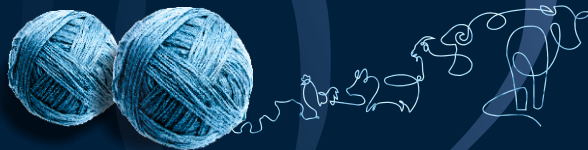


15th
INTERNATIONAL
SYMPOSIUM

MODERN
TRENDS
IN LIVESTOCK
PRODUCTION



P R O C E E D I N G S

29 - 31 October 2025, Belgrade, Serbia

Institute for Animal Husbandry
Belgrade – Zemun, SERBIA

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PROTEIN EFFICIENCY AND ANIMAL WELFARE – IS THERE A TRADE-OFF?

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Abstract: Improving nutrient efficiency in pig farming is essential for promoting environmental sustainability and reducing reliance on imported protein sources. Reducing dietary crude protein content, especially in conjunction with precise amino acid balancing, has been shown to reduce nitrogen excretion and ammonia emissions while maintaining growth performance. Furthermore, genetic selection is a promising way to increase protein efficiency and provide an additional approach to alleviating the environmental impact of swine production. However, such dietary modifications require careful monitoring, as deficiencies in essential amino acids can disrupt natural behaviours, increase stress susceptibility and endanger animal welfare. Neurophysiological pathways, particularly those associated with serotonin and cortisol regulation, highlight the welfare implications of amino acid imbalances. This brief review highlights the importance of taking a tailored, multidisciplinary approach to enhance protein efficiency and safeguard animal welfare under practical production conditions.

Key words: Nitrogen excretion, Amino acid balance, Genetic selection, Behavioural responses, pigs

Environmental Implications of Protein-Reduced Diets

Protein efficiency in pig production is gaining increasing relevance, both from an environmental and economic perspective (Pomar et al., 2021). Reducing dietary crude protein (**CP**) content has been shown to significantly increase protein efficiency and decrease nitrogen (**N**) losses through faeces and urine. In comparison with a standard diet, a 20% reduction in dietary CP supply from 20–100 kg body weight has been shown to reduce N losses by ~130–230 g/pig. Research has demonstrated that, upon attaining a body weight of 140 kg, the reduction increases to approximately 470–500 g of N per pig. This represents

approximately double the reduction previously observed in just 40 kg body weight gain (Ruiz-Ascacibar et al., 2017). This has substantial implications for mitigating the environmental footprint of pig farming.

Protein-restricted dietary protocols, particularly during the finishing phase, are a strategy for enhancing environmental sustainability by reducing reliance on imported protein sources such as soybean meal. Furthermore, these protocols are a viable approach within conventional production systems and do not adversely affect performance. Documented reductions in the use of protein-rich feed ingredients have been observed to range from 11 to 16% under these methodologies (Bee, unpublished).

Individual Variation and Genetic Potential for Nutrient Efficiency

Interestingly, individual variation in protein efficiency plays a major role in how pigs respond to low-protein diets. In the study of Ruiz-Ascacibar et al. (2017), around 30% of pigs fed low CP diets exhibited growth performance comparable to those on standard diets. This suggests considerable individual differences either in essential amino acid (EAA) requirements or genetic capacity for efficient EAA utilization. Subsequent genetic evaluations indicated that as much as 51% of the phenotypic variability in protein efficiency may be ascribed to genetic factors, yielding a heritability coefficient of $h^2 = 0.51$ (EwaoluwaGbemiga et al., 2023). Interestingly, apart from the considerable heritability related to protein efficiency, there was a notable genetic linkage with phosphorus efficiency (0.61 ± 0.16), as well as moderate genetic connections with the feed conversion ratio (-0.55 ± 0.14) and average daily feed intake (-0.53 ± 0.14), coupled with a rather low genetic association with average daily gain (-0.19 ± 0.19). These findings open realistic opportunities for breeding strategies targeting improved nitrogen efficiency without compromising performance.

Nutritional Challenges and Behavioural Consequences

However, feeding regimes characterised by reduced CP content can have unforeseen consequences for the welfare of pigs. Deficits in EAAs, such as methionine, threonine and tryptophan, can alter natural behaviors (Jensen et al., 1993). Reports of increased oral manipulation behaviours, such as tail and ear biting, have been linked to dietary protein restriction (Jericho & Church, 1972; Schröder-Petersen & Simonsen, 2001). Empirical investigations have demonstrated that pigs provided with *ad libitum* diets deficient in CP exhibit increased foraging-

related activities, including standing, walking and rooting (Jensen et al., 1993). This highlights the importance of unmet nutritional requirements in modifying natural behaviour.

The concept of the “ideal protein” serves as the foundation for formulating balanced pig diets. Typically, lysine is the first limiting amino acid in diets of growing-finishing pigs. The requirements for other EAAs are expressed in relation to lysine. A deficiency in EAAs such as methionine, threonine, or tryptophan can influence feed choice and intake patterns (Ettle & Roth, 2004, 2009; Roth et al., 2006). One possible behavioural consequence of such deficiencies is exploratory or manipulative behaviour, potentially escalating to biting and aggression (McIntyre & Edwards, 2009). These behavioural changes underscore the need for careful dietary formulation to ensure that nutritional requirements are met while still achieving the goal of reducing dietary CP levels.

Neurophysiological and Immune-Related Mechanisms

One plausible explanation is that EAAs act as precursors for neurotransmitters. Tryptophan, for example, is a serotonin precursor. Pigs exhibiting tail-biting behaviour have been found to have lower blood serotonin levels, which may indicate higher levels of anxiety or arousal (Ursinus et al., 2014). Furthermore, inadequate protein intake or imbalanced amino acid profiles can compromise immune function, especially when animals encounter immunological challenges that elevate their EAA requirements (Wu et al., 2014).

Contextual Considerations for Practical Implementation

It is important to recognise that controlled feeding trials are usually conducted under idealised conditions, such as strict hygiene measures and low stocking density, which may not reflect commercial production environments. Consequently, feeding recommendations are formulated with safety margins to account for variations in health, housing and management. Behavioural studies under different housing and dietary conditions revealed that pigs housed under suboptimal conditions and fed low-protein diets with unbalanced EAA profiles were more likely to exhibit abnormal behaviours (van der Meer et al., 2017). Conversely, the supplementation of methionine, threonine, and tryptophan (+20%) could mitigate some of these effects.

Several physiological explanations have been put forward. One theory posits that low dietary tryptophan reduces serotonin synthesis, thereby increasing stress sensitivity and aggression. Another considers the downstream effects on

cortisol and melatonin regulation, which further influence stress physiology and behaviour (Martínez-Trejo et al., 2009; Ursinus et al., 2014). Furthermore, pigs with high or low growth potential may both be susceptible to behavioural disorders due to a discrepancy between metabolic demand and nutrient supply, particularly when health is suboptimal or the immune system is activated.

Conclusions

In conclusion, reducing the amount of crude protein in pigs' diets can significantly improve protein efficiency and environmental sustainability by lowering nitrogen excretion and emissions. However, if not carefully managed, this approach may compromise animal welfare due to deficiencies in essential amino acids and related behavioural issues. A balanced, holistic approach that integrates precise amino acid formulation, robust animal health, appropriate housing and genetic selection is essential to ensure that improving protein efficiency and animal welfare are mutually reinforcing goals, not mutually exclusive ones.

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MULTIDIMENSIONAL ADAPTIVE STRATEGIES OF FARM ANIMALS FACING CLIMATE CHALLENGES

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Abstract: Modern livestock farming operates within increasingly challenging climatic boundaries, making adaptation the cornerstone of sustainable development. In confronting climate change, the innate adaptive abilities of domestic animals must not be underestimated or neglected, as they provide the essential biological foundation for survival and productivity in an ever more demanding environment. Alongside human interventions, understanding and enhancing animal adaptations lay the groundwork for developing resilient and sustainable livestock systems. The strategies through which animals respond to climate challenges encompass physiological, biochemical, immunological, morphological, and behavioral mechanisms—from panting and sweating, changes in blood composition and endocrine function, to modifications in body size, coat characteristics, and behaviors such as seeking shade or reducing feed intake. Interspecies and interbreed differences further underscore the critical importance of genetic diversity in fostering resilience. These insights offer a basis for designing strategies that support not only productivity but also animal welfare under global warming conditions. While human interventions may alleviate the immediate impacts of climatic stressors, it is the integration of animal adaptations that provides the long-term foundation for the stability and future of livestock farming.

Key words: climate change, livestock, adaptation, heat stress, biological mechanisms, animal resilience

Introduction

Climate change today poses one of the most significant challenges to agricultural sustainability, particularly for livestock production, which is directly influenced by climatic factors (Gaughan et al., 2019). Adaptation to these changes entails a dual strategy: spontaneous animal-based mechanisms and human-initiated practices and technologies. While the literature often highlights the importance of human interventions, the study of animal adaptations is equally, if not more, important, as the biological resilience of farm animals forms the basis of their survival and productivity under ever more challenging climate conditions.

The impacts of climate change on livestock are multifaceted, with heat stress standing out as one of the greatest challenges for farm animals (Henry et al., 2018). It is important to note that animals rarely encounter a single stressor; heat stress is frequently accompanied by combined influences such as inadequate nutrition, limited water access, or poor diet quality, where the synergy of multiple stressors can have a stronger effect than each acting alone (Cheng et al., 2022).

Animals possess innate capacities to cope with climatic challenges through the process of adaptation. Their response to stress includes an initial reaction to changing conditions, as well as deeper adaptive mechanisms, including activation of the endocrine system. In cases of prolonged stress, beneficial adaptations may become permanently established through genetic fixation (Collier et al., 2008).

This paper aims to present the significance and diversity of adaptive mechanisms in domestic animals, as well as key strategies for overcoming adverse environmental conditions. In the era of climate change, the resilience and adaptability of animals reflect the dynamic relationship between evolution and survival. To maintain homeostasis, animals activate a range of mechanisms—behavioral, physiological, biochemical, immunological, anatomical—or their combinations (Figure 1), with the effectiveness of these responses determined by previous exposure, duration and intensity of stress, physiological status, and environmental conditions (Ratnakaran et al., 2017; Sejian et al., 2018).

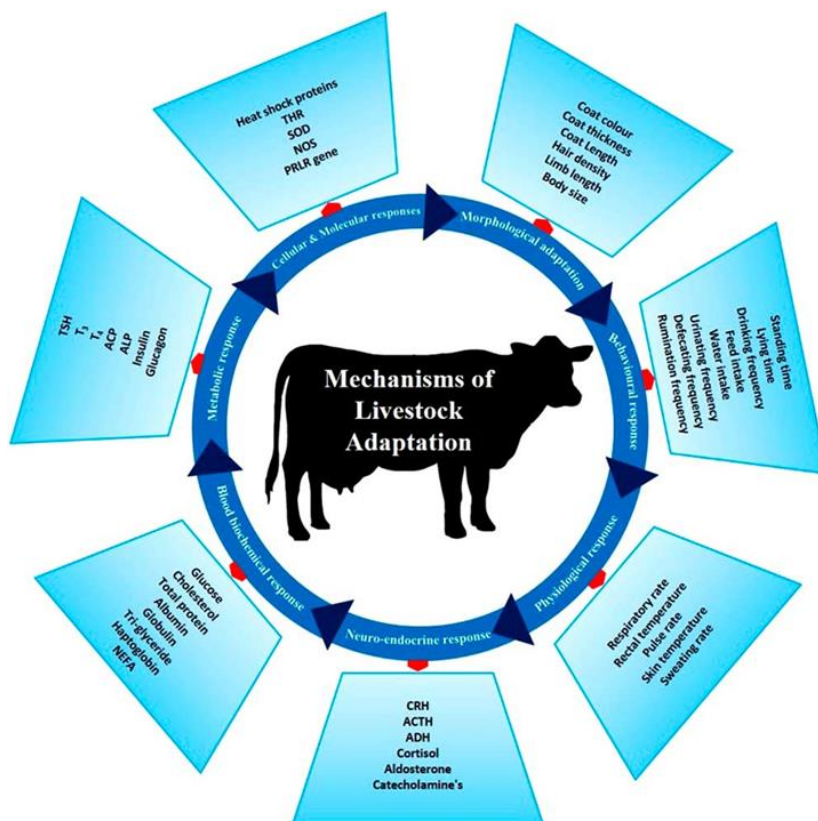


Figure 1. Adaptive strategies of farm animals coping with climate change (adapted from McManus et al., 2008)

THR=thyroid hormone receptor; SOD=super oxide dismutase; NOS=nitrous oxide synthase; PRLR=prolactin receptor; CRH=corticotropin-releasing hormone; ACTH=adrenocorticotropic hormone; ADH=antidiuretic hormone; NEFA=non-esterified fatty acids; TSH=thyroid-stimulating hormone; T₃=triiodothyronine; T₄=thyroxine; ACP=acid phosphatase; ALP=alkaline phosphatase.

Physiological strategies

Physiological strategies represent adaptive mechanisms by which domestic animals maintain homeostasis and mitigate the impact of climate stressors, regulating bodily, metabolic, and endocrine functions (Zhang et al., 2017). Mechanisms such as panting and sweating play a crucial role in thermoregulation, enabling efficient dissipation of excess heat (Bernabucci, 2019). Accelerated breathing increases moisture evaporation (Renaudeau et al., 2012), while an

elevated heart rate improves circulation and directs blood flow to the body surface, facilitating heat loss (Maibam et al., 2017). Increased rectal temperature (El-Tarabany et al., 2017) and adapted renal function help maintain fluid balance within the organism.

Pigs, due to the lack of sweat glands and relatively small lungs, struggle more with high temperatures and rely heavily on heat release through the skin. Goats, on the other hand, exhibit an exceptional ability to reduce metabolic rate and enter a state of torpor to conserve energy during extreme thermal conditions (Berihulay et al., 2019).

Among endocrine organs, the adrenal and thyroid glands play a central role in organismal adaptation to high temperatures (Romanello et al., 2018). Activation of the HPA and SAM axes leads to the release of several hormones essential for maintaining thermal homeostasis, including glucocorticoids, catecholamines, ADH, thyroid hormones, mineralocorticoids, growth hormone, and prolactin (Sheba et al., 2012). Increased cortisol concentrations support adaptive processes by stimulating hepatic gluconeogenesis, thus providing additional energy to animals under stress (Chen et al., 2015). In lactating cows and heifers exposed to heat stress (Zhang et al., 2014), reductions in TSH, T3, and T4 levels have been documented, representing a mechanism for reducing metabolic heat production.

Biochemical Strategies

Domestic animals employ biochemical adaptation mechanisms to climate change through alterations in blood composition and cellular adaptation. These mechanisms fall into two main groups: hematological responses and cellular-molecular responses.

Hematological Responses

Exposure to high temperatures leads to reduced erythrocyte counts and lower hemoglobin levels (Marai and Habeeb, 2010), as well as decreased packed cell volume (PCV) due to hemodilution, which enables more efficient heat dissipation (Garkal et al., 2016). Under heat stress, plasma albumin concentration increases, likely owing to its antioxidative role (Rasooli et al., 2004). Blood glucose level typically decreases as a result of diminished gluconeogenesis (Alberghina et al., 2013), while lower NEFA concentrations indicate increased utilization as an energy source (Skibieli et al., 2018). According to Baumgard and Rhoads (2013), the use of glucose to reduce metabolic heat production represents an important adaptive mechanism.

Cellular-Molecular Response

At the cellular level, one of the most important defense mechanisms is the increased production of heat shock proteins (HSP), which stabilize and protect cells from damage (Basirico et al., 2011). Heat stress conditions result in decreased DNA synthesis, transcription, and translation (Saxena and Krishnaswamy, 2012), as well as reduced fatty acid metabolism, increased glucose oxidation, and activation of the immune and endocrine systems (Collier et al., 2008). Genes involved in HSP synthesis and heat tolerance hold potential for use in selection and breeding of livestock lines resistant to climatic stressors (Kumar et al., 2017).

Immunological Strategies

Climate change significantly affects the health and welfare of domestic animals, promoting the spread of vector-borne diseases and weakening the immune system, which results in increased susceptibility to infections (Bernabucci, 2019). Heat stress disrupts immune cell balance, reduces antibody production, and impairs organs such as the thymus and spleen, thereby raising disease risk (Caroprese et al., 2021).

Different species and breeds display varying levels of immunological resistance, largely determined by genetics and environmental conditions. Cattle, especially dairy cows, are notably sensitive to the negative impacts of heat stress (Dahl et al., 2020). In contrast, dromedary camels exhibit heightened immune cell activity and superior antimicrobial responses (Hussen, 2021), while *Bos indicus* (zebu) are characterized by pronounced thermotolerance and a robust capacity to maintain immune function under elevated temperatures (Hansen, 2004).

Increased expression of immune genes, such as TLR2/4 and IL2/6, in Tharparkar cattle confirms their resilience (Bharati et al., 2017), and higher TLR gene expression in Osmanabadi goats under heat and nutritional stress indicates their adaptive capacity (Sophia et al., 2016).

For sheep, heat stress may weaken immune responses and raise infection risk; however, African Damaras maintain stable immune function and resistance (Almeida, 2011). Hungarian Cikta sheep exhibit superior thermotolerance compared to other breeds (Astuti et al., 2023), and goats have a higher lymphocyte-to-neutrophil ratio, indicating a well-developed immune system (Daramola and Adeloye, 2009).

Anatomical-Morphological Strategies

Anatomical changes are among the key ways in which domestic animals adapt to heat stress, revealing their remarkable plasticity and capacity for adaptation (Hall, 2004). These changes encompass variations in body size and specific body parts, hair color, type and density, as well as modifications in the respiratory system (McManus et al., 2008). Additionally, certain species develop unique morphological traits such as enlarged salivary glands, expanded absorptive mucosal surfaces, and an increased foregut volume when consuming fiber-rich diets (Renaudeau et al., 2012).

The physical structure of hair plays a crucial role in protecting the body from overheating. Attributes such as color, length, and density directly affect heat exchange with the environment, shielding against solar radiation and facilitating heat loss via convection and evaporation (McManus et al., 2008). In tropical regions, animals with light-colored coats can reflect a greater portion of solar radiation, while dark coats increase heat absorption. Thus, shades like white or light brown are advantageous in hot climates. Cattle in such environments also possess smooth, short, thin hair and fewer follicles per unit area, which significantly enhance heat dissipation (Henry et al., 2018; Bernabucci, 2019).

In sheep, thick wool acts as insulation during cold periods, but in high temperatures, long and dense fleece may lead to overheating (Renaudeau et al., 2012). Conversely, lower fiber thickness and density, combined with short and compact wool, enable more efficient heat loss and maintenance of homeostasis. Indigenous sheep breeds in arid and semi-arid regions often have a “carpet-type” fleece, which not only provides protection from solar radiation but also allows for more effective heat dissipation (Renaudeau et al., 2012). Furthermore, in some breeds, a fat tail represents a unique morphological adaptation that further facilitates thermoregulation (Berihulay et al., 2019).

Body Size

Body size directly affects the energy requirements of animals for maintenance, growth, and production, and changes in size have important implications for thermal biology. In warmer climates, reduced body mass may serve as a crucial adaptive mechanism, as a higher surface-area-to-volume ratio enables more efficient heat dissipation (Elayadeth-Meethal et al., 2021).

