrauterine transfer *(Foster et al., 2013; Garza et al., 2017)*. Once in the environment, PrP^{sc} can be found on multiple surfaces (particularly metal items), in airborne dust samples, and on pasture, persisting there for years. For example, in Iceland's cold, stable climate, environmental infectivity has lasted at least 16 years. PrP^{sc} has been found in semen, but so rarely and in amounts so small that breeding is still considered to pose a minimal risk of transmission *(Balch, 2022)*.

The transmission and incubation period of the disease depends on exposure to the infectious agent, the scrapie strain, and the host's genetic background *(O'Rourke et al., 1997)*. The *PRNP* gene that encodes PrP has been identified in a large number of species presenting a very high homology. A large number of polymorphisms have been described in the *PRNP* gene of different species which have a key role in the stability or conversion of PrP^{C} to the pathogenic isoform PrP^{Sc} *(Hunter, 1997; Bossers et al., 1997)*.

The prion protein of sheep is a protein of 256 amino acids. Over 15 polymorphisms of the *PRNP* gene in sheep have been reported *(DeSilva et al., 2003)*. Still, only the polymorphisms at codons 136, 154 and 171 are known to be closely linked to susceptibility to natural and experimental classical scrapie *(Bossers et al., 1996; Hunter et al., 1996; Dawson et al., 1998; Elsen et al., 1999; Thorgeirsdottir et al., 1999; Tranulis et al., 1999)*. The polymorphisms at codon

136 (Alanine/Valine/Threonine; A/V/T), codon 154 (Arginine/Histidine; R/H) and codon 171 (Arginine/Glutamine/Histidine/Lysine; R/Q/H/K) have been analyzed in many studies *(Hunter et al., 1996).* It has been shown that the VRQ haplotype is strictly associated with susceptibility in homozygosis and heterozygosis *(Belt et al., 1995; Hunter et al., 1996),* while the ARR/ARR genotype is correlated with resistance to scrapie *(Goldmann et al., 1994; Hunter et al., 1994; Baylis et al., 2002).* Variation of these codons results in a several genotypes identified in sheep. According to the United Kingdom National Scrapie Plan, these genotypes have been categorized into Types 1 to 5 regarding their association with scrapie resistance or susceptibility, with Type 1 being the most resistant to scrapie and Type 5 the most susceptible that should not be used for breeding. This information is used in European countries to implement national breeding programs to reduce the susceptibility to scrapie *(Arnold et al., 2002).*

Scrapie control relies mainly on surveillance and selective breeding of naturally genetic-resistant animals. Scrapie was first diagnosed in Greece in 1986 *(Leontides et al., 2000). Ekateriniadou et al. (2007)* described the alleles and genotype frequencies of healthy sheep from 13 rare breeds as well as from healthy and scrapie-affected sheep in the period 2003-2005. In our previous study *(Boukouvala et al., 2018),* 5815 blood samples of clinically healthy sheep that had been collected from 2012-2016, showed an increased percentage of the two genotypes, ARR/ARR - Type 1 and ARR/ARQ – Type 2, linked with resistance to the disease and relatively reduced percentage of the genotype ARQ/ARQ – Type 3 which is associated with disease susceptibility and was the most common genotype in the Greek flocks. In many countries, as mentioned above, but not in Greece, breeding programs have been officially implemented to increase genetic resistance. In Greece the implementation of the National Surveillance Programme, which do not include a breeding program reduced the percentage of scrapie positive cases. The purpose of the current study was to continue, in collaboration with farmers and veterinarians, the determination of the PrP genotypes –Types of clinically healthy sheep originated from all the Greek mainland during the period 2017-2022 to establish the selective breeding of naturally resistant animals and further increase the number of farms with an increased population of scrapie resistant rams.

Material and Methods

Samples

In total, 11.569 blood samples from clinically healthy sheep were analyzed from 2017-2022. The samples originated from all geographical regions of Greece mainland.

Genomic DNA extraction

Genomic DNA was extracted from the blood samples using the PureLink Genomic DNA kit (ThermoFisher Scientific) according to the manufacturer's instructions.

PrP genotype analysis

A Real-Time PCR method was used to detect the eight polymorphisms: 171Q/R/H/K, 136A/V and 154R/H. The analyses were performed by two tetraplex Real-Time PCR reactions as described by *Boukouvala et al. (2018).* The determined PrP genotypes were categorized into the 5 PrP Types as described by the UK National Scrapie Plan.

Results and Discussion

We determined the PrP Types from 11.569 clinically healthy Greek sheep in the present study. The blood samples that were analysed in the period 2017 to 2022 originated from all Greek mainland. The results (Figure 1) showed increased percentages of the resistant PrP Types 1 and 2, reduction of the susceptible Type 3 and nearly extinction of the most susceptible Types 4 and 5.

Our effort to determine the PrP genotypes of clinically healthy Greek sheep started in 2012 and continued until 2016 when 5815 blood samples had been fully genotyped *(Boukouvala et al., 2018).* The analysis showed that the percentage of the most resistant PrP Type 1 increased from 14.53% in 2012 to 30.19% in 2015 and slightly decreased to 22.67% in 2016.The continuation of these analyses showed an increasing trend of Type 1 percentage, starting from 30.07% in 2017 and reaching 43.09% in 2022. The resistant PrP Type 2 percentage in 2012 was 26.50% and, until 2016, had increased to 41.28%, being the most predominant PrP Type. In 2017 the PrP Type 2 percentage increased to 40.49%, while in 2022 was reduced to 36.12%, in parallel the percentage of the susceptible PrP Type 3 showed a reduction trend from 2012 to 2016 (58% to 35.11%) which continued in 2017, detected at 28.73% and stabilized in 2021 and 2022 to 20.6%. Finally, the most susceptible PrP Types 4 and 5 were detected at very low percentages in 2012-2016 and continued at similar levels in 2017-2022 where their percentages were lower than 1% (Figures 1 and 2).

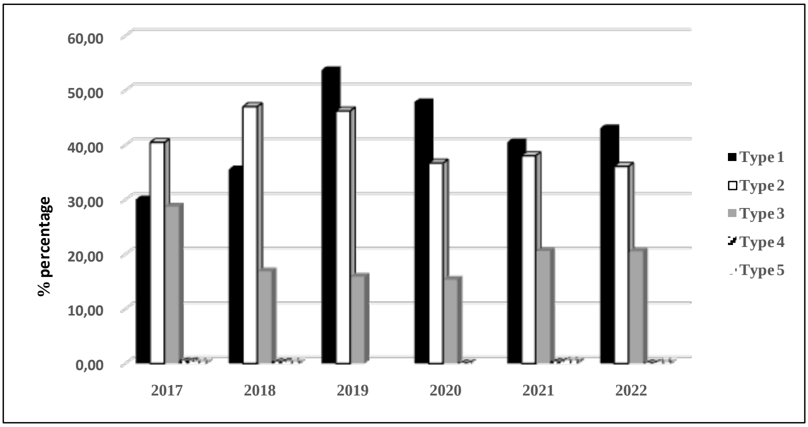

Figure 1. Percentages (%) of the PrP Types determined from 2017-2022.

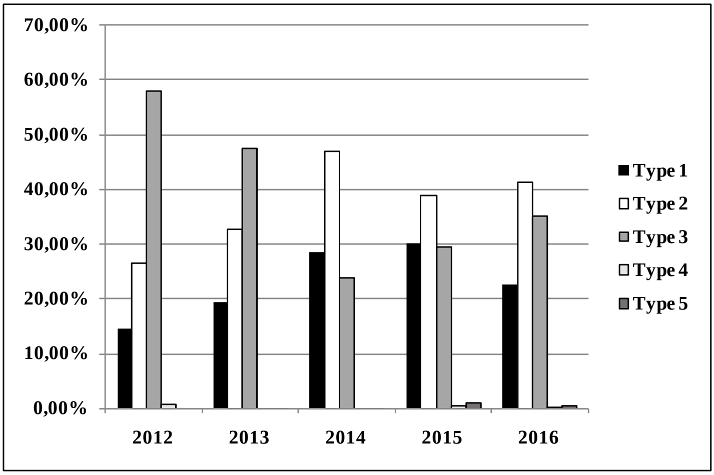

Figure 2. Percentages (%) of the PrP Types determined from 2012-2016 ***(Boukouvala et al., 2018).***

In 2019, the PrP Type 1 reached a high percentage of 53.76%, which could be attributed to the smaller number of samples analysed this year. Only 850 blood samples were analysed in 2019 due to the COVID pandemic and the government's bans forbidding movement and transport. It should also be mentioned that the slight decrease in PrP Type 1 percentage after 2019 was due some farmers' non-continuing selective breeding (due to COVID difficulties) or the new entrance of farmers starting the selective breeding program.

As mentioned above, the PrP Type 2 was the predominant one during the period 2012-2016, especially the ARR/ARQ genotype, which appears as the most frequent genotype in other countries/breeds that have begun an effort to increase the resistance to scrapie, such as the Sicilian sheep *(Reale et al., 2015)* and the Polish Pomeranian Landrace sheep *(Proskura et al., 2013).* The results from the period 2017-2022 showed the most resistant PrP Type 1 as the predominant one, followed by Type 2, which indicates that farmers and veterinarians followed the suggestions for selective breeding against scrapie and continued their efforts to increase the resistance of the Greek flocks to scrapie which were considered as susceptible ones according to the recorded scrapie cases *(Iqbal et al., 2020).*

Conclusions

The results of our study showed that the farmers and veterinarians successfully followed the suggestions for selective breeding against scrapie based on the *PRNP* gene analyses performed during 2017-2022.

The private initiatives of farmers and veterinarians, in collaboration with the Veterinary Research Institute for more than 20 years, have resulted in the establishment of a significant number of scrapie resistant sheep flocks.

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THE EFFECT OF THE APPLICATION OF MELATONIN IMPLANTS IN THE SPRING ON THE MANIFESTATION OF ESTRUS AND FERTILITY IN ILE DE FRANCE SHEEP

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Abstract: The aim of the present study was to determine the effect of the application of melatonin implants in the spring on the manifestation of estrus and fertility in Ile de France sheep. The experiment was carried out with 67 ewes (aged 3-6 years), divided in 2 groups – experimental (EG) (n=22) and control (CG) (n=45) during spring, 2021. The experimental group was treated with implants (Melovin, Ceva Animal Health) on 12th March and the breeding started two months later. Control group was not treated. The observation lasted 1 month (from 12th May to 10th of June). During the observed period, the ewes that came in estrus from the EG was 81.82 % vs. 71.11% from the control group. The fertility was 77.78% and 71.87%, respectively for EG and CG. The obtained results showed that melatonin implants, given solely, didn't have synchronization effect on estrus and fertility.

Key words: sheep, melatonin, estrus, fertility

Introduction

In sheep and goats, there are physiological mechanisms that limit reproduction to the autumn months and births to seasons that are favorable for survival and are characterized by the availability of quality food resources *(Scaramuzzi et al., 2006)*. With a view to year-round production of lambs and sheep's milk, and not only in certain periods of the year (spring-summer), it is necessary to induce estrus and fertilize the sheep during the anestrous period. In sheep breeding practice, two types of methods have been reported to control sex-cyclic activity: hormonal (pharmacological) and non-hormonal (natural), as hormonal methods are more effective for overcoming physiological anestrus. Three types of hormonal methods are known to synchronize estrus: 1.) By delaying ovulation and prolonging the luteal phase or methods based on the action of progesterone or its synthetic analogues (progestagens). The method is applicable both in the anestral and in the estrous season. 2.) By regression of the corpus luteum with Prostaglandin F2α or its synthetic analogues. The method is applicable only when there is a functioning corpus luteum or during the estrous season. 3.) By using melatonin to induce estrous behavior in anestrous ewes. As melatonin mediates day/night information, its administration is intended to mimic a short day and thereby starts the sheep cycling.

In sheep as seasonal breeding animals, melatonin secretion signals the neural structures, controlling pituitary gonadotropin secretion to stimulate their activity according to the season of the year *(Misztal et al., 2002)*. Activation of the gonadotropic system in anestrous sheep requires, however, several weekly exposures to melatonin, during which time the estradiol feedback loop that plays a major role in the GnRH/LH axis is altered *(Legan et al., 1977* cited in *Misztal et al., 2002)*. It takes at least 5 weeks to increase blood melatonin concentrations and accelerate the season, and to synchronize estrus, melatonin should be combined with the use of other methods, such as shortening the light day, ram effect or vaginal sponges *(Dankó, 2003).*

The aim of the present study was to determine the effect of the application of melatonin implants in the spring on the manifestation of estrus and fertility in Ile de France sheep.

Materials and Methods

The experiment was carried out during spring 2021, with 67 Ile de France ewes (aged 3-6 years, BCS 3.0-3.5), raised in experimental station of Institute of Animal Science, Kostinbrod. The sheep were fed on pasture and supplemented with concentrated mix (17% crude protein) in a dose of 250-300 g / per head per day. After the end of the controlled breeding campaign, the animals were sheared and transported to a high mountain pasture. The sheep were divided in 2 groups – experimental (EG) (n=22) and control (CG) (n=45) The experimental group was treated with implants (Melovin, Ceva Animal Health) on 12th March and the breeding started two months later. The implants were put subcutaneously on the ear with special gun Control group was not treated. The observation lasted 1 months (from 12th May to 10th of June). Every morning, the teasers were introduced to the ewes (ratio teaser/ewes 1:40) and those that were in heat were recorded. Ewes in heat were“hand” mated and each ram did up to 3 services per day.

The following parameters were studied:

1. Time of manifesting of estrus for the first time (in days) after the start of campaign - recorded daily for 30 days

2. Fertility - defined as the ratio of the number of ewes pregnant to the number of ewes with manifested estrus.

3. Fecundity - defined as the number of born lambs from pregnant ewes (included all born lambs – live and dead).

Fertility and fecundity were calculated after lambing. The data was presented in percentages and numbers. The significance of the differences between groups about the time of manifesting of estrus for the first time and fertility were established by the Fisher's exact test (http://graphpad.com/quickcalcs/contingency1/).

Results

During the observed period, the ewes that came in estrus from the EG was 81.82 % (18 of 22) vs. 71.11% (32 of 45) from the control group (Figure 1). If we divided the experimental period into three sub periods, each of 10 days, it was observed that in both groups the most sheep showed estrus for the first time were during the 2-nd decade (11-20 days) - 40.91% (9 of 22) for EG and 40.0% (18 of 45) for CG. Only in the first ten days, the percentage of ewes with manifested estrus was higher for EG - 31.82% (7 of 22) compared to 22.22% (10 of 45) for CG, but the difference was not significant ($P>0.05$). In the third ten days, the percentage of ewes with manifested estrus was also similar – 9.09 % (2 of 22) and 8.89 (4 of 45) for EG and CG, respectively. The percentage of sheep that did not show estrus for these thirty days was of interest - 18.2% (4 of 22) for EG and 28.89% (13 of 45) for CG, but the difference also was not significant ($P>0.05$). What this is due to, we can only speculate: either the ewes showed several quiet estruses and were not noticed by the rams, or the rams did not show interest in them, and therefore they were not detected, or these ewes were still in anestrus.

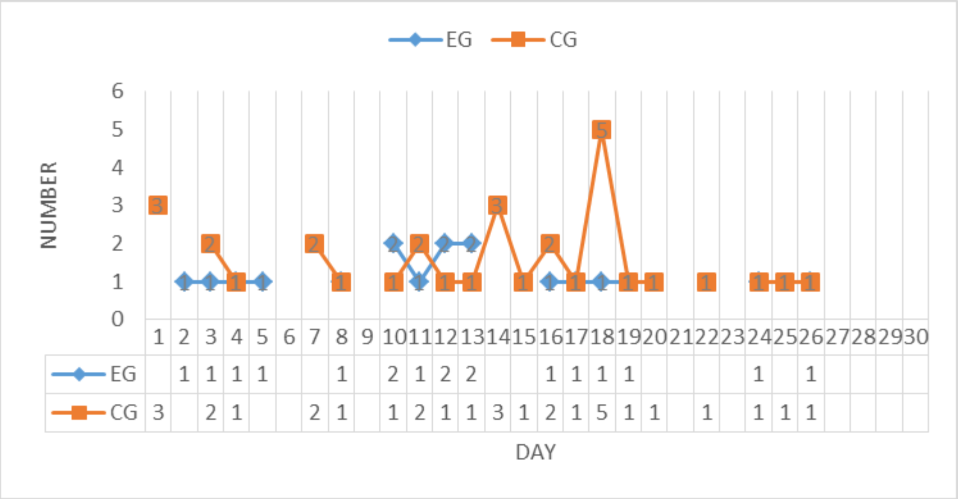

Figure 1. Distribution of ewes, that manifested estrus for the first time

The fertility was 77.78% and 71.87%, respectively for EG and CG, as the difference was not significant (P>0.05) (Table 1). Fecundity was 128.57% for EG and 113.04% for CG.

Table 1. Fertility and fecundity of the experimental groups.

Group	Fertility		Fecundity	
	%	ewes	%	lambs
EG (n=18)	77.78%	14	128.57	18
CG (n=32)	71.87%,	23	113.04	26

Discussion

Most often, melatonin is administered through subcutaneous implants. They are widely used to accelerate the breeding season in anestrous sheep and goats *(Abecia et al., 2011)*. The subcutaneous melatonin implant is placed 30-40 days before the rams are collected with the ewes *(Forcada et al., 2002).*

The obtained results from the present study showed that melatonin implants, given solely, didn't have synchronization effect on estrus and fertility. The results for both groups were similar and no statistical differences were found.