Research by Elayadeth-Meethal et al. (2021) has demonstrated that dwarf cattle breeds (DCB, *Bos taurus indicus*) possess superior thermotolerance compared to standard breeds (SCB, *Bos taurus indicus* × *Bos taurus taurus* crossbreds). While SCB can acclimatize to elevated temperatures in the short term,

DCB are much better adapted to living in hotter climates over the long term. Their higher hemoglobin concentration and smaller cell size further enhance their capacity to cope with heat stress.

Body Parts – Extremities

The size and shape of body parts such as the tail, beak, and legs play a significant role in regulating body temperature through heat exchange. Animals inhabiting warmer climates often develop larger extremities relative to their body size, consistent with Allen's rule, which remains underappreciated in the context of climate adaptation studies (Allen, 1877). Such extremities contribute to more efficient thermoregulation under high temperatures, whereas their impact is considerably less in cold environments.

In birds, the beak and legs serve as important "natural radiators"—well vascularized and not covered by feathers, they facilitate substantial heat loss. Mammals exhibit similar adaptations with ears and tails, as exemplified by Brahman cattle and Nubian goats, where these body parts play a crucial role in maintaining thermal equilibrium, fully supporting the predictions of Allen's rule.

Respiratory System

The respiratory system of domestic animals exhibits a degree of plasticity in response to climate change. Modifications such as increased lung capacity or altered breathing patterns (e.g., enhanced panting) enable animals to regulate body temperature more efficiently, thereby attenuating the negative effects of heat stress (Bernabucci, 2019).

Morphological Adaptations in Different Species

Goats stand out as the most adaptable species, capable of dissipating heat through their horns and surviving even with minimal vegetation. Burs and Nubian goats are particularly noteworthy, with long ears and wrinkled skin serving as efficient adaptations to arid conditions. In contrast, sheep show lower heat tolerance; while wool can provide protection, sheep with hair instead of wool, such as Red Maasai and Sahel sheep, as well as breeds like Dorper and Karakul, exhibit greater resilience. These breeds utilize unique survival mechanisms, such as fat storage in the tail, enabling them to withstand extended droughts and limited water availability.

Continental high-yield dairy cattle are more sensitive to heat stress compared to tropical types such as Zebu, Brahman, and Gir. It is therefore

recommended to improve their resilience by introducing genes characteristic of tropical populations, such as shorter hair or elongated body shape.

In poultry, morphological traits like naked necks (as seen in "naked-neck" chickens) or markedly larger combs and wattles in chicks significantly contribute to enhanced thermotolerance.

Pigs, on the other hand, are the most sensitive species to heat stress due to a lack of sweat glands, small lungs, and limited thermoregulatory capacity. While breeds such as Duroc show moderate resistance, certain Chinese breeds—Luchuan, Dianan, Bamaxiang, and Wuzhishan—possess genetic adaptations making them more robust and offering potential directions for genetic improvement of heat resistance in pigs.



Figure 2. Morphological characteristics of climate-resilient farm animal species

Behavioral Strategies

In addition to physiological, biochemical, and anatomical mechanisms, farm animals display a range of behavioral strategies to cope with climate challenges (Bernabucci, 2019). These adaptations, aimed at mitigating the effects of heat stress and maintaining thermal balance, include changes in movement, habits, and feeding behaviors (Renaudeau et al., 2012).



Figure 3. Behavioral patterns leading to cooling in different species of domestic animals

One of the most common forms of adaptation is seeking shade and cooler areas, bathing, or spraying water to cool the body (Zhang et al., 2017; Bernabucci, 2019). Cattle, for example, demonstrate a marked preference for natural tree shade, as dense foliage provides more effective evaporative cooling compared to artificial shelters (Ratnakaran et al., 2017). Poultry dissipate heat by spreading their wings and increasing heat loss through exposed body parts, while pigs—being particularly sensitive—find relief by wallowing in mud or lying on cool surfaces.

In cattle, increased heat load leads to reduced time spent resting and more time standing, which maximizes the body surface exposed to air and facilitates evaporative cooling, reducing the impact of radiant and convective heat from the ground (Sejian et al., 2018). Technological solutions such as sprinklers and fans have also proven effective in lowering heat stress, thereby increasing rest time and improving animal welfare (Henry et al., 2018).

Heat stress affects feeding habits as well. Animals often reduce food intake to lower metabolic heat production, and grazing cattle shift activity towards nighttime during the hottest days (Cheng et al., 2022). High temperatures also reduce the frequency of urination and defecation, depending on water availability, food supply, and environmental conditions (Sejian et al., 2018).

The need for water rises dramatically under such conditions; animals typically prefer moderate or cooler water, as it directly contributes to sustaining thermal stability.

All these behavioral adaptations reflect the innate ability of animals to recognize environmental cues and adapt their behavior accordingly. This interaction between ambient conditions and behavior serves as a crucial mechanism for survival amid climate change.

Conclusion

Climate change represents one of the greatest challenges for modern livestock production, directly affecting the health, productivity, and welfare of domestic animals. Although human interventions, such as technological and management solutions, can significantly mitigate the effects of climatic stressors, the long-term foundation of sustainability is primarily the biological adaptability of the animals themselves. Therefore, understanding and integrating animal adaptations is key to creating livestock systems that are resilient to climate change.

Analysis of physiological, biochemical, immunological, morphological, and behavioral mechanisms demonstrates that animals possess remarkable plasticity and the ability to maintain homeostasis under extreme conditions. These adaptations should not be seen merely as stress responses, but as a valuable biological basis for the selection and improvement of future populations. Their application in modern practice creates opportunities for the development of innovative strategies—from genetic programs aimed at increasing thermoresilience, to optimizing the management of nutrition, housing, and animal welfare.

While human adaptations are essential for immediate mitigation of climate-related impacts, only deeper understanding and utilization of animal adaptations can provide the foundation for the long-term stability and productivity of livestock production. In this way, the combination of the evolutionary strengths of animals and modern management practices may become the most effective response to the challenges of climate change—not only today, but also in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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ANTIMICROBIAL RESISTANCE: A GLOBAL CHALLENGE AND IMPLICATIONS FOR DAIRY CATTLE PRODUCTION

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Abstract: Antimicrobial resistance (AMR), especially antibiotic resistance, currently is one of the most important public health challenges, with serious consequences for human, animal and environmental health. In the context of dairy cattle production, the frequent and often unnecessary use of antibiotics, especially in the treatment of mastitis, significantly contributes to the development and spread of resistant bacteria. The aim of this review was to analyse the occurrence and mechanisms of AMR development in dairy production, with a special emphasis on mastitis as a main model for understanding the connection between veterinary practice, public health and food safety. Furthermore, the paper also provides an overview of current national and European strategic frameworks for AMR control, including selective therapeutic approaches, rapid diagnostics and preventive measures on dairy farms. In conclusion, the need for an integrated approach based on the *One Health concept*, which includes the collaboration of policy, science, education and practice, is necessary in order to preserve the effectiveness of antimicrobials and ensure sustainable dairy production.

Key words: antimicrobial resistance, mastitis, dairy cows, food safety, responsible antibiotic use

Introduction

Antimicrobial resistance (AMR) refers to the ability of microorganisms, including bacteria, viruses, fungi, and parasites, to survive and proliferate despite the presence of drugs designed to eradicate them or inhibit their growth (WHO, 2024). This phenomenon is not a result of human or animal adaptation but rather a natural evolutionary response seen predominantly in bacterial populations. AMR presents a formidable challenge in effectively treating infectious diseases in both humans and animals. Furthermore, the development of antimicrobial resistance is a naturally occurring process; however, it is significantly accelerated by the misuse and overuse of antibiotics in various sectors. In human healthcare, factors such as the unnecessary prescription of antibiotics for viral infections, patients not completing prescribed courses, and self-medication contribute to the emergence of resistant strains. In veterinary medicine, the routine use of antibiotics for disease prevention and growth promotion in livestock can create environments conducive to resistance development. Furthermore, the application of antibiotics in animal husbandry complicates disease management and impacts food safety. On a global scale, the implications of AMR extend far beyond treatment complexities; it threatens the effectiveness of essential medical procedures, such as surgeries and chemotherapy, which rely on effective antibiotics to prevent infections. In veterinary practice, the challenge is illustrated by conditions such as mastitis in dairy cattle, where resistant infections can lead to significant economic losses, decreased milk production, and deteriorated animal welfare. Addressing AMR necessitates a coordinated effort across healthcare systems, agriculture, and public health to ensure effective management and containment strategies are implemented.

This review aimed to analyse the mechanisms of AMR development in dairy production, with emphasis on mastitis as a model for understanding the connection between veterinary practice, public health and food safety. Furthermore, this paper also provides an overview of current national and European strategic frameworks for AMR control, including selective therapeutic approaches, rapid diagnostics and preventive farm measures.

Materials and Methods

A comprehensive literature search was conducted using electronic databases including Web of Science, Scopus, PubMed, and Google Scholar, as well as relevant websites of international organizations (WHO, WOA, EFSA, EU). The following keywords and their combinations were applied: “*antimicrobial*

resistance,” “antibiotic resistance,” “dairy cattle,” “mastitis,” “antimicrobial use,” “public health,” and “One Health.”

Publications considered included peer-reviewed articles, review papers, systematic analyses, official reports, and strategic policy documents published between 2000 and 2025, with particular emphasis on recent studies and the European regulatory framework. Literature was selectively included based on its relevance to antimicrobial resistance in dairy production, with mastitis serving as a primary model for exploring the interconnection between veterinary practice, public health, and food safety.

In total, more than 40 references were reviewed, of which those most directly contributing to the understanding of AMR development and spread, its economic and health implications, and potential strategies for prevention and control on dairy farms were cited in this paper.

Mechanisms of resistance development

Bacterial resistance to antibiotics primarily develops through two interconnected mechanisms: genetic mutations and the horizontal transfer of resistance genes (Ferrara et al., 2024). **Horizontal gene transfer** often occurs via mobile genetic elements such as plasmids and transposons, which can move resistance traits between different bacterial species. These genes equip bacteria with various defence strategies, such as breaking down or inactivating the antibiotic, avoiding its effects, or actively expelling it from the cell using specialized proteins known as efflux pumps. In the dairy sector, antibiotic resistance is a particularly pressing issue when it comes to pathogens associated with mastitis, an inflammation of the mammary gland in dairy animals. Mastitis is one of the most common diseases in dairy farming and is frequently treated with antibiotics, which increases selective pressure and contributes to the spread of resistant bacterial strains. Key bacterial causes of mastitis include *Staphylococcus aureus*, a pathogen known for its ability to form biofilms and resist many antibiotics; *Escherichia coli*, which can cause severe infections; *Streptococcus uberis*, frequently associated with environmental mastitis; and *Klebsiella spp.*, which are opportunistic pathogens that can lead to persistent infections. Research has indicated a troubling upward trend in the resistance of these bacteria to critical classes of antibiotics, including β -lactam antibiotics (such as penicillin), tetracyclines, and macrolides. This growing resistance poses a significant challenge for veterinary medicine, highlighting the need for careful antibiotic stewardship in agricultural practices (Alhassan et al., 2025).

The application of antibiotics within dairy cattle farms primarily targets the treatment of both clinical and subclinical mastitis. Additionally, these medications

are often utilized during what is known as "dry cow therapy," a preventative measure administered when cows are nearing the end of their lactation period, aimed at protecting their udders during the non-lactating phase to minimize the risk of infections. Historically, antibiotics have also been employed as growth promoters in livestock, a practice that has contributed to the alarming rise of antibiotic-resistant bacteria within agricultural settings. This misuse has profound implications for both animal health and public safety. In recent years, there has been a concerted effort to promote responsible antibiotic usage within dairy farming to mitigate the risks associated with antimicrobial resistance. However, certain regions continue to rely on blanket therapy, a controversial practice that involves administering antibiotics to all animals without confirming the presence of an infection. This indiscriminate approach fosters an environment conducive to the development of resistant bacterial strains. These resistant pathogens can infiltrate the food supply by contaminating milk and other dairy products, posing a significant public health risk as they can ultimately be transmitted to humans through consumption (WHO, 2024). *Staphylococcus aureus* is a significant pathogen associated with chronic mastitis, a condition that leads to inflammation and infection of the udder tissue. Notably, many strains of *Staphylococcus aureus* demonstrate resistance to commonly used antibiotics, particularly penicillin, and some have developed resistance to methicillin, classifying them as methicillin-resistant *Staphylococcus aureus* (MRSA). This resistance poses a significant challenge in treating infections effectively. On the other hand, *Escherichia coli* is often implicated as the primary pathogen in cases of acute mastitis. This bacterium can possess genes responsible for producing extended-spectrum beta-lactamases (ESBLs), enzymes that confer resistance to many beta-lactam antibiotics. The presence of these resistant strains complicates treatment options, making it critical for clinicians to identify the specific bacteria involved and determine the most effective antibiotic therapy. Effective management of both types of mastitis requires a thorough understanding of these pathogens and their resistance mechanisms.

Additionally, incorporating data on the spread of antibiotic resistance genes (ARGs) through manure and wastewater would provide valuable insights; for example, resistance genes such as blaCTX-M can persist in soil for months and facilitate horizontal transfer to environmental bacteria (Berendsen et al., 2015). Furthermore, related to human health, a study conducted Van Cleef et al. (2010) detected the identical MRSA strains in both humans and livestock, suggesting direct zoonotic transmission, confirming the potential for zoonotic transmission of MRSA and ESBL-producing *E. coli* from farms to humans

Mastitis as a model for understanding AMR

Mastitis represents a key model for investigating the dynamics of AMR, especially within the dairy sector. Characterized by inflammation of the mammary gland, mastitis is the most common disease affecting dairy cows and stands as the leading cause of antibiotic use in dairy farming. Treatment typically involves the frequent, and often empirical application of antibiotics, making mastitis a central reference point for examining and monitoring the development of antimicrobial resistance (Ruegg, 2017). The widespread occurrence of mastitis, particularly in its subclinical form, has led to an increased dependence on antimicrobial agents, often administered as a preventive measure. This is especially true during the dry-off period, a critical phase when cows transition into the next lactation. However, such preventive use exerts significant selective pressure on bacterial populations, encouraging the emergence and spread of resistant strains. The consequences are serious: not only is the efficacy of existing treatments diminished, but producers face rising costs, and potential risks to public health grow more pronounced (Oliver et al., 2011; WHO, 2024). Mastitis typically presents in two forms: clinical and subclinical. *Clinical mastitis* is characterized by visible symptoms such as changes in milk appearance (e.g., clots, discoloration), swelling or heat in the udder, and elevated body temperature. These clear indicators make clinical cases relatively easier to detect and manage. *Subclinical mastitis*, by contrast, is far more insidious and accounts for up to 90% of mastitis cases on some farms. It lacks obvious external signs, making diagnosis reliant on elevated somatic cell counts (SCC) or specialized laboratory analysis of milk samples (Halasa et al., 2007). This hidden nature poses a major challenge for herd management, allowing undetected infections to persist and spread within the population. Furthermore, chronic subclinical infections can lead to sustained inflammation, reduced milk yield, and a gradual build-up of resistance due to prolonged antibiotic exposure.

The pathogens responsible for mastitis are typically categorized into two major groups: contagious agents, which spread from cow to cow (often during milking), and environmental pathogens, which originate from the cow's surroundings, such as bedding or manure. Among the **contagious agents**, the most notable are *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma spp.*, *Staphylococcus aureus*, in particular, poses a significant challenge due to its multifaceted resistance mechanisms, including its ability to form biofilms and its characteristic intracellular localization. Moreover, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains complicates treatment, rendering β -lactam antibiotics largely ineffective (Barkema et al., 2009). Turning to **environmental pathogens**, notable examples include *Escherichia coli*, *Klebsiella spp.*, *Streptococcus uberis*, and *Prototheca spp.* While *E. coli* is frequently

associated with acute cases of mastitis, recent developments have raised concerns about its increasing acquisition of extended-spectrum beta-lactamase (ESBL) genes. These genes produce enzymes that can degrade a broad array of β -lactam antibiotics, thus severely limiting the treatment options available for affected herds (Oliver et al., 2011).

Treating mastitis in dairy cows involves numerous challenges that often lead to not optimal outcomes. One of the main complications is subclinical mastitis, a form of the disease that progresses without visible symptoms. Because it often goes unnoticed, affected cows can remain untreated for extended periods, allowing the infection to spread silently within the herd. Even when treatment is initiated, it is frequently based on empirical decisions, without identifying the specific pathogen, which not only reduces the likelihood of success but also contributes to the growing problem of AMR due to inappropriate antibiotic use (Ruegg, 2017). Another significant challenge is the limited efficacy of antibiotics during lactation. Many commonly used drugs fail to reach therapeutic levels in the mammary gland, especially when the infection involves biofilm-forming bacteria such as *Staphylococcus aureus*. Additionally, veterinary regulations often restrict the use of certain antibiotics during lactation, leaving veterinarians and producers with fewer options for effective treatment. The economic implications of treatment also present serious concerns. During the required withdrawal period, milk from treated cows must be discarded, which results in considerable financial loss, especially in high-yielding animals. Faced with these costs, some producers may shorten the duration of therapy or resort to unapproved treatments, actions that not only compromise animal health but also contribute to AMR (Oliver et al., 2011). Furthermore, pathogen identification remains a persistent obstacle. In the absence of detailed microbiological testing, it is difficult to distinguish between contagious and environmental causes of mastitis. As a result, treatment often involves broad-spectrum antibiotics used without precise targeting, which increases the likelihood of resistance development and reduces treatment effectiveness (Halasa et al., 2007).

To effectively combat antimicrobial resistance in veterinary medicine, especially regarding mastitis in dairy cows, several focused strategies have been developed. One key approach is *Selective Dry Cow Therapy* (SDCT), which replaces the traditional blanket antibiotic treatment during the dry period with a more targeted method. Instead of treating all cows with antibiotics, SDCT focuses only on those showing signs of infection or with a history of mastitis. This helps preserve antibiotic effectiveness and reduces overall antimicrobial use. *Rapid diagnostic tests* also play a crucial role in guiding treatment decisions. Tools like the California Mastitis Test (CMT) or PCR for identifying pathogens allow veterinarians and farmers to quickly detect the specific cause of mastitis. This

enables treatments to be tailored to the infection rather than relying on broad-spectrum antibiotics. Regular testing of mastitis pathogens and their antibiotic sensitivities through antibiograms provides valuable insight into local resistance patterns. Knowing which antibiotics remain effective against common pathogens helps farmers make informed treatment choices and minimizes inappropriate antibiotic use. *Biological and preventive measures* are equally important. Vaccination programs can boost animal health and resistance to infections. Improving hygiene during milking and maintaining clean living conditions for the cows significantly reduce infection risk by limiting pathogen exposure. Finally, education and training for farmers and veterinary staff are essential. Teaching the principles of responsible antibiotic use, effective mastitis management, and ongoing herd health monitoring encourages adoption of practices that support antimicrobial resistance reduction goals.

Public health and economic aspects of AMR in dairy farming

AMR in dairy farming poses a significant challenge that extends beyond veterinary medicine and animal health, becoming a critical public health issue as well as an economic concern. The close interaction between humans and livestock, coupled with the complexities of a globalized food supply chain, makes dairy cattle farming a key factor in the potential transmission of antibiotic-resistant microorganisms to people (WHO, 2024). Resistant bacterial strains emerging in animals can reach humans through several main pathways. One such route is through contaminated dairy products. Improper management of antibiotic use in dairy cows can result in residues persisting in milk at the time of processing. When antibiotics are given too close to milking or withdrawal periods are not properly observed, residues can contaminate raw milk. Additionally, inadequate pasteurization may fail to eliminate antibiotic residues and resistant pathogenic bacteria, which poses a serious risk when these products enter the human food chain (Oliver et al., 2011). Another pathway is direct contact with animals. People working on dairy farms, such as farmers, and veterinarians often have close and prolonged contact with livestock. This increases their risk of becoming colonized or infected with antibiotic-resistant bacteria, including strains like methicillin-resistant *Staphylococcus aureus* (MRSA). The occupational exposure heightens the chance of zoonotic transmission, where bacteria can jump from animals to humans (Dignard and Leibler, 2019).

Environmental contamination is also a significant concern. Antibiotic-resistant bacteria and antibiotic residues frequently enter the environment via the manure and urine of treated animals. When this waste is applied as fertilizer, it can contaminate soil and water sources, both surface and groundwater. Such

environmental spread facilitates the transmission of AMR to wildlife and human populations alike, broadening the public health threat (WHO, 2024). Furthermore, the presence of antibiotic residues in milk is particularly concerning for consumer safety. These residues may cause adverse health effects, especially in individuals sensitive to certain antibiotics like penicillins, who may suffer severe allergic reactions from contaminated milk. Moreover, antibiotic residues can disrupt the balance of intestinal microbiota, leading to digestive problems and other health issues. Importantly, these residues also play an indirect role in encouraging the development of AMR within human populations. According to the European Food Safety Authority (EFSA, 2024), this contributes to a wider public health problem, making infections harder to treat due to diminished antibiotic effectiveness.

To address these risks, strict regulatory measures have been implemented to monitor antibiotic residues in milk. Dairy processors are required to conduct routine testing using sensitive methods like the Delvotest, a biosensor test that detects antibiotic inhibitors in milk. Effective control of antibiotic residues depends on several critical factors, including strict adherence to withdrawal periods, ensuring sufficient time has passed after antibiotic treatment before milk is collected. Accurate and detailed record-keeping of all medications administered to livestock is essential to prevent mixing milk from treated and untreated animals prematurely. Additionally, ongoing education and training for dairy producers on responsible antibiotic use are vital. These programs foster best practices in medication management and encourage a culture focused on animal welfare and consumer safety.

From an economic perspective AMR in dairy cattle farming has extensive consequences that reach far beyond individual farms, impacting the entire agricultural value chain. One of the primary challenges for producers is the loss of milk production. Infections caused by resistant bacteria can significantly reduce both the quantity and quality of milk produced by affected cows. As a result, farmers may receive lower prices since buyers tend to prefer higher-quality products. Hogeveen et al. (2011) emphasize that the cumulative financial impact of these production losses can be substantial. Treatment costs also rise as managing antimicrobial resistance often requires more intensive veterinary care. This can involve multiple treatments, longer durations of therapy, and the use of more expensive or less effective antibiotics. Seegers et al. (2003) point out that these factors increase direct veterinary expenses and create logistical difficulties for farmers as they struggle to control resistant infections. Animal losses further exacerbate economic pressures. Chronic infections such as mastitis, particularly when treatments fail, can lead to the culling or euthanasia of affected cows. This not only results in immediate financial loss but also diminishes the herd's genetic diversity, which can negatively affect the long-term productivity and health of the

dairy operation. A serious economic risk arises when milk contains antibiotic residues above the maximum residue limits (MRLs). In such cases, contaminated milk must be discarded, causing significant financial damage to producers. Additionally, farmers may be temporarily excluded from milk purchasing networks, harming their market position and reputation among consumers and industry stakeholders.

Furthermore, it would also be beneficial to highlight the economic impact by quantifying the costs associated with AMR; for instance, the U.S. dairy sector incurs annual losses of approximately \$2 billion, while in the EU, mastitis-related AMR costs dairy farms around €1.7 billion each year due to milk waste and treatment expenses (EFSA, 2022). Additionally, presenting comparative data—such as the resistance rates in Croatia versus EU averages, noting 40% *S. aureus* penicillin resistance in Croatia compared to 25% across the EU—could further enhance the analysis.

Beyond individual farms, the socio-economic impact of AMR is even more far-reaching. The rising costs of human healthcare are a direct consequence of infections caused by resistant pathogens becoming more difficult and expensive to treat. The reduced effectiveness of antibiotics in human medicine due to multidrug-resistant organisms presents major public health challenges, as highlighted by the World Health Organization (WHO, 2024). Moreover, the threat AMR poses to global food security is especially concerning. In regions where agriculture forms the economic backbone, a decline in livestock production caused by AMR could have severe repercussions not only for farmers but also for entire communities dependent on agriculture. The seriousness of resistant pathogens underscores the urgent need for coordinated action to address AMR within agricultural systems, highlighting its critical links to public health and economic stability.

Strategies to mitigate antimicrobial resistance in dairy cattle

Mitigating the spread of antimicrobial resistance requires a multisectoral approach that connects human, animal and environmental health, by the concept of "One Health". Strategies include regulatory measures, changes in veterinary practice and the implementation of recommended hygienic-preventive procedures on farms (WHO, 2024, WOA, 2025).

The Republic of Croatia adopted the "*Nacionalni program za kontrolu otpornosti bakterija na antibiotike 2017. – 2021.*", which establishes priorities and activities in the sectors of public health, veterinary medicine, agriculture and environmental protection (Ministry of health, Republic of Croatia, 2017). Key guidelines include:

- strengthening supervision over the consumption of antibiotics in veterinary medicine,
- encouraging alternative therapies and preventive measures,
- promoting the responsible use of antimicrobials at farm level,
- strengthening the education of veterinarians and producers.

At the European Union level, the new Regulation 2019/6 (EU, 2019) on veterinary medicinal products has been in force since 28 January 2022, which clearly prohibits:

- the prophylactic use of antibiotics without clinical or laboratory evidence of risk (Article 107),
- group medication with antimicrobials except in exceptional situations with the authorisation of a veterinarian,
- the use of antibiotics to promote growth and improve production efficiency, which has been prohibited at EU level since 2006 (Regulation 1831/2003/EC).

Furthermore, the European Commission has introduced an ambitious target: to reduce the total sales of antimicrobials in the EU livestock sector by 50% by 2030 (EU Farm to Fork Strategy, 2020).

Mitigating AMR on dairy farms relies heavily on disease prevention, minimizing unnecessary antibiotic use, and adopting sustainable health practices. One effective approach is **Selective Dry Cow Therapy (SDCT)**, which replaces the routine use of antibiotics at the end of lactation, known as blanket dry cow therapy, with treatment only for cows that have confirmed infections based on indicators such as somatic cell count (SCC), the California Mastitis Test (CMT), or microbiological cultures. Research by Kabera et al. (2021) and Ruegg (2017) demonstrates that SDCT significantly reduces antibiotic use without compromising udder health or milk production. Improving milking hygiene is another crucial practice. This involves thorough udder preparation, disinfecting teats before and after milking (pre- and post-dipping), and ensuring proper maintenance of milking equipment. These measures help decrease the incidence of new intramammary infections, particularly those caused by environmental pathogens like *Escherichia coli* and *Streptococcus uberis*. Vaccination against key mastitis pathogens such as *Staphylococcus aureus*, *E. coli*, and *Mycoplasma bovis* also plays an important role. The use of commercial vaccines can reduce the occurrence of clinical mastitis and lessen the need for antibiotic treatments, especially in herds with a history of recurrent infections (Rainard et al., 2018). The application of rapid diagnostic tests directly on the farm further supports targeted therapy. Tools such as the California Mastitis Test, on-farm bacterial cultures, and PCR diagnostics enable quick

identification of causative agents, improving treatment effectiveness and minimizing unnecessary antibiotic use. Finally, educating farmers and veterinarians about responsible antibiotic use is essential. Training on antimicrobial stewardship fosters informed decision-making, reduces empirical antibiotic prescriptions, and encourages prevention through improved herd health management (Skjølstrup et al., 2020). All of these practices contribute to a comprehensive strategy for controlling AMR in dairy farming.

In addition to preventive measures and targeted therapeutic approaches, there is a growing focus on non-antibiotic strategies for managing bovine mastitis and mitigating antimicrobial resistance. These promising alternatives encompass the use of probiotics, bacteriophage therapy, and plant-derived bioactive compounds. For instance, non-antibiotic options such as probiotics, phage therapy, and herbal extracts like oregano or tea tree oil, along with immunomodulatory plant compounds like curcumin, may help decrease reliance on antibiotics in mastitis control (El-Sayed and Kamel, 2021).

Conclusion

Antimicrobial resistance (AMR) in dairy production constitutes a complex and multidimensional challenge with significant implications for animal health, public health, food safety, and the long-term sustainability of the dairy sector. The frequent and largely empirical use of antibiotics in mastitis therapy accelerates the development and dissemination of resistant strains, reduces treatment efficacy, and jeopardises the safety and quality of milk. Additional concerns arise from the persistence of antimicrobial residues and the emergence of multidrug-resistant pathogens, which increase production losses, elevate veterinary costs, and restrict access to both domestic and international markets.

Addressing this issue requires a comprehensive, evidence-based strategy embedded within the **One Health** framework, which recognises the interdependence of human, animal, and environmental health. Priority measures include limiting non-essential antimicrobial use, advancing rapid and reliable diagnostic methods, strengthening biosecurity protocols at farm level, and implementing targeted interventions such as selective dry cow therapy and immunoprophylaxis against major mastitis pathogens. Equally important are continuous education and awareness-raising activities among farmers and veterinary practitioners to promote prudent antimicrobial use.

Given the limited prospects for the development of novel antimicrobial agents, preserving the effectiveness of existing drugs depends on coordinated collaboration between the scientific community, policymakers, veterinary professionals, and industry stakeholders. Such synergy is essential to mitigate

AMR effectively, safeguard animal and human health, and maintain the resilience and competitiveness of the dairy sector.

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DIETARY PLANT-BASED BIOACTIVE COMPOUNDS FOR SUSTAINABLE MASTITIS CONTROL IN LIVESTOCK

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Abstract: The increasing antimicrobial resistance among mastitis pathogens necessitates alternative therapeutic approaches in dairy cattle. This study evaluated the antimicrobial efficacy of selected plant extracts against both reference and production strains of mastitis-associated microorganisms *in vitro*, and assessed the *in vivo* effects of an extruded feed additive (the wormwood–poplar extract additive) containing *Artemisia absinthium* and *Populus balsamifera* extracts, on udder health and milk quality in cows with subclinical mastitis. *In vitro* assays demonstrated significant antimicrobial activity of citrus peel (*Citrus* spp.) and spruce (*Picea abies*) extracts against *Candida* spp., *Escherichia coli*, and *Staphylococcus aureus*, including resistant field isolates. The 15-day *in vivo* trial involving 60 Holstein cows revealed a 68% reduction in somatic cell counts ($p = 0.017$) following supplementation, achieving compliance with top-grade milk standards without compromising milk composition or sensory attributes. These findings underscore the potential of phytogenic compounds as effective, natural alternatives for mastitis control, promoting sustainable dairy production and reduced antibiotic reliance.

Key words: Mastitis; *Artemisia absinthium*, antimicrobial activity, somatic cell count, milk quality, phytogenic feed additive

Introduction

Mastitis is one of the most common and economically detrimental diseases in dairy and meat-producing livestock worldwide, inflicting substantial losses due to decreased productivity, animal culling, and veterinary treatment costs (Chen et al., 2023). The disease is primarily caused by bacterial infection, with pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* frequently implicated (Kerro Dego and Vidlund, 2024). Conventional mastitis control relies heavily on antibiotics; however, this practice has contributed to a worrying increase in antimicrobial resistance (AMR), particularly among pathogens commonly associated with mastitis. Systematic studies have shown resistance rates exceeding 45 % for penicillin in *S. aureus*, with trends of increasing resistance noted across multiple antimicrobial classes over recent decades (Leon et al., 2025). In China, a study reported that 58.7% of *S. aureus* isolates from bovine mastitis were resistant to penicillin. Multidrug resistance was also observed, with 11.6% of isolates showing resistance to multiple antimicrobial classes (Yang et al., 2023). In Poland found penicillin resistance rates of 62.3% among *S. aureus* isolates from bovine mastitis, and studies in Turkey reported rates of 62.1% (Szweda et al., 2014). Given the global crisis of AMR and its potential impact on both animal and human health, there is a critical need to identify and implement viable alternatives to antibiotics. Dietary interventions using plant-derived bioactive compounds have emerged as a sustainable and promising strategy in this regard. These include phytochemicals such as essential oils, and polyphenols, which demonstrate antibacterial, anti-inflammatory, antioxidant, and immunomodulatory actions. Many studies have demonstrated inhibitory effects of compounds derived from *Eucalyptus globulus*, *Juglans regia*, *Schinus molle*, and *Taraxacum mongolicum* against mastitis pathogens, along with suppression of inflammatory pathways like NF- κ B and MAPK (Da Silva et al., 2023; Kaseke et al., 2023; Mezzasalma et al., 2025).

Moreover, local strategies such as intramammary administration of phytochemicals, probiotics, and bacteriocins are gaining traction, supported by growing evidence from biomimetic and plant-based therapeutic formulations. For example, intramammary flavonoid fractions have shown clinical efficacy against coagulase-negative *Staphylococci* in dairy cows (Gutiérrez-Reinoso et al., 2023). Such interventions not only target pathogens directly but also enhance host immune defenses and reduce inflammation, aligning with the One Health framework.

Plant-based bioactive compounds are naturally occurring compounds derived from herbs, spices, fruits, seeds, and other plant parts, known for their functional properties beyond basic nutrition. These compounds, often secondary metabolites, play crucial roles in plant defense and offer diverse pharmacological

benefits such as antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects (Wang et al., 2024). Their broad spectrum of biological activities has attracted increasing attention in veterinary science, particularly as sustainable alternatives to antibiotics in the management of livestock diseases, including mastitis.

Based on their biosynthetic origin and structural characteristics, plant bioactive compounds are commonly grouped into three main classes: polyphenols, terpenes and terpenoids, and alkaloids. Polyphenols, including flavonoids, phenolic acids, and tannins, exhibit strong antioxidant, anti-inflammatory, and antimicrobial activities, making them valuable in mitigating both pathogen load and host tissue damage in mastitis. For example, catechins and quercetin derivatives have demonstrated inhibitory effects on *S. aureus* and *E. coli*, alongside modulation of inflammatory pathways such as NF- κ B (Li et al., 2019; Wu and Brown, 2021).

Terpenes and terpenoids, particularly those found in essential oils, such as thymol, carvacrol, eugenol, and menthol, are volatile lipophilic compounds that disrupt microbial membranes, impair quorum sensing, and interfere with biofilm formation. Their rapid action and broad-spectrum efficacy make them especially promising in both dietary and topical applications.

Alkaloids, a diverse group of nitrogen-containing compounds like berberine and sanguinarine, show potent antimicrobial and immunomodulatory effects and are increasingly explored as feed additives or components in phytogenic formulations.

While other compounds such as saponins or coumarins may also contribute to antimicrobial activity, they are generally considered subclasses or functional extensions of the main groups above. The multifunctionality of these bioactives, particularly their combined antibacterial, anti-inflammatory, and antioxidant effects, makes them attractive candidates for integrated mastitis prevention strategies, especially in light of rising antimicrobial resistance and consumer demand for antibiotic-free production systems.

This study aims to assess current knowledge and identify effective dietary plant-based bioactives, especially essential oils, and polyphenols, for mastitis prevention and treatment in livestock. We focus on elucidating mechanisms, evaluating antimicrobial and immunomodulatory effects, and discussing pathways for integrating these compounds into sustainable livestock health management.

Material and Methods

In vitro study

Testing of traditional and alternative antimicrobial agents

To address the challenge of antimicrobial resistance among mastitis pathogens, the efficacy of commonly used udder treatment products, including chlorhexidine bigluconate, povidone-iodine, and other commercially available preparations sourced from two dairy farms, was evaluated against production (field) strains isolated from mastitic cows. Standard disk diffusion assays revealed that these traditional agents were largely ineffective against the production strains. While some products exhibited antimicrobial activity against reference laboratory strains (such as *Candida* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Serratia marcescens*), they failed to inhibit the growth of the field isolates. This finding underscores a marked decline in the effectiveness of conventional antimicrobials when used against contemporary, farm-derived pathogens.

Preparation and testing of plant extracts

In light of the limited efficacy of traditional antimicrobials, the study proceeded to investigate plant-based alternatives. Extracts were prepared from burdock leaves (*Arctium lappa folium*), citrus peel (*Citrus* spp. pericarpium), spruce (*Picea abies*), aloe vera (*Aloe vera folium*), and celandine (*Chelidonium majus*) using hot Soxhlet extraction with 70% ethanol as the solvent. The antimicrobial activity of these phytogenic extracts was assessed using the standardized disk diffusion method in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Both reference laboratory strains and production strains isolated from clinical samples were tested. The diameters of inhibition zones were measured to quantify the antimicrobial efficacy of each extract. This comparative approach allowed for a direct evaluation of plant extracts versus traditional agents in combating both standard and field-derived mastitis pathogens.

***In vivo* study**

Production of the feed additive

The extruded multi-functional feed additive was manufactured at the feed facility of “NFT-KATU” LLP, located within the Faculty of Veterinary and Livestock Technologies at the NCJSC “S.Seifullin Kazakh Agrotechnical Research University”. The additive was based on barley and oats as carrier materials, enriched with two phytogenic components: bitter wormwood (*Artemisia absinthium*) extract and balsam poplar (*Populus balsamifera*) bud extract. The wormwood extract, standardized to contain $\geq 1.2\%$ absintin (HPLC), was incorporated at 0.01% w/w, while the balsam poplar bud extract was included at 0.015% w/w. The production process of the feed additive involved barothermal extrusion, which combined high pressure and temperature to treat grain forages,

specifically barley and oats. This advanced processing method enhanced the digestibility and bioavailability of the nutrients in the grains. Following barothermal treatment, the mixture was formed into pellets, resulting in a uniform extrudate. The pelleted form facilitated convenient handling and feeding while ensuring consistent distribution of the active components throughout the animal feed. The extrusion line in the production and testing workshop utilized equipment produced in the Kostanay region of Kazakhstan, provided by LLP “Agrotekhservice-12”. The machinery included a PD-2000 pneumatic hammer crusher, an SG-800 kg mixer equipped with load cells and a cyclone, a frequency-controlled feed hopper, a PE-350 extruder, and a PG-600 flat matrix pelletizer.

For the purposes of this manuscript, the feed additive composed of barley and oats enriched with bitter wormwood (*Artemisia absinthium*) extract (0.01% w/w) and balsam poplar (*Populus balsamifera*) bud extract (0.015% w/w) is hereafter referred to as the wormwood–poplar extract additive.

Experimental design

The study was conducted at LLP “Agrofirma Rodina” in the Akmola region to evaluate the effectiveness of the extruded feed additive in the diets of dairy cows. The study involved 60 high-yielding Holstein cows of both black and brown varieties. A total of 120 milk samples were collected from animals diagnosed with mastitis. The affected cows received the wormwood–poplar extract additive for a 15-day treatment period. The details were previously described (Abakanova et al., 2025).