In Bulgaria, *Bonev (2008)* placed implants 40 days before the release of the rams at the end of the anestrous and the beginning of the estrous season (June to mid-July) in sheep from the North-East Bulgarian Thin-wool (NEBT) and the Synthetic Bulgarian Milk Population (SPBM), as peaks in the manifestation of estrus were observed on the 24-th day in dairy ewes and on the 28-th in thin-wool sheep, and the fertility for the studied 45-day period was 91.0% in NEBT and 62.0% in SPBM. A similar experiment was conducted by *Bonev and Yotov (2008)* with 250 sheep of the Ile de France breed. The use of melatonin implants has caused a withdrawal of the breeding season, thickening of conception (36 days), a high rate of conception from first (71.0%) and second (78.0%) estrus. In both studies, the authors didn't prepare control group (without treatment), so the actual effect of melatonin implants could not be seen.

The influence of melatonin on the reproductive axis is controversial. *Zarazaga et al. (2003)* demonstrated that changes in plasma melatonin content (day/night concentration ratio) did not affect the sex-cyclic performance of sheep. The authors reported that no relationship was observed between the variability in the onset and the offset of the annual breeding season of Ile-de-France sheep reared in France (45°N latitude) and the variability in the plasma melatonin concentrations, either absolute or relative to the summer or the winter solstices, respectively.

The results for fecundity is lower than those reported from other authors. *Achkakanova and Staykova (2019)* stated that Ile de France breed was successfully adapted in our country and mean prolificacy was 1.58 lambs per ewe, as the highest prolificacy had sheep at fourth parity – 1.72 lambs per ewe. *Laleva et al. (2020)* analyzed the phenotypic characteristics of breeding traits in sheep from two Ile de France flocks, reported the average fecundity was in the range of 1.573 to 1.717 lambs per ewe. It reaches its maximum at the third lambing for the animals reared in the Agricultural Institute – Stara Zagora and at the fourth in IAS – Kostinbrod.

The lowest values of fecundity, according to us, were due to several factors: transport and shearing stress; mistakes in nutrition during pregnancy – sheep were malnourished in some periods, because of errors of people, that looked after them. It is well known, that stressors, such as poor body condition, adverse temperatures or even common management procedures (e.g., transport or shearing) suppress normal oestrus behaviour and reduce ewe fertility *(Dobson at al., 2012)*. In general, a low BCS and the realization of a negative energy balance have a negative impact on the reproduction of sheep (reviewed by Scaramuzzi et al. 2006). There is a positive correlation between ovulation rate and live weight/BW, which affects later stages on fertility and fecundity *(Kleeman and Walter, 2005)*.

Conclusion

The obtained results showed that melatonin implants, given solely, didn't have synchronization effect on estrus and fertility. This method for estrus synchronization should be combined with others, such as ram effect or progestogens. During the observed period, the ewes that came in estrus from the experimental group (melatonin treatment) was 81.82 % vs. 71.11% from the control group (no treatment). The fertility was 77.78% and 71.87%, respectively for the experimental and control group.

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GENE POLYMORPHISM FREQUENCIES IN KRŠKOPOLJE PIG BREED

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Abstract: Genetic polymorphisms are associated with different production traits in pigs and some of them have major effects. In local pig breeds, which are usually characterised by small effective sizes, elimination of unfavourable alleles is more challenging because care must be taken to maintain genetic diversity. Therefore, the aim of the present study was to analyse the frequency of several causal mutations in the local breed Krškopolje pig associated with fatness and meat quality and to estimate the selected diversity parameters. A total of 253 DNA samples of Krškopolje pigs were analysed with Choice Genetics' custom SNP-chip. We observed a relatively high frequency (0.17) of stress syndrome mutation (mutated c. 1843T allele at *RYR1*). The mutated c.749A allele at *PRKAG3* gene (Arg250Gln or RN$^-$) associated with high muscle glycogen deposition was absent. The frequency of the variant at *PRKAG3* (Ile249Val) encoding 249Ile (associated with higher pH, fatness, darker colour, better water holding capacity) was 0.22, and of the variant encoding 102Ser was 0.50. The frequency of *MC4R* 892A allele associated with high lipid deposition and growth was 0.57. The average observed and expected heterozygosity values were 0.37 and 0.36, respectively, which is relatively high for a local pig breed. The present results demonstrate high frequencies of both unfavourable and favourable alleles affecting meat quality in Krškopolje pigs. Further monitoring of key polymorphisms and genetic diversity parameters is needed to develop conservation and breeding strategies.

Key words: local pig breed, candidate genes, SNPs, breeding program

Introduction

In recent decades, the main focus in pig breeding has been on improving carcass leanness, growth and reproductive performance, but this process was carried out only on a limited number of breeds *(Tribout et al., 2010; Rauw et al.,* *1998).* As a result, many local pig breeds experienced population decline or even extinction. Nowadays, local pig breeds are regaining importance due to increased societal awareness of the need to preserve phenotypic and genotypic diversity, better adaptive capacity to specific local environmental conditions and the potential for production of high-quality traditional meat products *(Čandek-Potokar and Nieto 2019).*

The Slovenian local Krškopolje pig is an example of such a breed, typically raised on small farms with only a few breeding sows *(Batorek Lukač et al., 2019).* As it has not been subjected to genetic selection, the breed exhibits higher carcass fatness, low growth potential and muscle development *(Poklukar et al., 2023).* An increased interest of farmers for selective breeding of local breeds (including Krškopolje pig) has been noted in a recent survey *(Mercat et al., 2022)* mainly in terms of reproductive performance and maintenance of genetic diversity, with meat quality also mentioned. However, breeding for meat quality in Krškopolje pig represents a great challenge due to the small population size and demanding organization of post-mortem sampling and measurements. Therefore, identification and characterization of molecular markers affecting these traits of interest is necessary for the development of breeding procedures aimed at managing genetic information. The main aim of the present study was to analyse the frequency of several causal mutations known to affect fatness and meat quality traits in the local Krškopolje pig, and to estimate the selected genetic diversity parameters which will help to develop conservation and breeding strategies.

Materials and Methods

Samples of ear tissue of 253 Krškopolje pigs were collected at slaughter for DNA extraction. Animals originated from 26 sires, 93 dams and 15 breeding farms (from 1 to 59 animals per farm). Genomic DNA was extracted using the Qiamp DNA Mini kit (Qiagen GmbH, Hilden, Germany) following manufacturer instructions. The DNA samples were genotyped using Choice Genetics' custom SNP-array containing 57811 single nucleotide polymorphisms (SNPs). For all samples, the average genotyping rate was more than 0.98. Genotype quality control and data filtering was performed using PLINK 1.9 software *(Purcel et al., 2007).* SNPs with minor allele frequencies less than 0.01 or with more than 10% of missing genotypes were excluded from the analysis. After filtering, 51304 SNPs remained, out of which 49651 SNPs were located on *Sus scrofa* 11.1 autosomes.

Allele frequencies and genetic diversity parameters, such as observed and expected heterozygosity, inbreeding coefficient of an individual relative to the subpopulation (F_{is}) and minor allele frequency with all SNPs were calculated. Principal component analysis (PCA) of genotyping data according to the breeding farm was performed using the R environment (version 4.3.1). Six causative SNPs associated with meat quality and fatness (Table 1) were extracted from the genotyping data and their allele frequencies were individually studied.

Table 1. Studied polymorphisms and their documented association with production traits

Gene	**Chr**	**Genome position (*Sus scrofa 11.1*)**	**Polymorphism**	**Trait**	**Reference**
RYR1	6	47357966	c.1843C>T	Meat quality	*Fujii et al. 1991*
PRKAG3 **(Arg250Gln or RN$^-$)**	15	120863533	c.749G>A	Meat quality	*Milan et al., 2000; Ciobanu et al. 2001*
PRKAG3 **(Ile249Val*)**	15	120863537	c.745G>A	Meat quality	*Milan et al., 2000; Ciobanu et al. 2001*
PRKAG3 **(Pro103Leu**)**	15	120864863	c.158C>T	Meat quality	*Milan et al., 2000; Ciobanu et al. 2001*
PRKAG3 **(Gly102Ser***)**	15	120865227	c.304G>A	Meat quality	*Milan et al., 2000; Ciobanu et al. 2001*
MC4R	1	160773437	c.892G>A	Backfat and growth	*Kim et al., 2000*

Chr = chromosome, * previously Ile199Val, ** previously Pro53Leu, *** previously Gly52Ser

Results and Discussion

Allele frequencies of six polymorphisms related to meat quality and carcass traits were studied in Krškopolje pig (Table 2). As previously shown (*Muñoz et al., 2018; Tomažin et al., 2021),* we confirmed a relatively high frequency (i.e. 0.17) of mutation 1843C>T in the ryanodine receptor 1 (*RYR1*) gene *(Fujii et al., 1991)*, of which the negative effects on technological quality of pork are well known *(Salmi et al., 2010)*. The mutated T allele is associated with uncontrolled loss of calcium from the sarcoplasmic reticulum to the cytosol, affecting the rate of pH decline and consequently meat water-holding capacity and colour *(Fujii et al., 1991)*. The presence of the *RYR1* mutated allele has been reported to be very low or absent in some European local pig breeds and in some cases, effective initiatives to eliminate this allele were taken *(Muñoz et al., 2018)*.