Isolation and identification of mastitis pathogens

To assess the current effectiveness of traditional mastitis control methods and to illustrate the severity of antimicrobial resistance, preliminary studies were conducted using commercial strains of microorganisms isolated from cows with subclinical and clinical mastitis. Pathogens identified included *Candida spp.*, *Escherichia coli*, as well as representatives of the general microflora, including staphylococcal and streptococcal bacteria and yeasts.

Assessment of dairy production and milk quality

Milk yield was monitored through controlled milking over the 15-day period. Milk samples were collected in sterile disposable bottles both prior to the introduction of the feed additive and throughout the 15-day experimental phase. The qualitative characteristics of the milk were assessed using the “Expert Super Plem Kombo” analyzer, which measured fat, protein, skimmed milk powder, lactose content, acidity, density, temperature, freezing point, and conductivity. The somatic cell count was determined with the “Ekomilk Scan” analyzer. Sensory

properties of the milk were evaluated according to the Republic of Kazakhstan standard 1732-2007, which outlines the organoleptic method for determining quality indicators. These assessments were conducted by university employees.

Diagnosis of mastitis

Latent mastitis in the cows was initially identified using the California test at LLP “Agrofirma Rodina” in the Akmola region.

Ethical approval

All animal procedures were reviewed and approved by the local ethical committee of the Faculty of Veterinary Science and Technology of Animal Husbandry at NCJSC «S.Seifullin Kazakh Agrotechnical Research University» (protocol №6, dated 28.03.2022).

Statistical analysis

Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC). The Kolmogorov–Smirnov test was used to assess the normality of the data distribution. Due to significant deviations from normality, all variables except for freezing point underwent logarithmic transformation. The differences between measurements taken before and after treatment were analyzed using the General Linear Model (GLM), with treatment as a fixed factor and individual animals as a random factor to account for variability among cows.

Results

***In vitro* antimicrobial activity of plant extracts**

The preliminary investigation revealed a high level of antimicrobial resistance among mastitis pathogens isolated from dairy cows. Traditional udder treatment agents, including chlorhexidine bigluconate, povidone-iodine, and other commercially available products, demonstrated low efficacy against production strains of microorganisms such as *Candida* spp., *Escherichia coli*, and various staphylococcal and streptococcal bacteria. Notably, some products that were effective against reference (laboratory) strains failed to inhibit the growth of field isolates, indicating a significant decline in the effectiveness of conventional antimicrobials. In contrast, several plant extracts exhibited promising antimicrobial activity. The disk diffusion assay showed that citrus peel extract (*Citrus* spp. *pericarpium*) was active against both reference and production strains of *Candida* spp., *E. coli*, and *Staphylococcus aureus*, and also reduced the overall microbial

load. Spruce extract (*Picea abies*) demonstrated broad-spectrum activity, particularly against *Staphylococcus aureus* and in reducing total contamination by production strains. Burdock leaf extract (*Arctium lappa folium*) was effective against *E. coli* and *Staphylococcus aureus* (reference strains), as well as against commercial strains of *E. coli* and in lowering total microbial counts. Aloe vera and celandine extracts showed some degree of antimicrobial activity, but their effects were less pronounced or more specific to certain strains. The comparative antimicrobial activity of the tested plant extracts is summarized in Table 1. The detailed results of the antimicrobial activity of each plant extract, including inhibition zones against both reference and production strains, are presented in Table 2. These results indicate that certain plant extracts, especially those from citrus peel and spruce, possess significant antimicrobial activity against both reference and field strains of mastitis pathogens, including those resistant to conventional treatments.

Table 1. Summary of antimicrobial activity of plant extracts against reference and production mastitis pathogens

Plant extract	Activity against reference strains	Activity against production strains	Notes on efficacy
Citrus peel (<i>Citrus spp.</i>)	Active against <i>Candida spp.</i> , <i>E. coli</i> , <i>S. aureus</i>	Active against <i>Candida spp.</i> , <i>E. coli</i> , <i>S. aureus</i> ; reduced total microbial load	Most broadly effective, potential universal agent
Spruce (<i>Picea abies</i>)	Active against <i>S. aureus</i> and others	Active against <i>S. aureus</i> ; significantly reduced total contamination	Broad-spectrum activity, especially strong vs. <i>S. aureus</i>
Burdock leaf (<i>Arctium lappa</i>)	Active against <i>E. coli</i> , <i>S. aureus</i>	Active against <i>E. coli</i> and reduced total microbial load	Effective against key bacterial strains
Aloe vera (<i>Aloe vera folium</i>)	Limited or no activity	Limited or no activity	Less pronounced antimicrobial effect
Celandine (<i>Chelidonium majus</i>)	Some activity against <i>E. coli</i> , <i>S. aureus</i>	Data limited	Moderate, strain-specific activity

Table 2. Inhibition zone diameters (mm) of plant extracts against reference and production strains of mastitis pathogens

Extract	Reference strains			Commercial strains		
	<i>Candida</i> spp.	<i>E. coli</i>	<i>Staph. aureus</i>	<i>Candida</i> spp.	<i>E. coli</i>	Total contamination
Burdock leaf extract (<i>Arctium lappa folium</i>)	–	6.0 ± 0.0	8.3 ± 2.3	–	8.7 ± 0.9	5.2 ± 0.4
Citrus peel extract (<i>Citrus</i> spp. <i>Pericarpium</i>)	12.3 ± 1.1	8.3 ± 0.9	11.5 ± 6.8	12.1 ± 1.8	8.8 ± 2.8	4.8 ± 1.4
Spruce extract (<i>Picea abies</i>)	6.9 ± 0.1	7.9 ± 0.1	17.3 ± 3.0	11.9 ± 1.5	11.9 ± 2.5	11.5 ± 1.8
Aloe vera extract (<i>Aloe vera folium</i>)	–	–	–	–	–	9.9 ± 0.0
Celandine extract (<i>Chelidonium majus</i>)	–	8.8 ± 0.2	10.5 ± 0.3	–	–	–

Note: “–” indicates absence of inhibition zones.

Case study: Efficacy of the wormwood–poplar extract additive in mastitis management

A 15-day trial involving 60 Holstein cows with subclinical mastitis showed that supplementation with the wormwood–poplar extract additive led to a significant improvement in udder health, as evidenced by a 68% reduction in somatic cell count (SCC) from 402,111 ± 106,680 cells/mL at baseline to 127,000 ± 6,360 cells/mL post-treatment ($p = 0.02$), thereby meeting the standard for top-grade milk ($\leq 200,000$ cells/mL) (Figure 1). No statistically significant changes were observed in key milk quality parameters, including fat, protein, lactose, and density, all of which remained within regulatory norms. The only significant change observed in milk physico-chemical parameters was an increase in temperature by 1.9°C (from 21.0°C to 22.9°C, $p < 0.001$).

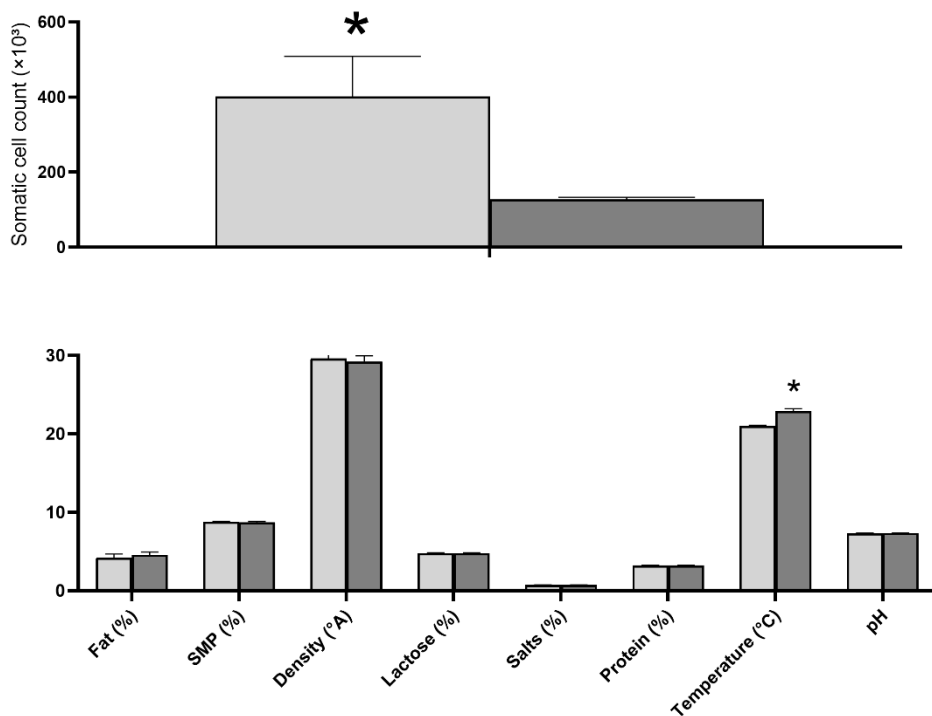


Figure 1. Physico-chemical parameters of milk from mastitic cows before and after treatment with the extruded additive. Data are presented as mean $\pm$ standard deviation (n=15). A significant reduction in somatic cell count was observed post-treatment (p=0.02)

Organoleptic properties (color, odor, consistency) also complied with established standards, indicating that the intervention effectively reduced inflammation without adversely affecting milk quality.

Discussion

The findings of our study highlight a critical issue in contemporary dairy practice: the increasing AMR among mastitis pathogens and the declining efficacy of conventional udder treatment agents. The high resistance observed in production strains of *Candida spp.*, *Escherichia coli*, and staphylococcal and streptococcal

bacteria aligns with global reports documenting the limited effectiveness of traditional antiseptics and antibiotics in mastitis management (Yang et al., 2023; Pires et al., 2024). The failure of agents like chlorhexidine bigluconate and povidone-iodine to inhibit field isolates, despite their activity against laboratory strains, underscores the urgent need for alternative therapeutic strategies (Okello et al., 2023; Rana et al., 2022). In our study, citrus peel extract exhibited broad-spectrum efficacy against both reference and commercial strains of *Candida spp.*, *E. coli*, and *Staphylococcus aureus*, as well as a reduction in overall microbial load. These findings are in line with recent studies that have shown citrus-derived extracts and eco-enzymes possess significant antibacterial properties against mastitis-associated pathogens, attributed to their content of flavonoids, essential oils, and polyphenols (Rizqan et al., 2023).

The present study demonstrated that dietary supplementation with the wormwood–poplar extract additive significantly reduced SCC in the milk of cows with subclinical mastitis, while maintaining the physico-chemical and organoleptic quality of milk. The observed 68% reduction in SCC is clinically meaningful, as SCC is a widely recognized biomarker of udder inflammation and milk quality (Ruegg, 2017). Achieving SCC levels below 200,000 cells/mL is particularly important, as it aligns with international standards for premium-grade milk and is associated with improved udder health and reduced economic losses (Ruegg, 2017; Sharma et al., 2011).

The anti-inflammatory and antimicrobial properties of wormwood and related phytochemical compounds have been previously documented. Wormwood extracts are rich in flavonoids, sesquiterpene lactones, and phenolic acids, which have demonstrated both in vitro and in vivo efficacy against mastitis-associated pathogens and in modulating immune responses (Juteau et al., 2003; Kordali et al., 2005). The efficacy of wormwood and other medicinal plants in mastitis management has been increasingly supported by recent research. Several studies have shown that plant extracts, including those from wormwood, possess significant antimicrobial activity against mastitis-associated pathogens such as *Staphylococcus aureus*, a major etiological agent in bovine mastitis (Coutinho et al., 2010). Moreover, a recent study evaluating the antimicrobial effect of plant-derived products found that these natural formulations were effective in reducing bacterial load and somatic cell counts in mastitic cows, in some cases outperforming conventional antibiotic treatments (Paşca et al., 2017; 2020). The antimicrobial efficacy of wormwood has also been demonstrated in a rat model, where topical application of its extract significantly reduced *Staphylococcus aureus* counts in infected surgical wounds compared to untreated controls (Moslemi et al., 2012).

The maintenance of milk quality parameters, such as fat, protein, lactose, and sensory characteristics, following supplementation with the wormwood–poplar extract additive, is in line with previous reports indicating that phyto-genic additives do not adversely affect the physico-chemical or organoleptic properties of milk (Paşca et al., 2020). This is an important consideration for the dairy industry, as it ensures that interventions aimed at improving udder health do not compromise product quality or marketability.

The only significant change observed in milk physico-chemical parameters was an increase in temperature. This rise is likely attributable to improved udder blood circulation following the resolution of inflammation, as increased local temperature is a recognized physiological response during recovery from mastitis and is associated with vascular dilation and enhanced tissue perfusion in the mammary gland (Gayathri et al., 2024). Additionally, daily fluctuations in udder temperature due to circadian rhythms and environmental factors, such as the timing of milking, may have contributed to this variation, as previous studies have shown that udder temperature can vary predictably throughout the day and in response to external conditions. Thus, the observed temperature increase likely reflects both post-inflammatory physiological changes and the influence of circadian or procedural variables.

The use of plant-based therapies for mastitis is gaining momentum as a strategy to address the growing problem of antimicrobial resistance associated with conventional antibiotic use in livestock (Coutinho et al., 2010). Plant extracts offer a multifaceted approach, combining antimicrobial, anti-inflammatory, and immunomodulatory effects, and are less likely to contribute to resistance development. The results of this study support the integration of the wormwood–poplar extract additive into mastitis control programs, potentially reducing reliance on antibiotics and contributing to sustainable dairy production. Future research should focus on elucidating the mechanisms of action of wormwood and poplar *in vivo*, optimizing dosing regimens, and evaluating long-term effects on udder health and milk production in larger cohorts.

Conclusion

This study demonstrated that traditional antimicrobial agents currently used for mastitis control are largely ineffective against field strains of mastitis-associated pathogens, underscoring the urgency for alternative strategies. *In vitro* experiments showed that plant extracts, particularly those derived from citrus peel (*Citrus spp.*) and spruce (*Picea abies*), exhibited notable antimicrobial activity against both reference and commercial strains of *Candida spp.*, *Escherichia coli*, and *Staphylococcus aureus*.

The *in vivo* trial further confirmed the practical benefits of phytogenic supplementation. Administration of the wormwood–poplar extract additive to cows with subclinical mastitis resulted in a significant reduction in SCC, indicating improved udder health. Importantly, this intervention did not adversely affect key milk quality parameters or organoleptic properties, maintaining compliance with regulatory standards.

Thus, these results support the integration of plant-based antimicrobials and feed additives into mastitis management protocols as effective, natural alternatives to conventional antibiotics. Further research should focus on elucidating the mechanisms of action, optimizing formulations, and evaluating long-term impacts on animal health and milk safety in commercial dairy herds.

Acknowledgment

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PREDICTION OF BW FOR GRAZING CATTLE FROM BODY MEASUREMENTS BY USING ARTIFICIAL NEURAL NETWORK LINEAR AND RANDOM FOREST REGRESSION APPROACHES

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Abstract: Prediction ability of Linear and Random Forest Regressions and Artificial Neural Network analysis was evaluated for relationships between body weight and body measurements such as Body Length (BL), Withers Height (WH), Heart Girth (HG), Body Depth (BD), Heart Height (HH), and Heart Width (HW), were studied using 232 data in total from 29 head Simmental beef cattle observed during a 4-month grazing performance in Kars in the east of Turkey. Body weight was regressed on the body measurements. Linear, quadratic and cubic effects of heart girth indicated that heart girth measurements would be useful in predicting body weight ($R^2 = 88.0, 89.8$ and 90.8% , respectively). However, quadratic effect was found statistically significant ($P < 0.05$). Similarly, regression of body length including linear, quadratic and cubic effects, R^2 values were obtained $82.2, 83.9$ and 84.0% , respectively and linear effect was found statistically significant ($P < 0.05$). The regression equation which is including all body measurements, R^2 value was found 95.1% . The regression of body weight on heart girth had high R^2 (88.0%), followed by body length (82.2%). The regression equations which is including both heart girth and body length was found high R^2 (93.8%). The correlation coefficient between body weight and body measurements were found the highest in HG and BL ($r = 0.94$ and 0.91 , respectively). Similarly, R^2 values for HG were found the highest among the other individual variables by RF, ANN and LR analysis while the determination coefficients of BL were lower for RF, ANN and LR ($50, 63$ and 62% respectively) while the same values for HG were higher and similar by RF, ANN and LR ($87, 87$ and 86% respectively). The results showed that heart girth and body length measurements were the best prediction parameters in predicting body weight in all methods. In terms of predictive ability of the methods used in this study, there was no superiority among them. However, it would be ideal to use the Random Forests algorithm with advantages in dealing

with data complexity and non-linear patterns for prediction of body weight based on measurements from digital images of the animals on grazing conditions.

Key words: prediction body weight, Simmental, beef cattle, body measurements, Random Forest Regression

Introduction

There are challenges in determining the body weights of animals, especially those raised in small commercial enterprises and pastures. Therefore, estimating body weight using body measurements has been practiced for many years. The estimation of body weights of animals using body measurements was first researched in the United Kingdom (Şekerden et al., 1991). Body weight plays a significant role in determining the economic characteristics of cattle and the condition of animals (Polgar et al., 1997; Ulutaş et al., 2001). The relationship between body measurements and body weight depends on the animals' level of nutrition, age, and breed (Çağlar and Şekerden, 1993). Body weight is used in ration preparation, determining body condition scores, and establishing the first mating time for heifers (Bozkurt, 2006; Willeke and Dürsch, 2002).

Researchers frequently use the multiple linear regression model (MLR) to examine the causal relationship between an animal's body weight and other body measurements. However, MLR only examines the linear relationship between the set of independent and the dependent variables. This may occasionally be complicated or nonlinear, which might lead to bias in the MLR predictions. Additionally, multicollinearity, a significant correlation between independent variables, can be a big problem for MLR.

The use of artificial intelligence for farming practices is rapidly becoming popular. However, the studies comparing the most popular supervised learning algorithms and ranking them are still scanty. Research for the comparison of various machine learning techniques in animal sciences for the prediction of disease, performance, hatchability lactation, genetic merits, body weights, disease diagnosis and predictions. For all the studies stated, algorithms like artificial neural networks, Support Vector Machines, K-nearest neighbours etc. have been found to be very useful and, in most cases, better than the conventional approaches due to the large amount of data (Pérez-Rodríguez et al., 2013; Kayri, 2016).

Random Forest model is a machine learning method that can be utilized for both classification and regression problems (Ersin and Yaz, 2023). The working principle of the Random Forest Regression model is similar to the decision tree structure. The dataset is randomly divided into small subsets, and decision trees are constructed based on these subsets. During the prediction phase, the average of the

predictions from the decision trees created from the dataset is taken (Arslankaya and Toprak, 2021). This algorithm is an ensemble learning method created by combining multiple decision trees.

Numerous studies have been conducted and continue to be conducted on predicting body weight using body measurements. However, there is limited research on predicting body weight using body measurements in Simmental breed animals, especially those raised in pastures (Heinrichs et al., 1992) and (Gilbert et al., 1993) indicated that body measurements can be used in body weight predictions and that there is a close relationship between body weight and body measurements. In many studies, it has been reported that the heart circumference parameter within the body measurements of animals can be used alone to predict body weight (Bozkurt, 2006; Willeke and Dürsch, 2002; Msangi et al., 1999; Poivey et al., 1980). Özkaya and Bozkurt (2007), in their study involving three different breeds (Holstein, Brown Swiss, and their hybrids), reported that body weight could be predicted using metric measurements. However, they noted a decrease in the predictive power of body weight using metric measurements in large-sized animals like Holstein when compared to Brown Swiss and hybrids.

The aim of this study is to predict the body weights of Simmental breed male calves raised under pasture conditions using body measurements and to determine the best prediction parameter. Numerous algorithms have been described by scientists as promising for resolving a range of animal science issues. Predicting future performance is an essential topic that, if done correctly, might assist in making critical decisions for raising income and productivity. Therefore, this study compared the most widely used machine learning algorithms and ranked them according to how well they could predict liveweights on a 12-month beef production system.

Materials and Methods

Material

In this study, 29 male calves raised in the pastures of Kars province located in the eastern part of Türkiye were used as the material. The study animals were Simmental breed male calves, commonly found in the region, with varying body weights (178.5–343.0 kg). The animals were exclusively grazed in the pasture approximately 4 months and were not fed with any commercial feed.

Body weight measurements

The performance of the animals (BW) was monitored fortnightly by using mobile weighing scale (TruTest) and a total of 232 data were collected. Additionally, certain body measurements (Body Length (BL), Withers Height

(WH), Heart Girth (HG), Body Depth (BD), Heap Height (HH), and Heap Width (HW)) were taken using a measuring stick and measuring tape. Since the animals were under pasture conditions, they were weighed and measured only once.

The parts of the body where measurements were taken are as follows:

Body Length: The portion measured from the shoulder point to the outside angle of the ischium, determined with a measuring stick.

Wither Height: The vertical distance from the highest point of the withers to the ground (vertical distance from the animal's shoulder to the ground), measured with a measuring stick.

Heart Girth: The circumference measurement taken just behind the shoulder blades with a measuring tape.

Body Depth: The vertical distance measured just behind the shoulder blades and immediately behind the front legs with a measuring stick.

Heap Height: The vertical distance between the front heap and the ground, measured with a measuring stick.

Heap Width: The distance measured between the prominences of the hip bones with a measuring stick.

Statistical analysis

A Ryan-Joiner normality test was applied to all data using the Minitab statistical software package. Descriptive statistics and regression analysis of body measurements with body weight were performed using the Minitab, statistical software package. Correlation coefficients between parameters were obtained. Polynomial regression analysis of body weight on body measurements was conducted. Body weight was obtained in kilograms, and body measurements were taken in centimeters.

The effect of the linear, quadratic, and cubic coefficients of the independent variables on body weight is shown in the following model:

$$y = b_0 + b_1x_i + b_2x_i^2 + b_3x_i^3 + e_i$$

y = the observed body weight of the i th animal, b_0 = constant, b_1 , b_2 , b_3 = linear, quadratic, and cubic coefficients, X_i = Body measurements (BL, HG, WH, BD, HH, and HW), e_i = Error.

A Random Forest model is a powerful machine learning algorithm that can be effectively used for predicting liveweight in animals based on various input features such as age, height, girth, breed, and other physiological or environmental factors.

Python was used for developing RF and MLR models, including linear models and used for comparisons with the results obtained from Minitab Statistical software program. In this study prediction ability of the models was evaluated within the scope of R^2 (determination of coefficient) instead of RMSE (Root Mean Square Error) or CV (coefficient of variation) to avoid cumbersome presentation of the results.

Below is an outline of the steps implemented a Random Forest model for bodyweight prediction:

1. Data Preparation

- Collection of Data: The data gathered on bodyweight and relevant features (body measurements).
- Cleaning of Data: The missing values, outliers handled, and data consistency ensured.
- Traits Engineering: Creating meaningful features (body measurements) and traits (e.g., ratios like Heart girth-to-wither height).
- Splitting of Data: The dataset divided into training and testing sets (e.g., 80% training, 20% testing).

2. Model Implementation in Python

the scikit-learn library:

Copy the code# Import necessary libraries

```
import pandas as pd
```

```
from sklearn.model_selection import train_test_split
```

```
from sklearn.ensemble import RandomForestRegressor
```

```
from sklearn.metrics import mean_squared_error, r2_score
```

```
# Load your dataset
```

```
data = pd.read_csv('liveweight_data.csv') # Replace with your dataset
```

```
X = data.drop(columns=['Bodyweight']) # Features
```

```
y = data['Liveweight'] # Target variable
```

```
# Split the data
```

```
X_train, X_test, y_train, y_test = train_test_split(X, y, test_size=0.2,  
random_state=42)
```

```
# Initialize and train the Random Forest model
```

```
rf_model = RandomForestRegressor(n_estimators=100, random_state=42)
```

```
rf_model.fit(X_train, y_train)
```

```
# Make predictions
y_pred = rf_model.predict(X_test)

# Evaluate the model
mse = mean_squared_error(y_test, y_pred)
r2 = r2_score(y_test, y_pred)

print(f"Mean Squared Error: {mse}")
print(f"R-squared: {r2}")
```

3. Model Optimization

- Hyperparameter Tuning: The techniques like Grid Search or Random Search used to optimize parameters such as `n_estimators`, `max_depth`, and `min_samples_split`.
- Feature (traits) Importance: Analyze body measurements importance to understand which variables contribute most to the prediction.

4. Interpretation and Deployment

- Interpret Results: Tools like SHAP (SHapley Additive exPlanations) and determination coefficients (R^2) used to interpret the model's predictions.
- Deploy Model: The trained model saved using libraries like scikit-learn or pickle for deployment in real-world applications.

Results

The averages of body weight and body measurements are shown in Table 1. Table 2 presents the regression equations that provide the best predictions using the body measurements taken from the animals. The regression results for the linear, quadratic, and cubic effects of each body measurement on body weight are shown in Table 3.

Table 1. Descriptive statistics of body measurements and body weight

Variables	Mean±SE
BW (kg)	236.76±6.23
BL (cm)	113.95±1.20
WH (cm)	108.43±0.70
BD (cm)	50.21±0.60
HH (cm)	113.98±0.68
HW (cm)	34.64±0.45
HG (cm)	141.07±1.20

BW: Body weight, BL: Body length, WH: Withers height, BD: Body depth, HH: Heart height, HW: Heart width, HG: Heart girth, SE: Standard error

As seen in Table 2, the regression equation including all body measurements yielded the highest R^2 value for predicting body weight (95.1%). It was observed that in regression equations where the heart girth measurement was absent, the R^2 value decreased (Table 2). The second-highest R^2 value was obtained in the regression equation that did not include HW (95.0%). The R^2 values for regression equations containing only BL and only HG were 82.2% and 88.0%, respectively (Table 2). The R^2 value was 93.8% in the regression equation containing both BL and HG. As shown in Table 2, the predictive power of body weight with WH, BD, HH, and HW measurements was found to be very low. A 1 cm change in HG measurement results in a 4.89 kg change in body weight (Table 2).

The polynomial regression analysis of body weight on body measurements (Table 3) revealed the highest values for BL and HG measurements (84.0% and 90.8%, respectively). The cubic coefficients for all body measurements showed a slight increase, and the R^2 value for the HH measurement remained constant. However, while the linear coefficient was statistically significant for all measurements, the quadratic coefficient was also significant for HG and BD measurements ($P < 0.05$).

Table 2. Regression equations to predict body weight using body measurements

Equations	R^2 (%)
$Y = -411 + 2.00 \text{ BL}^* + 0.86 \text{ WH} - 0.83 \text{ BD} - 1.38 \text{ HH} - 0.18 \text{ HW} + 3.67 \text{ HG}^*$	95.1
$Y = -476 + 4.37 \text{ BL}^* + 1.49 \text{ WH} + 1.14 \text{ BD} + 0.91 \text{ HH} - 3.12 \text{ HW}$	86.9
$Y = -414 + 2.04 \text{ BL}^* + 0.87 \text{ WH} - 0.78 \text{ BD} - 1.31 \text{ HH} + 3.63 \text{ HG}^*$	95.0
$Y = -469 + 2.31 \text{ BL}^* + 0.53 \text{ WH} - 0.69 \text{ BD} - 0.88 \text{ HW} + 3.19 \text{ HG}^*$	94.4
$Y = -406 + 2.14 \text{ BL}^* + 0.68 \text{ WH} - 1.29 \text{ HH} - 0.35 \text{ HW} + 3.43 \text{ HG}^*$	94.8
$Y = -385 + 2.02 \text{ BL}^* - 0.58 \text{ BD} - 1.10 \text{ HH} + 0.30 \text{ HW} + 3.79 \text{ HG}^*$	94.7
$Y = -363 + 0.96 \text{ WH} - 1.74 \text{ BD} - 2.51 \text{ HH}^* + 2.64 \text{ HW} + 5.51 \text{ HG}^*$	91.6
$Y = -465 + 2.14 \text{ BL}^* + 0.37 \text{ WH} - 1.01 \text{ BD} + 3.32 \text{ HG}^*$	94.2
$Y = -418 + 4.09 \text{ BL}^* + 1.52 \text{ WH} + 0.46 \text{ BD}$	84.9
$Y = -449 + 2.19 \text{ BL}^* - 0.002 \text{ WH} + 3.10 \text{ HG}^*$	93.8
$Y = -446 + 2.15 \text{ BL}^* - 0.83 \text{ BD} + 3.40 \text{ HG}^*$	94.2
$Y = -475 + 0.53 \text{ WH} - 1.33 \text{ BD} + 5.11 \text{ HG}^*$	88.7
$Y = -424 + 4.13 \text{ BL}^* + 1.75 \text{ WH}^*$	84.8
$Y = -331 + 4.36 \text{ BL}^* + 1.42 \text{ BD}$	83.6
$Y = -449 + 2.19 \text{ BL}^* + 3.09 \text{ HG}^*$	93.8
$Y = -301 + 4.72 \text{ BL}^*$	82.2
$Y = -390 + 5.75 \text{ WH}^*$	42.5
$Y = -58.3 + 5.88 \text{ BD}^*$	31.7
$Y = -352 + 5.17 \text{ HH}^*$	31.7
$Y = -14.6 + 7.26 \text{ HW}^*$	27.9
$Y = -453 + 4.89 \text{ HG}^*$	88.0

BW: Body weight, BL: Body length, WH: Withers height, BD: Body depth, HH: Heart height, HW: Heart width, HG: Heart girth, * It is statistically significant ($P < 0.05$).

While the linear coefficient for the HW measurement was statistically insignificant ($P > 0.05$), the quadratic coefficient was found to be statistically significant ($P < 0.05$).

Table 3. Regressions of body weight on the linear, quadratic and cubic effects of each body measurements

Variables	Constant	Linear	Quadratic	Cubic	R ² (%)
BL	-301.02	4.72*			82.2
	566.01	-10.15*	0.06		83.9
	3285.86	-80.00*	0.66	-0.00	84.0
WH	-390.21	5.78*			42.5
	3033.55	-57.60*	0.29		45.5
	-97956.90	2754.79*	-25.78	0.80	49.1
HH	-351.97	5.17*			31.7
	-36.50	6.67*	-0.01		31.7
	521.36	-18.96*	0.22	-0.00	31.7
HW	-14.64	7.26*			27.9
	934.66	-47.34*	0.78		31.1
	-26181.80	2309.38*	-67.20	0.65*	43.1
BD	-58.26	5.88*			31.7
	1519.85	-57.59*	0.64*		43.0
	-4501.04	308.23*	-6.73*	0.05	44.1
HG	-453.11	4.89*			88.0
	844.39	-13.25*	0.06*		89.8
	17719.20	-364.97*	2.50*	-0.01	90.8

BW: Body weight, BL: Body length, WH: Withers height, BD: Body depth, HH: Heart height, HW: Heart width, HG: Heart girth, * It is statistically significant ($P < 0.05$).

Table 4. Correlation coefficients between the body measurements and the body weight

Variables	BL	WH	BD	HH	HW	HG
BW	0.91	0.65	0.56	0.56	0.53	0.94

BW: Body weight, BL: Body length, WH: Withers height, BD: Body depth, HH: Heart height, HW: Heart width, HG: Heart girth,

All correlations were found to be statistically significant ($P < 0.05$). The highest correlation between body weight and body measurements was obtained in BL and HG measurements (0.91 and 0.94, respectively). All obtained correlations were statistically significant ($P < 0.05$).

The first stage of artificial intelligence-based data analysis is to adapt the data to the designed artificial intelligence model. In this context, the obtained data

has been standardized initially. The StandardScaler function from the sklearn library was used for this process. StandardScaler is the process of rescaling each feature value of the dataset so that the mean is zero and the standard deviation is one. This process allows machine learning algorithms to work accurately when the features in the dataset are of different scales. After standardizing the data, it was divided into a training set and a test set. This separation involves using a certain portion of the dataset for training and the remaining portion for testing. In the literature, this ratio is generally observed to be 70% for training and 30% for testing. Due to the small size of our dataset, 80% of the data was used for training and 20% for testing in our study.

The analyses were conducted using the Python programming language in the Spyder editor. For Random Forest, the max-depth parameter was set to 10. For classical artificial neural networks, only input and output layers were used without hidden layers, as the data size is small. The 'HWd' function was used as the optimizer for training the neural network, and 'mean squared error' was used as the loss function. The model was trained for 60 epochs, with data being sent one by one in each epoch. All hyperparameters were optimized and determined.

Six variables used in predicting the output in the dataset were employed in combinations from one to six, and the combinations that achieved the highest results were determined. These combinations and the success rates of the models (R^2 values) are presented in Table 5.

It was observed that Logistic and random forest Regression and classical neural network models produced similar results. The combination of HW BL WH HG HH parameters for the Random Forest model yielded the highest result with an R^2 value of 0.89. For Logistic Regression, the combination of HW BL WH HG BD produced the highest result with an R^2 value of 0.93. Similarly, for the classical Artificial Neural Network, the combination of BL WH HG resulted in the highest R^2 value of 0.93. Furthermore, when considering individual variables such as BL and HG their R^2 values were found the highest among the other individual variables. However, as seen Table 5 the determination coefficients of BL were lower for RF, ANN and LR (0.50, 0.63 and 0.62 respectively) while the same values for HG were similar in RF, ANN and LR (0.87, 0.87 and 0.86 respectively).

Table 5. The results of RF, ANN, and LR Analysis

Variables	RF	ANN	LR
HW BL WH HG HH BD	0.8645	0.8586	0.8467
HW BL WH HH BD	0.6608	0.6623	0.6888
HW BL WH HG BD	0.8808	0.9259	0.9353
BL WH HG HH BD	0.8752	0.8676	0.8831
HW BL WH HG HH	0.8977	0.8828	0.8876
HW BL HG HH BD	0.8894	0.8618	0.8792
HW WH HG HH BD	0.8762	0.8632	0.8718
HW BL HG BD	0.8696	0.9028	0.8990
HW WH BD	0.5505	0.6196	0.6398
BL WH HG	0.8835	0.9318	0.9328
BL HG BD	0.8599	0.9020	0.8998
WH HG BD	0.8328	0.9006	0.8935
BL WH	0.7738	0.7697	0.7371
BL HG BD	0.8640	0.8929	0.8995
WH HG BD	0.8381	0.8991	0.8935
BL	0.5027	0.6270	0.6262
HG	0.8686	0.8710	0.8563

BW: Body weight, BL: Body length, WH: Withers height, BD: Body depth, HH: Heart height, HW: Heart width, HG: Heart girth,

Discussion and Conclusion

Since there is limited research on predicting body weight using body measurements in Simmental cattle on grazing conditions, the obtained values were evaluated in comparison with results from studies on other breeds. The regression equation including BL, WH, BD, and HG measurements (Table 2) yielded an R^2 value of 94.2%. This value was higher than those reported by (Tüzemen et al., 1995; Tüzemen et al., 1993) and (Özkaya and Bozkurt, 2007) (90.7%, 90.7%, and 66.1%, respectively, and 91.8%). The R^2 value obtained for the regression equation including BL, WH, and HG measurements (93.8%) was higher than values reported by (Özkaya and Bozkurt, 2007), (Ulutaş et al., 2001), and (Çağlar and Şekerden, 1993) (66.1%, 91.3%, 82.5%, and 75.8%, respectively), but lower than the value reported by Şekerden et al. (1991) (97.7%) and similar to the value reported by (Bozkurt, 2006) (93.6%).

As seen in Table 2, the R^2 value obtained for the regression equation including only BL measurement (82.2%) was higher than those reported by (Ulutaş et al., 2001), (Çağlar and Şekerden, 1993), (Tüzemen et al., 1993), (Bozkurt, 2006), (Özkaya and Bozkurt, 2007) (68.3%, 55.4%, 67.0%, 70.4%, and 47.7%, respectively) but lower than values reported by (Heinrichs et al., 1992), (Şekerden et al., 1991) (93.0% and 96.1%). It was similar to the value reported by (Özkaya and Bozkurt, 2007) (79.2%). The R^2 value obtained for the regression equation

including only HG measurement (88.0%) was higher than values reported by (Goe et al., 2001), (NesamBLni et al., 2000), (Ulutaş et al., 2001), (Çağlar and Şekerden, 1993), (Özkaya and Bozkurt, 2007) (75%, 74%, 73.1%, 64%, and 60.7%, respectively) but lower than values reported by (Willeke and Dürsch, 2002), (Gilbert et al., 1993), (Şekerden et al., 1991), (Özkaya and Bozkurt, 2007) (94%, 95%, 97.3%, and 91.1%, respectively). However, the value obtained for the equation including only HG measurement (88.0%) was close to values reported by (Tüzemen et al., 1993), (Bozkurt, 2006), (Özkaya and Bozkurt, 2007) (87%, 89.9%, and 88.8%, respectively). The R^2 value obtained for the regression equation including both BL and HG measurements (93.8%, Table 2) was higher than values reported by (Ulutaş et al., 2001), (Çağlar and Şekerden, 1993), (Tüzemen et al., 1993) (80.6%, 74.8%, and 90%, respectively) but lower than the value reported by (Şekerden et al., 1991) (97.7%) and similar to the value reported by (Bozkurt, 2006) (93.2%).

In their study, (Nesamblni et al., 2000), (Bozkurt, 2006) suggested that the cubic coefficient (78% and 90.2%, respectively) could be a better predictor for HG measurement, while (Ulutaş et al., 2001), (Heinrichs et al., 1992), (Özkaya and Bozkurt, 2007) reported that the quadratic coefficient was a better predictor (95.1%, 98%, 94.4%, and 89.8%, respectively). The results of the study on Simmental male cattle (Table 3) concluded that the quadratic coefficient is a better predictor for estimating body weight compared to other body measurements. The obtained R^2 value (90.8%) was higher than that reported by (Nesamblni et al., 2000), similar to (Bozkurt, 2006), and lower than values reported by (Heinrichs et al., 1992), (Ulutaş et al., 2001), (Özkaya and Bozkurt, 2007) (98%, 94.4%, and 89.8%, respectively).