As for protein kinase AMP-activated non-catalytic subunit gamma 3 *(PRKAG3)* gene, four polymorphisms that affect meat quality have been studied in

Krškopolje pigs (Table 2). In its active form, the PRKAG3 enzyme inhibits glycogen synthesis and stimulates glycogen breakdown *(Milan et al., 2000*; *Ciobanu et al., 2001)*. The presence of the dominant *PRKAG3* c.749A mutated allele (Arg250Gln or RN) has been associated with high muscle glycogen levels resulting in low ultimate pH, poor water-holding capacity and pale meat colour (*Milan et al., 2000*). The mutated c.749A allele was absent in Krškopolje pig (Table 2) which is consistent with previous work *(Muñoz et al., 2018)*. The *PRKAG3* c.745G>A mutation (or Ile249Val) was associated with muscle ultimate pH, colour, fatness and water-holding capacity *(Ciobanu et al., 2001, Enfält et al., 2006)*. In Krškopolje pig, it was segregating with the frequency of favourable A allele of 0.22, thus we confirm the previously reported frequency of this allele on much smaller sample of Krškopolje pig *(Muñoz et al., 2018)*. In *PRKAG3* gene, there were two additional mutations reported that cause amino acid substitutions, i.e. Leu103Pro (c.158C>T) and Gly102Ser (c.304G>A) *(Milan et al., 2000)*, and have a potential effect on meat quality. In Krškopolje pig, C allele of *PRKAG3* c.158C>T substitution was fixed, while the frequency of *PRKAG3* c.G304A (Gly102Ser) was 0.5 (Table 2).

Table 2. Allele frequencies for six polymorphisms on candidate genes for meat quality, growth and fatness in Krškopolje pig breed

	Allele frequency (N = 253)
RYR1	
C allele	0.83
T allele	0.17
***PRKAG3* (Arg250Gln)**	
G allele	1.00
A allele	0.00
***PRKAG3* (Ile249Val)**	
G allele	0.78
A allele	0.22
***PRKAG3* (Leu103Pro)**	
C allele	1.0
T allele	0.0
***PRKAG3* (Gly102Ser)**	
G allele	0.50
A allele	0.50
MC4R	
G allele	0.43
A allele	0.57

The melanocortin-4 receptor (*MC4R*) is involved in the regulation of feed intake and energy balance. A missense mutation c.892G>A in *MC4R* gene replaces aspartic acid with asparagine and causes dysfunction of the protein. Its mutation (i.e. A allele) was previously associated with increased fatness, feed intake and with growth differences *(Kim et al., 2000; Piórkowska et al., 2010)*. In the present study, the frequency of the A allele was intermediate (i.e. 0.57), which corroborates with the previous study involving Krškopolje pig *(Muñoz et al., 2018)*.

The average minor allele frequency of all genotyped SNPs (Figure 1) in Krškopolje pig was 0.274, with 11669 highly informative SNP markers with frequencies between 0.4 and 0.5 and 7030 SNP markers with frequencies between 0.01 and 0.10.

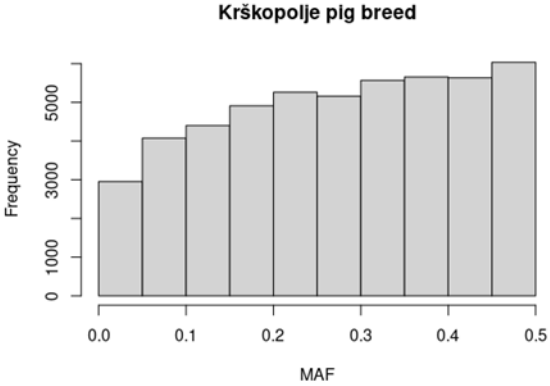

Figure 1. Frequency distribution of minor allele frequencies (MAF) in Krškopolje pig population

Genetic variability parameters (Table 3) within Krškopolje pig demonstrated relatively high expected and observed heterozygosity for local pig breeds, i.e. 0.360 and 0.370, respectively. Similar values for the Krškopolje pig were also reported by *Muñoz et al. (2019)* using different SNP-array and a smaller number of animals in the analysis. The observed and expected heterozygosity levels were higher than in other European local and similar to modern pig breeds *(Herrero-Medrano et al., 2014; Muñoz et al., 2019; Bordonaro et al., 2023)*. In the present study, the F_{is} value was observed to be negative (i.e. -0.03), indicating that there are fewer homozygotes than expected by random mating.

Table 3. Genetic diversity parameters of Krškopolje pig

Krškopolje pig (n = 253)	Mean
Observed heterozygosity	0.370
Expected heterozygosity	0.360
F_{is}	-0.030

F_{is} = inbreeding coefficient of an individual relative to the subpopulation

PCA was employed to explore the clustering of individuals from different breeding farms (Figure 2). The first and second principal components explained 10.44 and 8.38 % of the total variation, respectively. PCA separated two breeders of Krškopolje pigs (i.e. Breeder-A and Breeder-O) from the others. This could be a result of different breeding strategies or isolation of farm populations from the other farms. However, larger number of genotyped animals from different breeding farms is needed to see if or why these Krškopolje subpopulations are isolated.

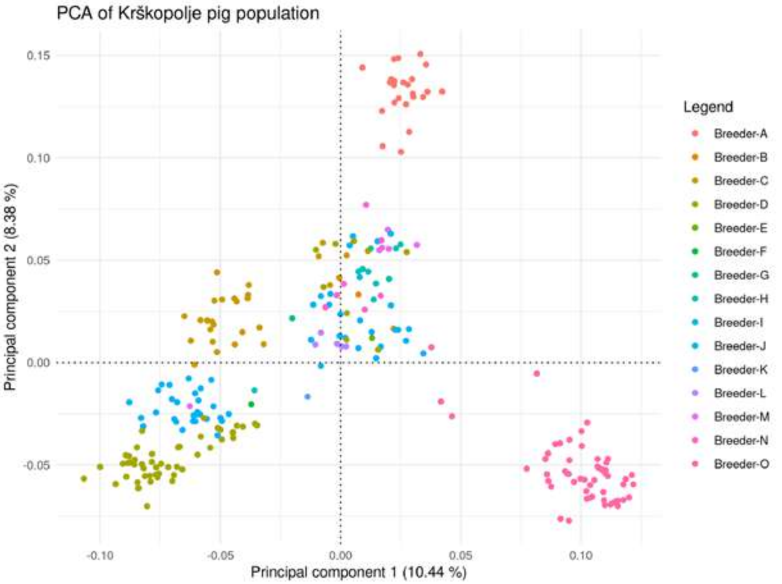

Figure 2. Genetic structure of different Krškopolje pig animals originating from 15 different breeders

Conclusions

The present study confirmed that the incidence of the mutant *RYR1* allele in Krškopolje pig is still relatively high, and therefore initiatives are needed to eliminate it, but with prudence not to jeopardise breed's genetic diversity. In addition, the segregation of the *PRKAG3* Ile249Val and *MC4R* c.892G>A alleles in the Krškopolje pig represents an opportunity for further association studies and/or possible practical applications in the breeding program. Maintenance of relatively high heterozygosity should also be considered in the development of conservation and breeding strategies.

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THE EFFECT OF *PRKAG3* AND *RYR1* GENES ON MEAT QUALITY TRAITS IN THE LOCAL KRŠKOPOLJE PIG BREED

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Abstract: Polymorphisms in *PRKAG3* (Ile199Val and Gly52Ser) or *RYR1* (Arg615Cys) genes have been reported to exert a major effect on pig meat quality. In the present study, the effects of these polymorphisms were investigated in 234 Krškopolje pigs genotyped with the Choice Genetics' custom SNP chip. The observed genotype frequencies for *RYR1* N/N were 66.2% and for N/n 33.8%. The frequencies of *PRKAG3* Ile199Val were 4.7%, 33.8% and 61.5% for Ile/Ile, Ile/Val and Val/Val, respectively, and Gly52Ser frequencies were 28.6%, 42.3% and 29.1% for Gly/Gly, Gly/Ser and Ser/Ser, respectively. Pigs carrying recessive "n" allele on *RYR1* exhibited paler colour with higher drip loss ($P<0.05$). As for *PRKAG3* polymorphisms, the Ile/Ile genotype (Ile199Val) was associated with lower muscle thickness, whereas Val/Val genotype was associated with higher monounsaturated fatty acids content ($P<0.05$). Gly/Ser heterozygotes (Gly52Ser) exhibited lower muscle pH 24h *post-mortem* ($P<0.05$) and brighter meat colour compared to Ser/Ser genotypes ($P<0.05$). By combining different genotypes of *RYR1* and *PRKAG3*, we could not confirm the hypothesis that the negative effects of the *RYR1* mutation could be counterbalanced by certain favourable *PRKAG3* alleles (like 199Ile) due to their low frequency.