As shown in Table 4, a high correlation was obtained between body weight and BL and HG measurements ($r=0.91$ and 0.94 , respectively). The correlation coefficient obtained between BL and BW was higher than values reported by (Yanar et al., 1995), (Tüzemen et al., 1995), (Özkaya and Bozkurt, 2007) (0.40 , 0.70 , and 0.69 , respectively, and 0.89), and similar to the value reported by (Özkaya and Bozkurt, 2007) (0.91). The correlation coefficient obtained between HG and BW was higher than values reported by (Yanar et al., 1995), (Tüzemen et al., 1995), (Özkaya and Bozkurt, 2007) (0.85 , 0.86 , and 0.78 , respectively) and similar to the values reported by (Özkaya and Bozkurt, 2007) (0.95 and 0.94).

Numerous research has evaluated artificial neural network models with linear and random forest regression to forecast domestic animal performance for various reproductive and productive variables (Saberioon et al. 2018; Van der Heide et al. 2019). The linear model in this investigation had a coefficient of determination ($R^2=0.93$) that was similar to Tsegaye et al. (2013) and our results were also in line with those of Huma and Iqbal (2019) ($R^2=0.92$). In a study done

with other domestic farm animals, the weight of Baluchi sheep was estimated using RF by Huma and Iqbal (2019), and the coefficient of determination (R^2) was similar to the current study ($R^2=0.98$). But the R^2 values of the nonlinear models in this investigation were greater than those in the study by Ali et al. (2015), which employed decision tree algorithms and artificial neural networks to predict the live weight of Harnai sheep with an accuracy of 0.81 to 0.84. Simmental cattle are intensively raised, especially in the Eastern Anatolia region of Turkey. Regression equations are recommended for predicting body weights of Simmental cattle on grazing conditions. The equations obtained have a high predictive power for estimating body weight. Predicting body weight is important for determining the needs of animals and monitoring their performance. Although using a scale to determine body weight is a more accurate method, especially during the summer months when animals are grazed and housed in pastures, it is recommended to use body measurements, especially heart girth measurement, due to the difficulty of using a scale to track body weights. Moreover, weighing animals under grazing conditions is a stressful activity and reduces their welfare.

In conclusion, the results showed that heart girth and body length measurements were the best prediction parameters in predicting body weight in all methods. In terms of predictive ability of the methods used in this study, there was no superiority among them. However, it would be ideal to use the Random Forests algorithm with advantages in dealing with data complexity and non-linear patterns for prediction of body weight based on measurements from digital images of the animals on grazing conditions.

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VIEW AT SUSTAINABILITY IN DAIRY SECTOR: CURRENT STATE AND PERSPECTIVES

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Abstract: Milk and dairy products are nutritionally rich foods, providing energy and high-quality proteins along with a number of essential micronutrients. In this way, the dairy sector supports the existence of people in food chains around the world. Human activities affect the Earth's climate because they cause the release of huge amounts of greenhouse gases, which are retained in the Earth's atmosphere along with the gases that are naturally present in it. Food and drink are considered to be responsible for 20 to 30% of the impact on the environment, in which meat and dairy products are the most important. Research and methodologies are mostly focused on the emission of greenhouse gases (GHG), but numerous other impacts on the environment must also be taken into account, such as impacts on land and water through various types of pollution. In addition, the consumption of scarce water reserves and energy resources is also taken into account. Therefore, a sustainable approach to food and beverage production has no alternative. The dairy sector faces challenges to which it responds with numerous organized and synchronized actions and projects in order to successfully restructure and fit into the overall human activities to reduce pollution. Sustainability itself has 3 dimensions, ecological, economic and social. The aim of this paper is to present the current situation of sustainability in the dairy sector in the world and numerous activities undertaken by various actors in the dairy sector, from farm entities and dairy companies to various associations and umbrella organizations such as the International Dairy Federation, the European Dairy Association as well as non-specific dairy-strictly unrelated bodies. All activities are carried out in coordination and under the auspices of the United Nations and its organizations, primarily the Food and Agriculture Organization.

Key words: milk, dairy products, sustainability, pollution, gas emissions

Introduction

Milk and dairy products - a food that we are sure we know well. However, can we consider this to be true? This is a frequent question from consumers, but also from food experts (Rogelj, 1998). On the other hand, today we are witnessing fierce criticism and pressure on the production and processing of milk as a food sector that significantly contributes to environmental pollution and the production of greenhouse gases. Let's consider these two, seemingly different aspects, yet very related.

It is a proven and tested fact that milk is a food that provides the young organism with all the necessary ingredients in the right quantity, in the right proportion and in the right form that the body best absorbs (Sarić and Brenjo, 2022). It is also necessary for the normal functioning of the adult organism, so it is an important food during the entire life period and therefore occupies a special status among all types of food (Sarić, 2018). Milk and dairy products are a good source of high-quality protein, numerous micronutrients and natural bioactive components (Barać and Sarić, 2023).

Sustainability is a term that has a very broad meaning. The modern concept of sustainable development includes a balance between human activities and social, economic and environmental factors (Grujić and Andrejaš, 2013). The 2030 Agenda for Sustainable Development, adopted by all United Nations member states in 2015, provides a common blueprint for peace and prosperity for people and the planet, now and in the future. The 17 goals of sustainable development are at the center (sustainable development goals - SDG) (UN, 2015). The European Green Deal is aimed at sustainable and inclusive growth in which there will be no net greenhouse gas emissions in 2050. It is the main strategic development document for the European Union (EU) in the period up to 2030 (Boromisa, 2020). The Green Plan is an integral part of the Commission's strategy for the implementation of the United Nations Program until 2030 and the goals of sustainable development (European Commission - EC, 2019). In 2020, the European Parliament debated about EGD (European Green Deal) and decided to support it, partly because of the circumstances at the time (Covid-19) to set 2050 as the target year for net carbon neutral EU (Fetting, 2020). Of course, the focus is on the entire food chain (from Farm to Fork) so European Commission (EC) adopted „Farm to Fork“ strategy (European Green Deal) which provides the main strategic directions for the development of food chains in order for Europe to become the "first climate-neutral continent" by 2050 (EC, 2020). In the EC report (2025) the progress of EU members in achieving national goals in the implementation of CAP strategic plans (2023-2027) for the period 2023-2024 is stated. Scientists of the Intergovernmental Panel on Climate Changes (IPCC) have warned that global

warming of 1.5 °C (predicted in the period 2030-2052) will have serious and even irreversible consequences on our environment and societies (IPCC, 2018). According to Crippa et al. (2023) total GHG emissions in 2022 reached 53.8 Gt CO₂-eq, and the share of the food production sector is about ¼ of total GHG emissions. It was found that food and drink, including tobacco and narcotics, are responsible for 20-30% of the environmental effects of total consumption, and in the case of eutrophication, even over 50%. Meat and meat products contribute the most to this. The estimated contribution of these products to global warming is in the range of 4 to 12% for all products. This includes the entire production and distribution chain „from farm to fork“. The second most important product group is dairy products, while other food products have a lesser effect (EC, 2006). Globally, animal protein production is associated with a total of 6.2 Gt CO₂-eq emissions, which accounts for approximately 12% of the estimated 50 to 52 Gt CO₂-eq total anthropogenic emissions in 2015 (FAO, 2022a). According to IPCC report (2022) the sector of agriculture, forestry and other land uses (AFOLU) in the period 2010-2019 carried 13-21% of the total anthropogenic GHG emissions. At the same time, food systems account for 23-42% of global GHG emissions. In the same report, a diet rich in vegetable proteins and poorer in meat and dairy is recommended. The highest GHG intensity is shown by ruminant meat. Cattle from dairy systems have a lower intensity of emissions than those from meat farming (8-23 and 17-94 kg CO₂-eq/100g of protein resp.).

Current State in Dairy Sector

Dairy Sector is very huge and probably the most comprehensive among all food chain systems. World milk production is forecast to grow by 1.5% to just under 981 million tonnes in 2024 (estimation for 2024, FAO, 2024a). Growth is expected to be driven by significant volume increases in Asia, with increases in Europe, South America, Oceania, Central America and the Caribbean. For the period 2024-2033, global milk production is projected to grow at a rate of 1.6% per year over the next decade, supported by higher yields per animal than most other major agricultural commodities. Global per capita consumption of fresh dairy products is expected to grow by 1.0% per year over the next decade, primarily driven by higher per capita income growth (OECD/FAO, 2024). On average, the calculated per capita consumption of dairy products increased by 1.36% in 2023, i.e. from 117.5 kg in 2022 to 119.1 kg in milk equivalent (Van Herdeeh, 2024; OECD/FAO, 2024).

The dairy sector is quite well institutionally organized at the world and regional level, including the aspect of sustainability. In its studies and activities, the FAO (Food and Agriculture Organization) has activities through many projects in

the sustainable dairy sector and through its reports with data from the entire food chain in the dairy sector with recommendations to governments and institutions with an important emphasis on sustainability (2010, 2022a, 2023, 2024a,b) as well as in cooperation with the OECD (Organization for Economic Co-operation and Development - forum and knowledge hub for data) (OECD/FAO, 2024). IFCN (International Fast-Checking Network) is a politically neutral, global dairy industry supply chain research and advisory network focusing on milk production, milk prices and other dairy topics (IFCN, 2024).

The International Dairy Federation (IDF), founded in 1903, is the leading source of scientific and technical expertise for all stakeholders in the dairy chain. With more than 1,200 highly qualified dairy experts in 39 member countries worldwide, IDF represents 74% of global milk production (<https://fil-idf.org/about-us/>). The EDA (European Dairy Association) publishes data on the EU dairy sector in its 2023-2024 report under the slogan “The future is in dairy – milk and dairy products are nutritional treasures, economic powerhouses and true leaders in environmental sustainability”.

Dairy as sustainable food

Consumption of dairy products and milk is often included as important elements of a healthy and balanced diet (Pereira, 2014). It is an ideal food because it contains all the necessary ingredients, properly balanced and free of toxins, in a form that is easy to digest (Sarić, 2018; Fox and McSweeney, 1998). In cow's milk, water makes up about 7/8, and dry matter about 1/8 of milk. Cow's milk is dominant and makes up about 81% of world milk production (Sarić and Brenjo, 2022). Its composition consists of dry matter (12.50-13.00%), milk sugar (4.70-5.20%), fat (3.50-4.00%), proteins (3.10-3.50%) and minerals (0.80-0.95%) (Miletić, 1994; Bijeljic and Sarić, 2005). As incomes and population grow, it is expected that more dairy products will be consumed globally in the medium term (OECD/FAO, 2024). Biologically active components of milk protect newborns and adults from pathogens and various diseases (immunoglobulins, antibacterial peptides, antimicrobial proteins, oligosaccharides and lipids) (Barać and Sarić, 2003). Among other things, milk and dairy products are a good source of calcium, essential amino acids and zinc (Havranek and Antunac, 1993; Badshah et al., 2021). Fermented dairy products are particularly noteworthy, as they provide the human body with microorganisms that modulate the intestinal microflora (Okoniewski et al., 2023).

Plant-based alternatives to dairy products (e.g., soy, almond, rice, and oat beverages) are becoming more popular in many regions, especially in North America, Europe, and East Asia. Key drivers of expansion include health concerns

and greater consumer awareness of the environmental impact of dairy production. The sustainability of popular substitutes, such as almond and soy beverages, is being questioned as more and more consumers consider other environmental issues besides greenhouse gas emissions, such as water consumption and deforestation (OECD/FAO, 2024). For comparison, milk and soy drink can be observed. The soy drink does not contain cholesterol, but it contains phytochemicals (isoflavones), so we can really talk about the health aspect (Keshun Liu, 1999). Despite the well-balanced protein structure of soy milk, which some claim is even better than cow's milk, it cannot be a real substitute for cow's milk. The protein utilization ratio (PER), a measure of protein quality relative to casein (cow's milk, standard of 2.5), in raw soybeans is quite low (Gupta, 1997). Soy milk does not contain lactose, the main carbohydrate in cow's milk. The natural carbohydrates in soybeans are sucrose, stachyose and raffinose. The latter two can cause bloating and stomach discomfort (APV, 1997; Gupta, 1997). Compared to cow's milk, soy milk is poorer in vitamins and minerals except iron and niacin. Soy milk contains only 1/5 of the calcium of cow's milk (Sarić et al., 2006; APV, 1997).

Table 1. Vitamin content in soymilk and cow's milk compared with recommended daily intake (Sarić et al., 2006. adapted to APV, 1997; Bylund, 1995; Schaafma, 2003)

Vitamins	Recommended Daily Intake (mg/day)	Soy drink (mg/litre)	Cow's Milk (mg/litre)
A	0,8	50 i.u.	0,2-2,0
B ₁	1,2	0,6	0,4
B ₂	1,8	0,5	1,7
B ₁₂	0,005	0,0	0,004
Niacin	15,0	5,0	9,0 NE*
C	70,0	5,0	5,0-20,0
D	0,002	0,0	0,002

*Niacin Equivalent

Some studies confirm that malnutrition remains a major and continuing problem in developing countries. Namely, many poor people in countries with low personal incomes suffer from micronutrient deficiencies due to a diet based mainly on cereals. Diet with food products of animal origin is particularly suitable for combating malnutrition and a wide range of nutritional deficiencies (Randolph et al., 2007).

Smedman et al. (2010) made a comparison of carbon footprints in gCO₂-eq for 100g of different types of drinks, where it turned out that milk was the worst choice, and mineral water the most sustainable. It is clear that mankind cannot live on mineral water, so they concluded that the amount of CO₂-eq cannot be the only criterion. Based on the fact that humans need a certain amount of nutritional

ingredients, they defined the so-called nutrient density and climate impact index - Nutrient Density to Climate Impact Index (NDCI) = nutritional density (kcal, proteins, vitamins, minerals)/GHG emission (CO₂-eq) (Table 2). From this point of view, dairy products are among the best sustainable food products (Smedman et al., 2010; de Jong, 2013).

Table 2. Carbon footprint in g CO₂-eq/100g, NDCI index and nutrient density of several types of beverages (adapted to Smedman et al., 2010)

Indicator	Milk	Soft drink	Orange juice	Beer	Red wine	Mineral water	Soy drink	Oat drink
GHG emissions	99	109	61	101	204	10	30	21
Nutrient density	53.8	0.0	17.2	0.0	1.2	0.0	7.6	1.5
NDCI index	0.54	0.00	0.28	0.00	0.01	0.00	0.25	0.07

An interesting approach is provided by the work published by Đekić et al. (2025). They applied the calculation of the carbon footprint for 13 types of cheese using the factor 1 kg CO₂-eq per kg of milk taking into account the amount of milk required for 1 kg of cheese (FAO, 2022a). They took into account the weight of the cheese as well as the nutritional values (proteins, fats, energy). The results differed and showed that the nutritional value of the product must be taken into account when assessing the carbon footprint of a food.

Environmental sustainability of dairy production

According to many opinions, sustainability encompasses three dimensions: economic, social and environmental. These dimensions are also referred to as profit, people and planet (the 'triple P') (Jongeneel and Slangen, 2013). The first ecological question is how to quantify environmental impacts.

Quantification and allocation of environmental impacts in dairy production

Environmental impacts in dairy production are those from the farm (farm-gate), including impacts on soil, water, air, biodiversity, landscape preservation, etc. (Hind, 2010).

Table 3. Total reported carbon footprints for the entire chain of milk production, processing and transport in kg CO₂-eq* (Sarić, 2024, adapted to Carlsson-Canyama et al., 2003; Sevenster and de Jong, 2008; IDF, 2009; Smedman et al., 2010; FAO, 2010; US Dairy, 2011; de Jong, 2013; van Asselt and Weeks, 2013; IPCC, 2007)

Total emissions of CO₂ - eq of dairying (production, processing and transport of milk)**	0,3-2,5 kg CO ₂ -eq/kg milk (average 1,4)
Milk production (to the farm gate)	0,65-1,65 kg CO ₂ – eq/kg milk (average 1,13); Share in total emissions of the entire milk production chain ~ 81%
From farm gate to warehouse before transport to retail	0,155 kg CO ₂ – eq/kg milk; Share in total emissions of the entire milk production chain ~ 14%
Dairy processing	0,117 kg CO ₂ – eq/kg milk; 5,7% ; 0,086 kg CO ₂ – eq/kg milk; 7,6%
Share of milk processing in emissions from farm gate to warehouse before transport to retail	13-14% (0,17 kg CO ₂ – eq/kg milk)

* Only emissions from milk production that go into the further chain of milk from the exit from the farm are taken into account, and meat is further calculated in other or special carbon footprint assessment cycles

** Calculations were made on the basis of data from various literature sources, so the sum does not necessarily have to be 100% (note authors)

When there is talk about the further procedures of transport, processing and sale (post-farm-gate), it primarily means the impact on water, soil and air, environmental pollution through the generation of different types of waste, and the consumption of scarce reserves of natural resources (water, energy sources). The usual measure of quantifying the impact on the environment is the carbon footprint, the carbon dioxide equivalent (CO₂-eq) of emissions per product quantity, and the LCA (Life Cycle Assessment) technique. In the case of milk, many factors are unknown or vary from farm to farm and even from cow to cow (Rotz et al., 2010; de Jong, 2013).

The main strengths of LCA lie in its ability to provide a holistic assessment of production processes, in terms of resource use and environmental impact, as well as to take into account multiple parameters (ISO, 2006). IPCC issued Guidelines for National Greenhouse Gas Inventories (IPCC, 2006), and the following year provided data on GHG quantities and the participation of dairy production in global anthropogenic emissions. The amount of milk produced in the world in 2007 was about 553 million tons (FAOSTAT, 2009). Greenhouse gas emissions from dairy farming, including emissions from deforestation and milk processing, have been calculated at 1,989 million tonnes CO₂-eq. Milk and meat production from dairy farming (including dairy cows, replacement calves, surplus

calves and culls) plus dairy processing, packaging and transport contribute 4.1% of total anthropogenic greenhouse gas emissions estimated at 49 gigatonnes (IPCC, 2007). Milk production, processing and transport alone contribute 2.7% of total anthropogenic greenhouse gas emissions. The average global emissions per kg of milk and kg of meat from cull dairy cows and bulls and kg of meat from surplus calves were 2.4 kg CO₂-eq, 15.6 kg CO₂-eq and 20.21 kg CO₂-eq resp. In the same year, IDF (2010) published a guide to LCA methodology, which was later supplemented (IDF, 2015; IDF, 2022). Bengoa et al. (2014) also mention milk and dairy products in their guide to LCA methodology for food products. The system boundaries are set “from cradle to farm gate” and “from farm gate to retail”. The functional unit used to report GHG emissions was defined as 1 kg of CO₂-equivalents per kg of fat and protein corrected milk (FPCM), at the farm gate (Vellinga et al., 2013). FPCM is mainly used on the farm, as a basis for comparing farms, it ensures a fair comparison between farms with different breeds or feeding regimes. FPCM is based on standard milk with 4.0% fat and 3.3% true protein content. In processing, the recommended unit is 1 kg of product (IDF, 2015), but in practice a conversion factor is also used, i.e. conversion into kg of FPCM. Allocation factors are important, so on the farm allocation is made to the produced milk and meat from separated cows and male calves, while in processing it depends on the dairy product (e.g. milk and cream or cheese, whey and cream) (IDF, 2010). Of course, data on GHG emissions depend on production conditions and vary by region. India, as the largest milk producing country, has an emission rate of 1.98 kg CO₂-eq. per kg of FPCM. This is approximately 0.5 kg CO₂-eq. per kg of FPCM above the world average of 1.50 kg CO₂-eq per kg of FPCM. In contrast, the USA as the world's second largest milk producer has the lowest average GHG emissions per kg of FPCM among the 10 milk producing countries and the second lowest of all countries analyzed (Hagemann et al., 2012). According to FAO (2018), in 2015 there was a marked difference in the intensity of GHG emissions between different countries. The lowest emission intensity at the farm level was found in countries with a developed milk production sector and ranged between 1.3 and 1.4 kg CO₂-eq per kilogram of FPCM. In developing countries, the emission intensity ranged from 4.1 to 6.7 kg CO₂-eq per kilogram of FPCM. In the FAO study (2022), where it was assumed that the global emissions intensity of cow milk for the year 2020 was 1 kg CO₂-eq per kg of milk, Africa was marked as the region with the highest emission of kg CO₂-eq/kg of milk, and looking at the countries with the largest individual milk producers, they are Pakistan, India and Brazil. Life cycle assessment is a quantitative method used as a decision support tool. It provides insight into which parts of the production chain have a major impact on the environment and what kind of interdependencies exist between the different phases of the life cycle (Sevenster, 2013). LCA is not only a technique that monitors GHG

emissions, but also other environmental factors such as land or water use and related effects. Thus, the IDF created a guide to Water Footprint Methodology for the Dairy Sector (2017) and Life cycle assessment guidelines for calculating carbon sequestration in cattle production systems (2022). LCA is an important tool in industry strategy and government policy making. Major dairy producers worldwide such as FrieslandCampina, Arla Foods and Fonterra evaluate many of their products and packaging using LCA (Sevenster, 2013).

Sustainable milk production on dairy cow farms

To organize sustainable milk production, farmers' focus should be on all three aspects of sustainability: (i) economic (through increasing milk production), (ii) ecological (through reductions in resource use, nutrient excretion and greenhouse gas emissions per unit of product) and (iii) social (ensuring social acceptability through maintaining good health and well-being of cattle, producing safe and high-quality food and caring for the local and wider community) (Capper and Cady, 2020; Džomba and Čengić-Džomba, 2012).

Milk production on farms, primarily through the production of animal feed and animal excreta, largely contributes to soil, air and water pollution (expansion of arable land, deforestation, reduction of biodiversity). In the production of animal feed, soil and water pollution occurs primarily as a result of the application of mineral fertilizers and pesticides, while undigested parts of food are introduced into the soil and water through animal excrement; nitrogen, phosphorus, microelements, but also various drugs (antibiotics, hormones...) and pathogens. In addition to pollution, water consumption in the chain of animal production is also significant. Highly productive cows need to be provided with 82 liters of water/day (14 to 171 L/day) (Monteiro et al., 2023) and any reduction in feeding has a negative impact on milk production. Nevertheless, this direct water consumption amounts to only about 3% of the total water consumption on the farm, while the largest share of water consumption comes from crop irrigation (Capper and Cady, 2020). Of the total ammonia emissions, which are a significant contributor to acid rain and ecosystem acidification, about 64% originate from animal production. The welfare and performance of animals in dairy production are directly affected by climate change, while the indirect impact relates, to a large extent, to the production and quality of animal feed (Baumgard et al., 2017). At the global level, the emission of greenhouse gases from animal production amounts to 14.5% or 7.5 Gt CO₂-eq (FAO GLEAM, 2022b), where about 30% of GHG is emitted from milk production. According to FAO (2022a), for 2015, the dominant source of GHG was cattle production, which accounted for 62% of the total emissions from the animal production sector, namely 42% (2.9 Gt CO₂-eq) from meat production and

20% (1.4 Gt CO₂-eq) from milk production. The contribution of other types of domestic animals to the total emissions of gases was significantly lower and ranged between 7 and 14%.

In milk production, GHG emissions are the result of various complex biological and chemical processes. Excluding animal feed as an indirect source of emissions, direct greenhouse gas emissions from animal production are linked to CH₄ emissions from enteric fermentation and manure and N₂O emissions from manure (manure management, manure applied to the soil and manure left on pastures) and in 2021 amounted to 4.2 Gt CO₂-eq or 54% of total emissions from agricultural production (from farms) (FAO, 2023). Enteric CH₄ and CH₄ from the manure of dairy cows and heifers have the largest share in the total CO₂-eq from dairy farms (Capper and Cady, 2020). Other emissions include carbon dioxide, which is mainly associated with energy use, production and transport of inputs, and land use and land use changes. When considering the share of individual greenhouse gases in the total emissions from the milk production sector, methane from enteric fermentation participates with 58.5%, CO₂ and N₂O from the production, processing and transport of animal feed with 29.4% and CH₄ and N₂O emissions from manure management with 9.5% (FAO, 2018). In the production of one kilogram of milk, 3.15 kg of CO₂-eq is emitted, and the largest part of the emission comes from biological processes and activities related to the farm (1.51 kg CO₂-eq), land use change (0.51 kg CO₂-eq), animal feed (0.24 kg CO₂-eq), retail (0.27 kg CO₂-eq), processing (0.15 kg CO₂-eq), packaging (0.10 kg CO₂-eq), transport (0.09 kg CO₂-eq) and other losses (0.27 kg CO₂-eq) (Poore and Nemecek, 2018). Methane emissions from enteric fermentation contribute to more than half of the total emissions in milk production, which puts this source of methane emissions in the first place from the point of view of mitigating emissions. In second place is manure management. Methane production is also affected by the amount of food consumed, as well as the chemical composition of the food. Increasing food consumption also increases methane production in the rumen (Ramin and Huhtanen, 2013). Increasing the productivity of animals reduces methane production expressed per product unit, but in most cases the absolute emission on a daily basis (g CH₄ /day) increases. This is precisely why, in addition to increasing productivity, it is important to increase the efficiency of feed conversion or reduce the number of animals in the herd (Cassandro et al., 2013).

Manure is a source of CH₄, N₂O and NH₃ emissions, and the amount of emitted gases depends on the method of manure management, environmental conditions and manure composition. It is estimated that the total annual production of methane from manure amounts to 17.5 million tons (FAO, 2023b). The amount of methane originating from manure is significantly lower compared to the

emission of methane originating from rumen fermentation and depends primarily on the way animals are kept (Hristov et al., 2013).

To ensure ecologically sustainable milk production, proper management of nutrients on dairy farms, primarily nitrogen and phosphorus, is of crucial importance. For the most part, nutrients enter the farm through animal feed and fertilizers (nitrogen also through fixation by legumes and precipitation). Nutrients leave the farm in the form of products - milk, meat and plant products (Čengić-Džomba and Džomba, 2021).

Sustainable dairy processing

Milk processing in general can be energy and water intensive, depending on the end product and the process chosen. During its life cycle, milk is heated and cooled several times. At the farm, milk is cooled to a temperature of 4°C. After milk is received at the dairy, it is heated and cooled several times (depending on the type of end product into which the milk is processed, e.g. cheese, powder, yoghurt, etc.). After processing, the milk will leave the factory at temperatures between 4°C (e.g. fresh products) and 20°C (e.g. milk powder, products stable at ambient temperatures) (van Asselt and Weeks, 2013). FAO (2010) published an estimated greenhouse gas (GHG) emission of 0.155 kg CO₂-eq per kg of milk from the farm gate to the warehouse before it reaches the retail outlet; of this, 0.086 kg CO₂-eq. per kg of milk (or 86 kg per 1,000 kg of milk) is attributed to the processing of milk in the factory. These are average values for Europe and vary depending on the region and product. For example, fermented milk has a rather high emission of 0.304 kg CO₂-eq per kg of milk while cheese and whey have a lower rate of 0.126 kg CO₂-eq per kg of milk at the farm gate. When calculated on a finished product basis, the emission rate for cheese is higher, approximately 1.260 kg CO₂-eq per kg of cheese (based on 10 kg of milk for 1 kg of Gouda cheese) (van Asselt and Weeks, 2013).

Table 4. Total reported carbon footprints for dairy products in kg CO₂-eq/kg product for the entire production chain „from cradle to grave“ (Sarić, 2024, adapted to IDF, 2009)

Drinking milk	Fermented milk	Cheese and whey
1,2 kg CO ₂ – eq/kg drinking milk	1,1 kg CO ₂ – eq/kg fermented milk	8,8 kg CO ₂ – eq/kg cheese

It is significant that processing contributes a fairly small share of the total amount of GHG. When comparing the contribution of emissions from processing to emissions at the farm level they are much lower (van Asselt, 2013). The share of

the farm represents about 80% of GHG emissions in the total life cycle of 1 kg of milk. The milking participates with a negligible ~ 0.02 CO₂-eq per kg of milk. GHG emissions in milk processing mainly come from the use of energy. The share of transport is variable but generally not significant. The main effect from the stages of storage, sale and consumption comes from cooling devices. Also, significant amounts of energy are consumed in cleaning processes. The main contribution to energy and water consumption comes again from the farm. The same applies to eutrophication and acidification (IDF, 2009).

Energy consumption and GHG generation certainly depends on the type of dairy product. Dryers and steaming stations are big consumers of energy, as well as heat treatment devices. Cheese production consumes less energy but generates large amounts of whey. All dairy products consume significant amounts of energy and water for washing and cleaning processes. Water consumption and the amount of waste water depend on the type of plant. Depending on the type of installation and the cleaning system and its management, the total amount of water used in the process can reach several times the amount of processed milk. Consumption is usually 1.3-3.2 liters of water / kilogram of milk received, but it can reach 10 liters of water / kilogram of milk received. Regardless, it is possible to optimize this consumption to 0.8-1.0 liter of water / kilogram of milk by using modern equipment and proper management (UNEP, 2000). The highest consumption of water occurs during secondary operations, especially in cleaning and disinfection, where 25-40 percent of the total amount is consumed. Data on the amount of wastewater varies, but some common values are 1-5 lit./kg of milk, although higher values are also recorded (Integrated pollution and prevention control, 2006).

To improve sustainability, a combination of technological solutions that are technically, economically and environmentally acceptable are applied. Of course, the social aspect is important because any actions must be transparent and be voluntarily accepted by the entire company or factory staff. Various techniques are available, including those from the set offered by BREF (Best reference documents): techniques to increase energy efficiency, techniques to reduce water consumption, techniques to avoid the use of harmful substances, techniques to increase the use of resources, wastewater treatment techniques, techniques to reduce air emissions, techniques to reduce noise, new techniques in the FDM (food, drinks and milk) sector (Santonja et al., 2019).

Also, IDF has developed guides with innovative technologies in wastewater treatment (IDF, 2019). In addition, there are case studies and various other techniques that will help in innovative sustainable technological solutions. Which of the techniques will be applied depends on the company's policy. Other sets of measures and actions include those related to economic and social activities.

Economical and social sustainability of dairy production

Improving the economic and environmental sustainability of dairy production requires a holistic approach that integrates technological innovations, management practices, policy interventions, market mechanisms and collaborative partnerships (Brkić and Puvač, 2024). Precise planning (both on and off the farm) is gaining importance, especially when considering and adopting circular economy strategies (Bhat et al., 2022). The dairy sector provides people with a means of livelihood, status, income and employment. Policies can play an important role in improving the profitability of the dairy sector. Since dairying is usually a commercial activity, the economic aspect is worth studying in its own right (Joengeneel and Slangen, 2013). A common description of economic sustainability is the ability of a system, company or sector to survive in the long term. It refers to the ability of a system, company or sector to maintain its structural and functional capacities after a disturbance or shock. Such a shock may be due to changes in physical conditions (e.g. weather shock), market conditions (e.g. price declines and increased price volatility) or policy changes (Perrings, 1998).

Over time, the EU dairy sector has experienced some changes in its policy framework, the most significant being the abolition of the milk quota system in April 2015. Following the abolition of milk quotas in 2015, EU milk production increased in five Member States. Market-based incentive payments provided by milk processors could help improve the carbon footprint of dairy products. CAP support payments and risk management tools are important to better cope with high price volatility in margins. Milk production in disadvantaged regions faces particular constraints, justifying a targeted policy approach (Jongeneel et al., 2023). The EU Green Deal "Farm to fork strategy" (EC, 2020) is a new comprehensive approach to the way Europeans value food sustainability. Creating a favorable food environment that makes it easier to choose a healthy and sustainable diet will benefit the health and quality of life of consumers and reduce healthcare costs for society.

The progress report on the EU28 CAP objectives for 2023-2024 outlines progress towards sustainability. The measures include: supporting farmers operating in difficult conditions (e.g. mountainous areas), managing these risks (adverse weather conditions, extreme events), improving carbon sequestration and storage in soils and biomass, reducing greenhouse gas (GHG) emissions associated with livestock farming, supporting young farmers, improving animal welfare and combating antimicrobial resistance (EC, 2025).

Small milk processors are important especially for developing countries and rural areas. The development of value-added products, a direct marketing approach improves short food supply chains and relationships between producers

and consumers. Family participation, high standards of animal welfare improve the circular economy (Miočinović et al., 2024). In developing countries, besides providing nutritional ingredients to the population, cattle provide dairy products for the market that bring income to the household and are often a sign of social status (Murphy and Allen, 2003; Kitalyi et al., 2005; Randolph et al., 2007).

Activities on improving sustainability in dairy sector

The dairy community responds to the challenges of sustainable development with numerous activities. Following this, the Dairy Sustainability Framework (DSF) was founded in 2013, a non-profit organization that covers about 30% of global milk production (<https://www.dairysustainabilityframework.org/>). The Global Dairy Platform (GDP) is a body established by the world's largest Dairy companies (<https://globaldairyplatform.com/>). In 2016, the IDF signed a Declaration together with the head of the FAO in Rotterdam stating that "the dairy community accepts sustainability challenges" and that it undertakes to take a full part in promoting sustainability in the dairy sector (<https://fil-idf.org/dairys-global-impact/dairy-declaration-of-rotterdam-3/>). In 2021, the dairy community launched the Pathways to Dairy Net Zero initiative (<https://pathwaystodairynetzero.org/>), which expressed its commitment to reduce GHG dairy emissions by 2030. More than 200 organizations representing about 40% of global milk production have signed this declaration and given their support (<https://fil-idf.org/about-us/>). GDP (2022) in its rather critical review of the IPCC report on climate change (2022) says that the authors (IPCC note author) "suggest that increasing sources of plant-based protein can directly reduce greenhouse gas footprints and improve animal welfare, land use and human health. However, the report does not consider the nutritional implications of a diet rich in plants and low in meat and dairy products, especially in countries where meat and dairy products are scarce or too expensive for many people". EDA (2023-2024) declares in its report that it strongly supports the ambitions and goals of the European Agenda for the Environment and that it wants to build the future of the dairy industry. There are numerous examples of projects and concrete actions that are carried out in many regions in cooperation with the DSF, GDP and the International Fund for Agricultural Development (IFAD). Some examples are: case study - Supporting the development of the sustainability of the dairy sector for small dairy processors in India, in Rwanda in cooperation with Inyange Industries', in Kenya in cooperation with Palmhouse Dairies (DSF, 2023, 2021-2022, 2020-2021).

Even in the first decade of this century, dairy companies clearly emphasized their sustainability goals. Here are some examples: "halve the

ecological footprint of our products and sources and that 100% of agricultural raw materials are sustainable" (Unilever, 2011); "CO₂ neutral 2020 and global number one partnership criteria" (Danone, 2010), "in 2020 40% of energy used on dairy farms from renewable sources, 30% reduction in water consumption, 50% recycled packaging material" (Dairy UK, 2008) (de Jong, 2013).

Today, dairy companies publish their annual sustainable reports or these are part of general annual reports. In its sustainability reports for 2023 and 2024, the world's leading company Lactalis, regarding climate actions, set one of the goals "to reduce the group's greenhouse gas emissions by at least 50% by the end of 2033 (reference year 2019) (Lactalis, 2023, 2024). Nestlé, in its reports for the year, sets the main goals for climate change: "Nestlé aims to reduce greenhouse gas emissions gases (GHG) by 20% by 2025 and 50% by 2030 compared to 2018 levels, on the way to net zero by 2050 at the latest." (Nestlé, 2023, 2024). Royal FrieslandCampina N.V. also in its annual report for 2024, gives results in the field of sustainability (e.g. GHG emissions reduced by 1.4% in production and transport and by 9.8% in milk production on farms compared to 2023) (Royal Friesland Campina, 2024). The American cooperative Dairy Farmers of America states in the report for 2024 that the DFA is a science-based GHG reduction point of 30% by 2030 (https://issuu.com/dairyfarmersofamerica/docs/sust23007_ssr_fnl_issuu). The Chinese company Mengniu states in its 2024 report that "Mengniu's Ningxia factory has been successfully certified as a 'Lighthouse Factory' (solar-powered), which makes it the first dairy plant in China and the first liquid milk factory in the world to receive this prestigious title (China Mengniu Dairy Company Limited, 2023, 2024). Finally, two positive examples related to dairies in Bosnia and Herzegovina can be highlighted. The first is the biogas plant on the "Spreča" dairy farm near Kalesija in Bosnia and Herzegovina, which supplies milk to dairy Milko's d.o.o. Sarajevo. (<https://rtvbk.ba/farma-spreca-u-kalesiji-prva-farma-u-bosni-i-hercegovini-koja-proizvodi-struju-iz-biomase/>). Another example is the dairy Lactalis BH/Inner, which has been part of the Lactalis group since 2007. With its activities, the dairy follows the group's main directions (Carbon Net Zero until 2050). Inner undertakes activities: continuous process of plant modernization, fight against deforestation, appropriate packaging, consumer awareness, animal care, education, audits, raising awareness. Concrete actions at the local level are the procurement and planting of seedlings for 11 schoolyards in Bosnia and Herzegovina. Surely this is not the end of the list and similar examples in the region can be found.

Conclusion

Dairy sustainability is a difficult question to answer. It is true that there is strong evidence that significant GHG emissions are attributed to animal production, including the production of milk and milk products. Things seem to be like that if they are viewed unilaterally. However, looking at things comprehensively, the situation is not so simple. Bearing in mind other aspects of the sustainability of the dairy sector, it plays an important role in many segments of sustainability: it is a necessary source of valuable nutrients, especially for the children's population, for the malnourished population in developing countries, a huge number of people derive their income from dairying. On the other hand, the dairy community, which is very organized, is aware of the label it likes and the situation humanity is in, so it takes all possible steps in its segment and at all levels to be an active participant in achieving Global Net Zero and to be a leader in its achievement. Dairy science and practice has already found and is finding new opportunities every day to reduce the emission of greenhouse gases, pollution, and for a better economic and social policy. Many studies are undertaken to help both decision makers and policy makers to make the right decisions and take the right moves. It is difficult to say what the future holds and the fate of the dairy sector will depend on many things, but at the moment it can be said that the sector is very conscious of taking actions to achieve all sustainability goals.