Key words: local pig breed, SNPs, meat quality, breeding program

Introduction

Published research has identified a number of polymorphisms that affect carcass properties and meat quality in cosmopolitan pig breeds. However, in many local pig breeds, including the Slovenian Krškopolje pig, the effects of some major polymorphisms known to affect meat quality have not yet been studied. The exception is Arg615Cys mutation on *RYR1* gene, which has undesirable effect on meat quality *(Salmi et al., 2010)* and has been found in relatively high frequency (i.e. 0.20) in Krškopolje pig population *(Tomažin et al., 2021).* An interesting polymorphism (Ile199Val substitution in *PRKAG3* gene; *Milan et al., 2000*) has been reported to have beneficial effect on meat quality and could counterbalance the negative effect of *RYR1* allele in cosmopolitan breeds *(Škrlep et al., 2010).* The variant 199Ile has been associated with lower glycolytic potential and consequently higher pH24, while another polymorphism, the Gly52Ser substitution, has been shown to affect ham pH24 and loin muscle lightness *(Ciobanu et al., 2001).* In Krškopolje pig, the frequency of 199Ile has been reported to be 0.22, thus having the potential for application in the breeding program *(Muñoz et al. 2018).* Therefore, the main objective of the present study was to associate *PRKAG3* genotypes (Ile199Val and Gly52Ser) with meat quality traits in local Krškopolje pig breed and to relate their impact to *RYR1*genotype.

Materials and Methods

The samples of Krškopolje pig breed (n=237) were collected for DNA extraction and meat quality analysis. At the age of 400.5 ± 7.8 (mean ± SE) days, pigs were slaughtered, eviscerated and carcasses were weighed. Measurements of muscle thickness and backfat thickness above GM muscle were taken on split carcasses. The value of pH after 45 minutes *post-mortem* (pH45) was measured in *longissimus dorsi* muscle using MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland). One day after slaughter, the samples of *longissimus dorsi* muscle were taken for meat quality. Briefly, pH (after 24 hours) was measured using pH meter Mettler-Toledo MP120 (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) and objective colour (i.e. CIE L*, a*, and b* colour parameters) using Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan). Water holding capacity was determined by measuring drip loss (after 24 hours of storage) according to the EZ method *(Christensen, 2003).* Warner-Bratzler (WB) shear force was measured on three to four cooked cylindrical cores excised perpendicular to the direction of fibres using a TA Plus texture analyser (Ametek, Lloyd Instruments Ltd., Fareham, UK). Fatty acid composition of backfat (i.e. saturated, monounsaturated and polyunsaturated fatty acid content) and intramuscular fat content were determined using the near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using in-house calibrations.

DNA was extracted from ear tissue using Qiamp DNA Mini kit (Qiagen, (Qiagen GmbH, Hilden, Germany) following manufacturer instructions. The DNA samples were genotyped using Choice Genetics' custom SNP-array containing polymorphisms on *RYR1* (Arg615Cys) and *PRKAG3* (Ile199Val, Gly52Ser, Leu53Pro, Arg200Gln) genes. These genotypes were extracted using PLINK 1.9 software *(Purcel et al., 2007).*

Analysis of variance was performed using sasLM package in R software (version 4.3.1). The model included fixed effects of *RYR1* ("NN" and "Nn"), *PRKAG3* Ile199Val (Ile/Ile, Ile/Val, Val/Val) or Gly52Ser (Gly/Gly, Gly/Ser, Ser/Ser), farm (n = 9) and two-way interaction (*PRKAG3* x *RYR1*). Carcass weight was included as a covariate. Significant differences between the estimated marginal means were evaluated using the PDIFF function.

Results and Discussion

The observed frequencies of *RYR1*and *PRKAG3* genotypes are presented in Table 1. Almost 34% (n = 79) of Krškopolje pigs were carriers of *RYR1* recessive allele (Nn). The genotypic frequencies of *PRKAG3* Ile199Val SNP were 4.7, 33.8 and 61.5% for Ile/Ile, Ile/Val and Val/Val genotypes, respectively, while for *PRKAG3* Gly52Ser the genotypic frequencies were 28.6, 42.3 and 29.1% for Gly/Gly, Gly/Ser and Ser/Ser, respectively. The *PRKAG3* Leu53 and Arg200 were fixed in Krškopolje pig. In line with previous studies *(Muñoz et al., 2018; Tomažin et al., 2021),* a relatively high frequency of the *RYR1* recessive ("n") allele has been confirmed. It is a consequence of the uncontrolled introgression of the mutation from the modern breeds in the past and a lack of systematic elimination of this allele from the present population of Krškopolje pigs *(Kastelic and Čandek-Potokar, 2013).* Regarding *PRKAG3* genotypes, similar frequencies of 199Ile (i.e. 0.22) and 200Arg (i.e. 1.00) have been reported for Krškopolje pig by *Muñoz et al. (2018),* while frequencies for Gly52Ser and Leu53Pro are reported here for the first time.

The effect of *RYR1* genotype (Table 2) was significant for several meat quality traits. For example, lower water-holding capacity (higher drip loss) in "Nn" animals ($P < 0.05$) is consistent with results of our previous studies on Krškopolje pig *(Tomažin et al., 2021)* and on numerous other cosmopolitan breeds using different measurement methods *(Salmi et al., 2010).* The reduced water holding capacity in animals carrying recessive "n" allele is the result of the loss in regulation of calcium release from the sarcoplasmic reticulum which accelerates the rate of pH decline *(Monin et al., 2004).* In the present study, a tendency for decreased pH45 ($P = 0.06$), lighter, more red and more yellow colour of meat, larger drip loss ($P < 0.05$) and higher WB shear force ($P = 0.05$) were observed in

“Nn” animals which is consistent with many studies on cosmopolitan breeds *(see meta-analysis of Salmi et al., 2010; Oliván et al., 2018; McPhee and Trout, 1994).*

Table 1. Frequency of *RYR1* and *PRKAG3* genotype in Krškopolje pigs

	RYR1		**Total**	**%**
	NN	**Nn**		
PRKAG3				
Ile199Val - Ile/Ile	5	6	11	4.7
Ile199Val - Ile/Val	50	29	79	33.8
Ile199Val - Val/Val	100	44	144	61.5
Gly52Ser - Gly/Gly	39	28	67	28.6
Gly52Ser - Gly/Ser	65	34	99	42.3
Gly52Ser - Ser/Ser	51	17	68	29.1
Leu53Pro - Leu/Leu	155	79	234	100
Arg200Gln - Arg/Arg	155	79	234	100
Total	155	79	234	
(%)	66.2	33.8		

We did not detect any significant differences between “NN” and “Nn” genotypes for the backfat and muscle thickness, although “Nn” pigs usually exhibit higher carcass leanness or lower fatness *(Salmi et al., 2010).* There was no difference in backfat fatty acid composition, which could be due to different diets that pigs received.

Table 2. Estimated marginal means for carcass and meat quality traits as affected by *RYR1* genotypes

	RYR1		**RMSE**	**P-value**
	NN	**Nn**		
Carcass traits				
Backfat above GM muscle, mm	42.9	41.6	7.38	0.292
Muscle thickness, mm	71.8	73.5	6.66	0.131
Meat quality traits				
pH45	6.32	6.23	0.250	0.063
pH24	5.46	5.45	0.078	0.719
CIE L*	53.1	55.4	3.99	< 0.001
CIE a*	10.2	10.8	1.78	< 0.05
CIE b*	6.3	7.1	1.53	< 0.01
IMF, %	5.03	4.70	1.532	0.183
Drip loss, %	4.4	5.3	1.98	< 0.05
Shear force, N	45.9	49.1	9.49	0.054
Backfat fatty acid composition				
SFA*	43.0	42.6	1.88	0.153
MUFA*	46.8	46.6	1.55	0.983
PUFA*	10.9	10.4	1.68	0.109

IMF = intramuscular fat content, GM = *gluteus medius*, SFA = saturated fatty acid content, MUFA = polyunsaturated fatty acid content, PUFA = polyunsaturated fatty acid content,
Results on fatty acid composition are presented in g/100 g of total fatty acids
$^{a, b}$ Values within a row with different a and b superscripts differ significantly at $P < 0.05$.

As for the *PRKAG3* Ile199Val (Table 3), animals carrying Ile/Ile genotype exhibited lower muscle thickness compared to Val/Val and Ile/Val genotypes ($P < 0.05$). In addition, a tendency for greater backfat thickness was observed in animals carrying Ile/Ile compared to heterozygous Ile/Val genotype ($P = 0.06$). The Ile/Ile compared to Val/Val genotype was associated with lower monounsaturated fatty acid content compared to Val/Val, with the Ile/Val closer to Val/Val ($P < 0.05$). In agreement with previous reports on cosmopolitan breeds, the 199Ile allele was at least favourable for muscularity *(Enfält et al., 2006; Škrlep et al., 2010)*. However, no differences in meat quality traits were observed between *PRKAG3* alleles on codon 199 which is contrary to literature reports. In cosmopolitan pig breeds it was previously shown that Ile199 genotype affected colour, pH and water holding capacity *(Ciobanu et al., 2001; Škrlep et al., 2010; Otto et al., 2007)*. Possible reason for discrepancies could be low number of Ile/Ile pigs or that genotype effect can be also breed-dependent *(Ryan et al., 2012)*.

Table 3. Estimated marginal means for carcass and meat quality traits as affected by *PRKAG3* Ile199Val genotype

	***PRKAG3* Ile199Val**			**RMSE**	**P-value**
	Ile/Ile	**Ile/Val**	**Val/Val**		
Carcass traits					
Backfat above GM muscle, mm	46.5	40.9	43.1	7.30	0.062
Muscle thickness, mm	66.8^{a}	71.9^{b}	72.7^{b}	6.61	< 0.05
Meat quality traits					
pH45	6.13	6.31	6.29	0.252	0.436
pH24	5.48	5.44	5.46	0.078	0.213
L*	54.9	54.1	53.6	4.12	0.543
a*	10.6	10.5	10.3	1.82	0.763
b*	7.1	6.6	6.6	1.58	0.599
IMF, %	5.0	4.9	5.0	1.54	0.892
Drip loss, %	4.4	4.8	4.6	2.02	0.717
Shear force, N	40.9	46.2	47.9	9.50	0.115
Fatty acid composition					
SFA	42.9	42.9	42.8	1.89	0.915
MUFA	45.9^{a}	46.6^{a}	47.1^{b}	1.53	< 0.05
PUFA	10.9	10.4	10.0	1.68	0.191

GM = *gluteus medius*, IMF = intramuscular fat content, SFA = saturated fatty acid content, MUFA = polyunsaturated fatty acid content, PUFA = polyunsaturated fatty acid content,
Results on fatty acid composition are presented in g/100 g of total fatty acids
$^{a,\,b}$ Values within a row with different a and b superscripts differ significantly at $P < 0.05$.

Considering the *PRKAG3* Gly52Ser polymorphism (Table 4), animals with Ser/Ser genotype exhibited higher pH24 values and darker colour of *longissimus* muscle compared to Gly/Ser genotype (P < 0.05). Water-holding capacity tended to be increased in Gly/Ser genotypes (P = 0.09). The study of *Ciobanu et al. (2001)* showed that this polymorphism affected ham pH (higher value in Ser/Ser compared to Gly/Gly genotype) and loin CIE L* (lightness) parameter (lighter meat in Ser/Ser than in Gly/Ser and Gly/Gly genotypes).

Table 4. Meat quality of *longissimus dorsi* muscle according to PRKAG3 Gly52Ser genotype

	PRKAG3 Gly52Ser			RMSE	P-value
	Gly/Gly	Gly/Ser	Ser/Ser		
Carcass traits					
Backfat above GM muscle, mm	42.3	41.6	44.2	7.33	0.122
Muscle thickness, mm	71.3	73.1	71.4	6.67	0.260
Meat quality traits					
pH45	6.24	6.32	6.28	0.252	0.459
pH24	5.46^{ab}	5.44^{a}	5.47^{b}	0.077	< 0.05
L*	53.3^{ab}	54.8^{b}	52.7^{a}	4.02	< 0.01
a*	10.8	10.3	10.0	1.80	0.112
b*	6.5	6.8	6.3	1.56	0.116
IMF, %	5.1	5.0	4.6	1.53	0.216
Drip loss, %	4.4	5.1	4.4	1.99	0.088
Shear force, N	45.8	46.8	48.6	9.57	0.367
Fatty acid composition					
SFA	43.0	42.8	42.8	1.89	0.781
MUFA	46.6	46.8	47.1	1.54	0.228
PUFA	10.3	10.4	9.9	1.68	0.243

GM = *gluteus medius*, IMF = intramuscular fat content, SFA = saturated fatty acid content, MUFA = polyunsaturated fatty acid content, PUFA = polyunsaturated fatty acid content,
Results on fatty acid composition are presented in g/100 g of total fatty acids
$^{a, b}$ Values within a row with different a and b superscripts differ significantly at $P < 0.05$.

In order to assess potential counterbalancing of negative effects of recessive “n” allele, genotype *RYR1* was combined with *PRKAG3* (Ile199Val) and effects on carcass and meat quality traits were assessed (Figure 1). Despite several differential effects observed for carcass and meat quality traits in regard to the combination of *RYR1* and *PRKAG3* Ile199Val, we could not confirm our hypothesis on counterbalancing effect between the genotypes. Namely, in our study we detected just 11 animals carrying *PRKAG3* Ile/Ile allele (see Table 1) originating from 4 different farms. Still, the combination of genotype “Nn” at *RYR1* with Ile/Val at *PRKAG3* (Figure 1) indicated smaller backfat thickness, the combination of N/n at *RYR1* with Ile/Ile the most tender meat and the lowest polyunsaturated fatty acids content ($P < 0.05$).

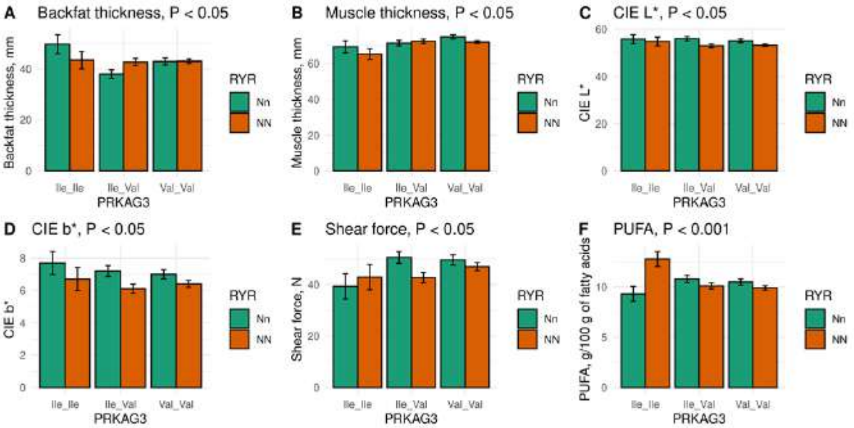

Figure 1. Carcass and meat quality traits affected by the combination of *RYR1* (NN and Nn) and *PRKAG3* (Ile199Val) polymorphisms

Conclusions

In the present study, the *RYR1* genotype in Krškopolje pig affected meat quality traits (i.e. objective colour parameters, drip loss and pH45), while the *PRKAG3* Ile199Val genotype was associated with muscle thickness and backfat monounsaturated fatty acid content. The combination of both alleles (i.e. *RYR1* “Nn” and *PRKAG3* Ile/Ile) could not confirm the hypothesis on counterbalancing effect on meat quality traits due to low number of detected *PRKAG3* Ile/Ile animals of Krškopolje pig. Therefore, additional animals carrying *PRKAG3* Ile/Ile genotype should be included in the analysis.

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FUSARIUM AND DEOXYNIVALENOL CONTAMINATION OF WINTER WHEAT DEPENDING ON GROWING SEASON AND CULTIVAR

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Abstract: Wheat is the most important staple food in the world and the main source of carbohydrates, fibre, proteins, vitamins, minerals and phytochemicals for human consumption. The nutrients from wheat kernels can also be used as livestock feed. Fusarium head blight (FHB), caused by fungal species of the *Fusarium* genus, is one of the most important wheat diseases worldwide. The lack of FHB management strategies results in significant economic losses in yield and quality of wheat kernels. In this study, the influence of growing season and wheat cultivar on some FHB and yield component traits, as well as on the trichothecene mycotoxin deoxynivalenol (DON) levels during the harvest period in 2014 and 2015, was investigated. Significant influence of growing season (year) and wheat cultivar on disease (FHB index – FHBI, incidence of *Fusarium*-damaged kernels – FDK and levels of DON) and yield parameters (spike weight – SW, kernel weight per spike – KWS, and 100-kernel weight) was found. FHBI, FDK, and DON were higher in 2014, while SW, KWS, and 100-kernel weight were lower in 2014 than in 2015. The mid-early wheat cultivar Simonida had lower FHBI, FDK, and levels of DON and significantly higher SW, KWS, and 100-kernel weight than the mid-late cultivar NS 40S. There was a significant effect of year × cultivar interaction on FHBI, FDK and 100-kernel weight.

Key words: Fusarium head blight, *Fusarium* and deoxynivalenol contamination, yield component traits, winter wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. In Serbia, wheat is grown on about 600,000 ha, with a total production of 3.4 million tonnes and an average yield of 5.7 t ha^{-1} in 2021 *(Statistical Yearbook of the Republic of Serbia, 2022)*. It is used in the food industry to produce bread, pasta, cakes, crackers, cookies, pastries, starch, alcohol, flour, etc. The nutrient composition of wheat kernels varies depending on genotypic characteristics, yields, climate and soil. Wheat grown in dry climates is usually a durum type with a protein content of 11-15% and a strong structure of protein gluten (elastic protein). The durum type provides the most suitable flour for bread making. Wheat from humid areas is softer, with a protein content of about 8-10% and a weak gluten structure, ideal for cakes, cookies, pastries, etc. *(Lafiandra et al., 2022)*. Due to the high proteins (on average 10-12%), carbohydrates (75%) and fibre (13.4%) contents, wheat is also used for animal feed, in feeding ruminants, pigs and poultry, especially in developed countries *(Grote et al., 2021; Tóth et al., 2022; Kartseva et al., 2023)*. Fusarium head blight (FHB) is a devastating fungal disease in wheat crops that causes yield loss with destructive effects on grains, shrivelled and discoloured kernels, and the formation of the trichothecene mycotoxin deoxynivalenol (DON) *(Jones, 2000; Wegulo et al., 2011)*. *Fusarium graminearum* sensu lato (teleomorph: *Gibberella zeae* (Schwein.) Petch.), also called *F. graminearum* species complex (FGSC), is the major causal agent of FHB. The FGSC includes more than 16 phylogenetically distinct species *(Sarver et al., 2011)*, of which *F. graminearum* sensu stricto (s.s.) is the most widespread. In Serbia, in addition to *F. graminearum* s.s., *F. vorosii* was recently identified as a member of the FGSC *(Obradović et al., 2022)*. Several *Fusarium* species, *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. poae*, are considered the predominant species causing FHB. Among them, *F. graminearum* and *F. culmorum* are the most aggressive to winter wheat and the most important DON-producing fungal species *(Bottalico and Perrone, 2002; Tan et al., 2021; Balducci et al., 2022)*.

Wheat straws and chaffs are used in pig and turkey production and can be contaminated with secondary metabolites of fungi (mycotoxins) as forage of wheat kernels for cattle feed. Mycotoxins have toxic effects on animal and human health, causing acute and chronic diseases (mycotoxicoses). Animal toxicoses occur through mycotoxin-contaminated feed and bedding *(Cowger and Arellano, 2013)*. Intoxication of animals through DON-contaminated feed can cause anorexia, emesis, and feed refusal. DON has also neurotoxicity and immunotoxicity effects *(Haidukowski et al., 2005)*.

FHB and DON contamination are influenced by many factors such as climatic, agroecological and environmental conditions, especially during flowering stages, which is the most sensitive host stage for *Fusarium* infection, and by agrotechnical measures. Agricultural practices such as tillage, crop rotation, crop residue management, irrigation management, resistant cultivars, chemical and biological control, and disease forecasting are preharvest strategies against FHB and to reduce DON accumulation in wheat kernels *(Yuen and Schoneweis, 2007; Blandino et al., 2012; Wegulo et al., 2015)*. Since of the economic importance of FHB in wheat crops, the main objective of this study was to evaluate the effect of the growing season (year) and wheat cultivar on *Fusarium* and DON contamination during harvest in 2014 and 2015 in Serbia, taking into account the infection with *Fusarium* spp. under natural conditions.

Material and Methods

Field trials. A field trial was conducted at the Institute of Animal Husbandry, Belgrade-Zemun, Serbia (44°84' N, 20°40' E; 88 m a.s.l.) under dry farming conditions in response to natural precipitation. During the two harvest seasons, 2014 and 2015, FHB and yield traits and DON levels of two Serbian wheat cultivars, Simonida and NS 40S, were evaluated. These cultivars were selected due to widespread cultivation around the experimental area in Serbia. The sowing dates were in the third decade of October, with seven days between the sowing of mid-early and mid-late cultivars. The soil type was carbonate chernozem. The previous crop was maize. The field trials were arranged as split-plot randomized block designs with four replications. The size of the subplot was 5 m^2. Fertilization with ammonium nitrate was applied at the end of February at the rate of 250 kg ha^{-1} (33.4% N). At the tillering wheat stage, weeds, fungal leaf diseases and insects were controlled by adequate chemical treatments. Wheat kernels were harvested in the first decade of July in both investigated years.

Disease and wheat yield component traits. FHB severity of naturally infected plants was estimated on 20 randomly selected wheat spikes within each subplot (80 wheat spikes per treatment tested) at the end of the flowering stage (GS 69). The evaluation was based on the scale of *Blandino et al. (2012)* with seven evaluation classes (1 = 0 to 5%, 2 = 5 to 15%, 3 = 15 to 30%, 4 = 30 to 50%, 5 = 50 to 75%, 6 = 75 to 90%, 7 = 90 to 100% spike area infected). Following the methods of *Wegulo et al. (2011)*, the FHB index (FHBI) was calculated using the formula = (incidence (%) × severity (%))/100, where FHB incidence was the percentage of infected spikes and FHB severity was the percentage of infected spikelets, while the incidence of *Fusarium*-damaged kernels (FDK) was visually evaluated in wheat-harvested subsamples of 10 grams.

Wheat kernel subsamples were ground by an analytical mill (IKA A11, Germany) and analysed by competitive direct enzyme-linked immunosorbent assay (ELISA) for the detection of deoxynivalenol (DON). According to the instructions of the manufacturer of the Celer DON ELISA kits (Tecna, Italy), the assay procedure was performed. The absorbance values of the samples were measured optically at a wavelength of 450 nm using an ELISA reader (Biotek EL x 800TM, USA). The detection limit for DON in wheat was 0.04 mg kg^{-1}.

Spike weight (SW), kernel weight per spike (KWS) and 100-kernel weight as yield components were also determined.

Meteorological data. Weather data for the Surčin area (Belgrade) were provided by the Republic Hydrometeorological Service of Serbia (Table 1).

Table 1. Meteorological data (mean monthly temperature, total precipitation and mean monthly relative humidity (RH)) in two growing seasons (October 2013 – July 2014) and (October 2014 – July 2015) in the Surčin area (Belgrade)

Growing season	Month	Temperature (ºC)	Precipitation (mm)	RH (%)
2013–2014	October	14.6	45.8	72
	November	9.4	28.7	80
	December	2.1	6.7	85
	January	4.6	24.6	80
	February	7.0	12.9	72
	March	9.9	44.5	69
	April	13.1	86.9	73
	May	16.8	233.4	72
	June	20.8	85.6	68
	July	22.4	181.0	71
	Seasonal mean temperature	12.07		
	Total season precipitation		750.1	
	Seasonal mean RH			74.2

Table 1. (continue)

Growing season	Month	Temperature (ºC)	Precipitation (mm)	RH (%)
2014–2015	October	13.6	57.1	76
	November	9.2	9.9	78
	December	3.8	61.4	82
	January	2.9	48.3	78
	February	3.2	50.0	77
	March	7.0	102.0	73
	April	11.8	30.9	62
	May	17.7	58.9	70
	June	20.6	84.2	70
	July	25.1	1.7	58
	Seasonal mean temperature	11.49		
	Total season precipitation		504.4	
	Seasonal mean RH			72.4

Data analyses. The effects of two growing seasons on FHB variables (FHBI and FDK), DON levels and yield component traits (SW, KWS, and 100-kernel weight) in two wheat cultivars were analysed using a general linear model (Multivariate Analysis of Variance) with SPSS software (IBM SPSS Statistic 20). Tukey's test was used to compare treatment means at P≤0.05 and P≤0.01 significance levels. Linear relationships between tested variables were calculated using Pearson's correlations.

Results

FHB traits and deoxynivalenol levels. *F* values of the year and cultivar and their interaction are shown in Table 2. The year and cultivar had a highly significant (P≤0.01) effect on FHBI, FDK, and DON levels. FHBI, FDK and DON were higher in 2014 than in 2015. The mid-late cultivar NS 40S had a statistically significantly higher (P≤0.01) FHBI, FDK and DON than the mid-early cultivar Simonida. High significance (P≤0.01) showed by year × cultivar interaction for FHB variables, FHBI and FDK (Table 2).

Yield component traits. The influence of year and cultivar treatments was significant (P≤0.05) for SW and KWS and highly significant (P≤0.01) for 100-kernel weight. The *F* values of the year × cultivar interaction were highly significant (P≤0.01) for 100-kernel weight. SW, KWS and 100-kernel weight were statistically significantly lower in 2014 and in the mid-late cultivar NS 40S compared to 2015 and the mid-early cultivar Simonida (Table 3).

Table 2. Year and cultivar effects on FHB index (FHBI), the incidence of *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) levels in wheat kernels

Factor	FHBI (%)	FDK (%)	DON (mg kg^{-1})
Year effects (Y)			
2014	27.67^{a}	17.46^{a}	3.238^{a}
2015	17.08^{b}	7.28^{b}	1.916^{b}
F-test	**	**	**
Cultivar effects (C)			
Simonida	20.47^{b}	5.94^{b}	1.640^{b}
NS 40S	24.28^{a}	18.80^{a}	3.514^{a}
F-test	**	**	**
Interactions (F-test)			
Y × C	**	**	ns
Means	22.38	12.37	2.58

Y, Year; C, Cultivar;
Means followed by the same letter within a column are not significantly different by *Tukey's test* at P ≤ 0.05 level. ns, not statistically significant; **significant at the 0.01 level of probability.

Table 3. Year and cultivar effects on wheat yield component traits, spike weight (SW), kernel weight per spike (KWS) and 100-kernel weight

Factor	SW (g)	KWS (g)	100-kernel weight (g)
Year effects (Y)			
2014	1.79^{b}	1.42^{b}	3.63^{b}
2015	2.09^{a}	1.72^{a}	4.37^{a}
F-test	*	*	**
Cultivar effects (C)			
Simonida	2.10^{a}	1.70^{a}	4.43^{a}
NS 40S	1.78^{b}	1.45^{b}	3.57^{b}
F-test	*	*	**
Interactions (F-test)			
Y × C	ns	ns	**
Means	1.94	1.57	4.00

Y, Year; C, Cultivar;
Means followed by the same letter within a column are not significantly different by *Tukey's test* at P ≤ 0.05 level. ns, not statistically significant; **significant at the 0.01 level of probability.

Correlations between tested variables. In correlation analyses, there were highly significant ($P \leq 0.01$) positive correlations between FHBI with FDK ($r = 0.84$) and DON levels ($r = 0.80$), SW with KWS ($r = 0.99$) and 100-kernel weight ($r = 0.65$), and KWS with 100-kernel weight (0.70). FDK had a highly significant

(P≤0.01) positive correlation with DON levels (r = 0.90). Significant negative correlation coefficients were also obtained for the relationships between disease variables (FHBI, FDK, and DON level) and yield component traits (SW, KWS, and 100-kernel weight) (Table 4).

Table 4. The correlation coefficients (r) between tested variables

	FHBI	FDK	DON	SW	KWS
FDK	0.84**				
DON	0.80**	0.90**			
SW	-0.61**	-0.51**	-0.68*		
KWS	-0.69**	-0.57**	-0.72*	0.99**	
100-kernel weight	-0.85**	-0.95**	-0.92**	0.65**	0.70**

FHBI, FHB index; FDK, the incidence of *Fusarium*-damaged kernels; DON, deoxynivalenol; SW, spike weight; KWS, kernel weight per spike. *significant at the 0.05 level of probability; **significant at the 0.01 level of probability

Discussion

This study evaluated year and cultivar effects on wheat kernel quality and yield. During the observation period, the 2013-14 growing season was wetter, with total precipitation of 750.1 mm compared to 2014-15 (504.4 mm). Mean temperatures and RH were similar in both investigated growing seasons, October 2013 – July 2014 (12.07 °C and 74.2%) and October 2014 – July 2015 (11.49 °C and 72.4%) (Table 1). During the most susceptible stage for *Fusarium* infection (flowering), in May, total precipitation was higher in 2014 (233.4 mm) than in 2015 (58.9 mm), with mean monthly temperatures of 16.8 °C and 17.7 °C, respectively. In June, at the milk and maturity wheat stages, total precipitation and mean temperatures were similar in both years (2014 – 85.6 mm and 20.8°C, and 2015 – 84.2 mm and 20.6°C). In May and June 2014, the mean relative humidity (RH) was 72 and 68%, respectively, and in May and June 2015, they were similar (70%).

Considering the meteorological data during flowering in both investigated growing seasons, the weather conditions for FHB development on wheat spikes were more favourable in 2014 than in 2015. FHBI, FDK, and DON were significantly higher by 1.6, 2.4, and 1.7 times, respectively, in 2014, while SW, KWS, and 100-kernel weight were significantly lower by 1.2, 3.2, and 2.1 times, respectively, in 2014 than in 2015 (Tables 2 and 3). In similar studies in Serbia, *Jevtić et al. (2021, 2022)* reported higher FHBI, FDK, and yield losses in 2014 than in 2015, emphasizing to some extent the influence of climatic factors during wheat anthesis in May. According to the results of *Cowger et al. (2009)*, prolonged fog in the post-flowering period affected FHB severity, FDK, and DON in winter wheat. *Kriss et al. (2010)* also found that year-to-year variation in FHB intensity was related to environmental conditions (moisture and wetness), especially two months before wheat maturity and indicated a strong influence of wheat cultivar resistance, fungicide control, and agronomic practices such as crop rotation and tillage type on FHB epidemics, yield loss, and DON contamination. In this study, levels of DON were higher in treatments with higher FHBI and FDK, which is in agreement with reports by *Jones and Mirocha (1999)*, *Jones (2000)*, *Wegulo et al. (2011)*, and *Mesterházy et al. (2003, 2011, 2015)*. In treatments of years and cultivars, DON levels exceeded the maximum level (MPL) prescribed by the European Commission (1881/2006/ EC) for unprocessed cereals, except for durum wheat, oats, and maize (1,250 mg kg^{-1}) (Table 2), consistent with previous reports by *Krnjaja et al. (2015)*.

Although resistant cultivars are critical for reducing FHB and DON contamination of wheat, most commercial cultivars are susceptible to FHB in practice. In this study, the mid-late cultivar NS 40S showed higher susceptibility to *Fusarium* and DON contamination than the mid-early cultivar Simonida. *Jevtić et al. (2021)* identified sets of moderately resistant cultivars with FHBI of 25% or less and moderately susceptible or susceptible cultivars with FHBI of 26 to 54% based on cultivar response to FHB in 2014. Since FHBI was 20.47% (Simonida) and 24.28% (NS 40S), both wheat cultivars studied could be classified as moderately resistant.

In correlations between disease variables (FHBI, FDK, DON), FHBI and FDK, FHBI and DON, and FDK and DON were significantly positively correlated (Table 4). According to the reports of *Paul et al. (2005)* and *Weguło et al. (2011)*, the correlation coefficient between FDK and DON and FHBI and DON was above 0.50, while in this study, it was between FHBI and FDK, FHBI and DON, and FDK and DON. *Jevtić et al. (2021)* found a higher correlation coefficient between FHBI and FDK in moderately resistant wheat cultivars (r = 0.732) than in susceptible/moderately susceptible wheat cultivars (r = 0.362). *Mesterházy et al. (2015)* reported that FHB levels had a lower effect on DON contamination than FDK in 40 winter wheat cultivars from Hungary (20) and Austria (20). There were also significant negative correlations between yield component traits (SW, KWS, 100-kernel weight) with FHB variables (FHBI and FDK) and DON values (Table 4). These results were in line with those of *Wegulo et al. (2011)*. In addition, using statistical analysis with multiple stepwise regression, *Jevtić et al. (2022)* found a more significant influence of FDK than FHBI on thousand kernel weight (TKW) in moderately resistant cultivars, while both FHBI traits, FHBI and FDK, influenced TKW in moderately susceptible and susceptible cultivars.

Conclusion

It can be concluded that in this study, growing season and cultivar had a significant effect on both disease and yield variables in winter wheat. Contamination of wheat kernels with *Fusarium* and deoxynivalenol was higher in the wetter year of 2014 than in 2015, while yield component traits (SW, KWS and 100-kernel weight) were lower in 2014 than in 2015. The mid-early wheat cultivar Simonida showed lower susceptibility to FHB and DON than the mid-late cultivar NS 40S.

These results indicate the need to improve breeding programs by creating wheat cultivars from an earlier maturing group. Since environmental conditions in Serbia are very convenient for FHB development and DON accumulation in wheat crops, cultivar selection should be recommended as a primary agricultural measure to achieve high and healthy wheat yields.

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