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FORTIFICATION WITH NATURAL PLANT-BASED ADDITIVES IN CHEESE PRODUCTION: SUSTAINABLE CONCEPTS

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Abstract: Fortification of cheese involves the addition of micronutrients, including them in the casein matrix and proper improvement of the cheese, making the final product more attractive to consumers. In the Agri-Food sector, it is possible to enrich cheeses by adding plants secondary metabolites and/or using by-products from the processing plants. Interest in fortification of cheeses with antioxidants and phenolics derived from plant extracts (PE), spices, and plant by-products has been increasing significantly and encourage the development of a sustainable, circular economy and create “greener” environmentally friendly compounds that improve consumer health. Synthetic additives have become undesirable in the food due to potential harmful effects on human health. Consumers are trying to consume healthier or functional foods, safer alternatives to foods with synthetic additives. Numerous plant-based preservatives have been tested for use in the food industry. These natural molecules increase the microbiological stability of food and the levels of bioactive ingredients (BAI), while exhibiting antimicrobial (AM) and antioxidant (AO) effects. Cheeses are generally susceptible to contamination by pathogens and spoilage agents. In the dairy industry (more often on smaller farms), several sources of contamination have been observed: use of raw milk as a raw material, the environment where milk is obtained, stored and processed, or due to the practice of milk curdling with vegetable coagulants. The use of preservatives in cheese production is imperative, but they can degrade sensory properties, which consumers will not accept. This

review discusses an innovative approach, primarily focuses on the frameworks and procedures for cheese fortification.

Key words: natural plant additives, cheese making, functional properties, antimicrobial effects, antioxidative activity, shelf-life

Introduction

When we consider the physiological well-being and biochemical processes in the human body, the consumption of dairy products is of essential importance, as they represent a significant source of essential vitamins (A, D and B₁₂), fatty acids, high-quality protein, minerals (calcium, magnesium, potassium, selenium and zinc) and the primary source of certain BAI that protect and promote health (polar lipids, proteins and peptides, oligosaccharides, live and active cultures of microorganisms in fermented milk products). To address the challenge of lactose intolerance in milk and digestive problems in children/adults that lead to reduced milk consumption, the most appropriate alternatives are lactose-free or lactose-reduced milk products (the most accessible and affordable source of essential nutrients).

Following more modern trends, there is an increase in interest in dairy products fortified with plant PE and plant-based antioxidants, necessary for the neutralization and removal of free radicals, which are constantly generated in the human body. They can damage vitally important molecules and predispose to the occurrence of chronic non-infectious diseases (cardiovascular diseases, diabetes, and cancer). The goals of food fortification are: improving its chemical and nutritional quality and techno-functional properties, increasing the content of essential micronutrients in food, and providing health benefits, with minimal risk or completely no consequences. Fortification of cheeses with dietary herbs, spices, and PE may lead to AM, AO, antimutagenic, anti-inflammatory, and immunomodulatory effects, and contribute to better public health (Picciotti et al., 2021; Kurćubić et al., 2015; Ritota and Manzi, 2020; Kurćubić et al., 2023a; Comerford et al., 2024; Kurćubić et al., 2024; Wazzan, 2024; Ferreira et al., 2025).

The food market is abundant in fortified dairy products (42.9%) (Watson et al., 2006), as they are the most famous carriers of functional BAI and the most widespread nutraceutical foods. Dairy products are of such a composition that they provide a simple way to incorporate the fortifiers into these matrices, and are therefore significantly represented in many diets (Choi et al., 2006). In order not to reduce the acceptability and palatability of the final product by consumers, one of the most important guidelines is that cheese fortification must not degrade the sensory and physicochemical properties of the cheeses (Lacroix et al., 2013). The incorporation of BAI gives cheeses numerous nutritional advantages, as they are a

suitable means for their transport (Kumar, 2015). During ripening and aging, more attention must be paid to the preservation and protection of the activity of BAI in the matrix.

A large number of studies on natural herbal supplements for the fortification of cheeses and other dairy products are available, aligned with current trends, cost-effective principles and circular economy, and in environmental sustainability and human well-being (“safer and greener alternatives”, which can be easily purchased and incorporated directly to the milk, cheese curd, or final product) (Ansari and Kumar, 2012; El-Aziz et al., 2012; Dwyer et al., 2015; Bukvicki et al., 2018; El-Sayed and Youssef, 2019; Dupas et al., 2020; Ritota and Manzi, 2020; Kumar Paswan et al., 2021; Muñoz-Tebar et al., 2021; El-Loly et al., 2022; Difonzo et al., 2023; El-Aziz et al., 2023; Kandyliari et al., 2023; Silva et al., 2023; Ferreira et al., 2024; Tarchi et al., 2024; Fernandes et al., 2025; Maddaloni et al., 2025).

Aim of our review is to consider the possibilities and potential for further, future research: a) optimization of extraction procedures; b) more precisely determining the concentrations required to achieve the desired health benefits; c) the impact of fortification on the absorption of macronutrients present in cheese (proteins, calcium); d) testing solubility and possible interactions with other milk constituents; e) achieving stability and functionality of cheeses during processing and storage; f) the potential for fortification while preserving the sensory quality of the final product (Wazzan, 2024).

Biopreservation strategy: the use of herbs and spices like source of plant-derived BAI to prevent the growth of pathogenic microorganisms in cheese and improve his functional and nutritional properties

Preparation of herbal supplements for use in food - basic postulates

Fortification of cheeses can be achieved by incorporating plants/spices into the milk before cheesemaking, adding them to curdled cheese, or rolling the cheese in chopped herbs (Dupas et al., 2020). PE as food additives are made from a large number of different plants, using different methodologies and solvents (non-toxic, approved for the industrial production of food ingredients and food products) (EU, Directive 2009/32/EC). To achieve uniform quality of PE, it is necessary to take into account the fact that variations in their chemical composition are contributed by plant genotype, geographical location, environmental conditions and agronomic conditions (Martillanes et al., 2017). The extraction method, temperature, solvent

and pressure affect the chemical composition of the prepared PE, so it is necessary to choose the optimal extraction parameters to obtain the desired compounds and their bioactivity (Sasidharan et al., 2010; Ferreira-Santos et al., 2020). Today, modern extraction techniques are used, which include subcritical water extraction, supercritical fluid extraction, enzyme-assisted extraction, microwave and ultrasound, pulsed electric field and accelerated solvent extraction. Conventional extraction methods (maceration, percolation, infusion, decoction, reflux extraction, Soxhlet extraction and hydrodistillation (steam and water distillation or their combination) are still successfully used (Azwanida, 2015; Zhang et al., 2018; Pisoschi et al., 2018; Cheng et al., 2021). Today, it is top priority to take into account the environmental impact of extraction methods while favoring more sustainable and “greener” processes that optimize the recovery of high-value BAI, minimizing the amount of solvents used and the resulting waste (Ferreira-Santos et al., 2020).

Application of PE as a AM preservative in cheeses

Contamination with various pathogenic bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* species) is a critical point in cheese production, since cheese (especially in artisanal processing) is produced from unpasteurized milk. The unique and consumer-attractive sensory characteristics of artisanal raw milk cheeses are the result of the use of unpasteurized milk. Pasteurization reduces bacterial load and extends the shelf life of milk, but denatures whey proteins and leads to complex interactions with casein micelles, fat globules, and minerals (Singh and Waungana, 2001). Above mentioned bacteria can be treated as a significant health risk, but by producing enzymes that degrade proteins and lipids, they also seriously affect the physicochemical properties of cheese (texture, taste and aroma). The general public is concerned about the production of thermostable enterotoxins by *Staphylococcus aureus* (*S. aureus*), which persist even after cheese ripening, leading to spoilage and potential foodborne diseases (Johler et al., 2018; Oliveira et al., 2022). Controlling the presence of the above-mentioned bacteria in cheeses is essential for preserving their safety and quality (Kyrykbaeva et al., 2025).

Preservatives are food additives that extend the shelf life of food by protecting it from spoilage caused by microorganisms (most commonly as AM and antifungal agents, fungistatic agents, and agents for preventing exponential multiplication of bacteriophages). Waste from the processing of plant raw materials (by-products like grape skins and seeds, grape flour and seed extracts, pineapple peel, pomegranate seeds) have been shown to exhibit strong AM activity, without

detrimental effects on bacterial starter cultures and endogenous bacterial strains, not incorporated into the starter culture (Costa et al., 2018).

The results of the study by Bermudez-Beltrán et al. (2020) showed that the design of Petit Suisse cheese from gooseberries, incorporated into moringa leaf powder and gelatin, created an innovative dairy product (cheese) with desirable physicochemical, microbiological, and bromatological quality. Petit Suisse can become more sensorially acceptable and desirable after the addition of gelatin, which is a significant improvement, especially when considering its specificities (fresh and smooth cheese) and already good acceptance by consumers, especially children but also other age categories. Enrichment with moringa leaf powder reveals higher values for ash, protein, fiber and fat content, i.e. better, improved nutritional value of the fortified cheese.

The aim of the study of Kurćubić et al. (2015) was to assess the impact of cheese enrichment with polyphenols from ethanolic PE of the *Kitaibelia vitifolia* (Pirotski kachkaval) applied by adding to the cheese lump after texturization or by spraying on the surface of the cheese. The number of colonies of aerobic bacteria, coagulase-positive staphylococci, *S. aureus* and anaerobic sulfite-reducing bacteria did not differ significantly ($p > 0.05$) between the control and experimental group samples, as confirmed by microbiological testing. The AO activity of conventional and enriched cheese was assessed using 5 modern methods and revealed a slight increase associated with phenols in enriched cheese samples compared to control samples. Enriched cheese samples showed better sensory quality compared to control one, as assessed by a five-member expert panel. Statistically significant ($p < 0.05$) changes were observed in moisture content and total solids of the control and modified cheese batches, but other parameters did not differ significantly ($p > 0.05$).

The abundance of phenols, dietary fiber (DF) and high AO capacity of the fruit of the *Arbutus unedo* L. plant interested Masmoudi et al. (2020) to evaluate the effect of enriching soft cheese “Sardaigne” with an PE obtained by applying an aqueous decoction. LC-MS analysis of the PE revealed quinic acid and catechin as the dominant phenolic compounds. DPPH radical activity detected an IC_{50} value at the level of ~ 0.31 mg/mL. Fortified cheese showed a higher crude protein content and higher firmness. Fortification at an amount of 0.3 g/L of PE increases cheese yield, improves (after 5 days of storage) DPPH radical scavenging activity, without changing color and sensory properties, which qualifies it as a useful functional ingredient, with potential health benefits.

Solhi et al. (2020) reported recently that asparagus powder (AP) can be a quality source of BAI for the enrichment of processed cheeses (it reduces pH and lipolysis indices, and increases phenolic content, AO power and proteolysis of processed cheeses). The evidence was revealed by investigating the qualitative

properties of enriched processed cheese (PC) with different levels (0.5%, 1% and 1.5% w/w) during the storage period. Technologically, AP contributed to the structure of PC being more elastic and rigidity, with reduced spreadability compared to the control sample (determined by dynamic oscillatory rheometry).

One of the most recent pieces of evidence is that hops represent a promising herbal natural supplement for improving both the microbiological and physicochemical characteristics of cheese, as hop extract effectively inhibits the pathogenic bacteria *S. aureus* and *Listeria monocytogenes* (*L. monocytogenes*) (Kolenc et al., 2022; Plaskova and Mlcek, 2023). Studies are constantly being deepened, considering the balance of antimicrobial (AM) effectiveness and efficiency with sensory acceptability, by examining the interactions of hop extracts with cheese matrix components (lipids and proteins), optimal doses and methods of application, and the impact on sensory properties, texture, and overall quality (Betancur et al., 2023). Hop extract effectively reduced bacterial growth, especially *S. aureus*, while improving the physicochemical properties of cheese (firmness and texture) during a 90-day ripening period. Future trials of the application of hop extract in commercial cheese production are planned to reveal the practical viability and parameters of economic success. In cheeses treated with hop extract, a significant increase in AO activity was demonstrated by the DPPH test. The improved AO profile, along with the high phenolic contribution of hop extract, highlights its candidacy as a functional additive for improving the nutritional value and shelf life of cheese (Kyrykbaeva et al., 2025).

Since we stated in the Abstract that one of the most important sources of contamination is the environment where milk is obtained, we cite the significant results of El-sawy et al. (2024), who, in their research, determined that four tested ethanol extracts from medicinal plants (cinnamon bark, chamomile flower, calendula flower and sage leaf) exhibit antibacterial and anti-biofilm efficacy (against gram-positive and gram-negative food-borne pathogenic bacteria). Mixtures of the tested low concentrations of extracts exhibited synergy (more effectively inhibited biofilm than each of the extracts individually). In an *in vitro* safety study of pasteurized milk and white soft cheese, the PE cocktail used to control *L. monocytogenes* ATCC7646 biofilms on stainless steel surfaces significantly improved the safety and quality of the cheese produced and achieved a significant reduction in *L. monocytogenes* counts without compromising sensory quality and without showing toxicity (even during cheese storage at cold chain temperatures). From all of the above, it can be concluded that the tested extracts are a quality alternative for mitigating *L. monocytogenes* infections.

As we mentioned in the Introduction chapter, the valorization of agricultural by-products is of great importance, and a good example is the most recent research by Ferreira et al. (2025) on onion peels (OP), a major agricultural

by-product that negatively impacts the environment. The study presents sustainable innovations in food - the development of foods (cream cheeses) enriched with natural compounds that improve their nutritional value and shelf life. The strategy of waste management and reducing their impact on the environment is recognizable. As a solution to the above challenge, phenolic compounds from onion peels, which exhibit AO power, are extracted and added to food, while extending their stability through the microencapsulation process. The extract was found to exhibit antidiabetic effects (significantly inhibits α -amylase and β -glucosidase activities, at levels of 91.9% and 95.8%). Cheese samples enriched with the extract have improved technological properties (excellent water retention and reduced syneresis). A comparison of the effects of encapsulated and free extract indicates the favorable effects of microencapsulation in maintaining stable total phenol content and AO capacity, i.e. the best oxidative stability (controlled release of the extract from microparticles). The incorporation of organic enhancers (OP) in microencapsulated extracts can be a winning combination for prolonging the shelf life and stability of fortified foods.

Muñoz-Tébar et al. (2019) investigated whether oil extracted from the seeds of *Salvia hispanica* L. could be a sustainable source of omega-3 fatty acids for the fortification of sheep cheese. The oil was prepared in two concentrations (3 and 5 g/L) as Ca-caseinate-stabilized emulsions, incorporated into pressed sheep cheese, and comprehensive quality were determined during a 32-day ripening period. Sheep milk coagulation was not degraded by fortification, the ripening process was preserved, with improved fat, dry matter, and α -linolenic acid content. Cheeses enriched with chia oil showed good sensory acceptance by panelists. Fortification positively contributed to the physicochemical quality of the cheeses.

Farrag et al. (2017) investigated the application of hibiscus soft drink on the characteristics and quality of white soft cheese, with AM and hypertensive treatment by adding hibiscus soft drink (prepared by soaking and blanching, at 0, 2, 4, 6, 8 and 10%). Total solids, protein, fat and pH values decreased with increasing hibiscus PE addition. Cheese hardness was reduced, as were gumminess and chewiness, in cheeses where the soft drink was added by soaking, and were even lower in cheese enriched by blanching. Cheese color parameters (L^* , a^* , b^* ,) were higher in cheese samples enriched with blanched hibiscus soft drink compared to those enriched by soaking. Anthocyanin values (polymer color, color density, tannin contribution and total anthocyanin concentration) were lower in blanched hibiscus cheese samples than in other types of cheese. Color was rated as acceptable for those cheese samples that contained up to 6% of soaked or blanched hibiscus soft drink. Cheese with more hibiscus was unacceptable to the evaluators. Total counts of filaments, yeasts and molds were lower in blanched hibiscus cheese samples than in soaked hibiscus cheese, compared to control cheese samples.

Therefore, the application of hibiscus addition as an AM agent and in the treatment of hypertension in the consumption of fortified white soft cheese can be analyzed commercially, for the benefit of producers and consumers.

Pomegranate peel and grape seeds, as vegetable by-products from the Agri-Food sector, are proven effective natural preservatives against *L. monocytogenes*, *S. aureus* and *Salmonella enterica* in cheese (Jayathilakan et al., 2012). Their polyphenols have AM effects - changes that lead to bacterial death are achieved by penetrating the cell walls with membrane rupture, damaging proteins and membrane enzymes (Cowan -M.-M. 1999; Daglia, 2012).

The study by Kandyliari et al. (2023) focused on the analysis of dairy products fortified with aqueous PE from plant-based by-products (*Citrus aurantium* peel, *Citrus limon* peel and *Rosa canina* seeds) and herbs (*Sideritis* spp., *Hypericum perforatum*, *Origanum dictamnus*, *Mentha pulegium* L., *Melissa officinallis*, *Mentha spicata* L. and *Lavandula angustifolia*). Fortified cream cheese, kefir, yoghurt and vegan yoghurt were tested for AO content, phenolic profile and sensory characteristics. Fortification of cream cheese with St. John's wort extract contributed to higher antioxidant and phenolic content (0.53 ± 0.16 mmol Fe^{2+} /L and 56.36 ± 7.13 mg GAE/g, respectively) than when fortified with BAI from other prepared and tested plant materials. AO capacity and phenolic content also differed statistically significantly ($p < 0.05$). Overall acceptability ranged from 4.0 ± 1.1 for the control group to 2.6 ± 1.0 (the lowest value, for cream cheese enriched with mountain tea extract). A statistically significant difference ($p < 0.05$) was found between the samples for texture, taste and overall acceptability, but not for color and aroma ($p > 0.05$). The use of phytocomplex cocktails from PE originating from different plants leads to higher bioavailability of BAI (Malongane et al., 2017). In line with the findings of this study, in study of Kandyliari et al. (2023), the cocktail of plants and/or processing by-products also showed higher concentrations of antioxidants and total phenols before and after *in vitro* digestion.

Mahajan et al. (2016) investigated and concluded that aqueous extracts of pine needles (*Cedrus deodara* (Roxb.) Loud.) improve the oxidative stability of lipids in low-fat *Kalari* cheese, a typical Indian hard and dry cheese, as well as the microbial load, due to the AO and AM properties of pine needle phytochemicals.

Ansari and Kumar (2012) in their review report on cheese as a quality and suitable medium for the delivery of oil-soluble natural colorants and phytochemicals (carotenoids, bixin, lutein, omega 3-fatty acids, docosahexanoic acid and eicosapentanoic acid).

Possibilities for improving the sensory properties of cheese with natural herbal additives

The addition of plant extracts to dairy products must result in products that are accepted by consumers. Therefore, attention must be paid to the sensory evaluation of the quality of the fortified products. Attention must be given to the types of plants from which the extracts are derived, the concentrations of the applied extracts, as well as the stages at which the extracts are incorporated during the cheese production process (Ritota and Manzi 2020).

The concentration of added green tea aqueous extract in cottage cheese plays an important role in its sensory acceptability. Panelists noted that concentrations of 8% and 9% resulted in a pleasant cheese taste and flavor, while concentrations above 10% led to a bitter taste, which became more pronounced with increasing extract concentration (Evstigneeva et al., 2016). Foda et al. (2010) investigated the sensory properties of white cheese enriched with spearmint essential oil. The best results were observed in cheeses fortified with spearmint essential oil at concentrations of 0.5, 0.75, and 1.0 mL/kg retentate, respectively. On the other hand, cheeses with higher concentrations of spearmint essential oil (1.5, 2.0, and 2.5 mL/kg of retentate) received significantly lower scores from the panelists. According to the study by Tarchi et al. (2024) the addition of varying concentrations of olive leaf extract to Cantal cheese resulted in noticeable color changes. These changes were directly dependent on the concentration of the added extract, leading to alterations in the L^* , a^* , and b^* values. The fortified cheese appeared darker, redder, and more yellow compared to the control sample. The hardness and cohesiveness of Cantal cheese increased significantly ($p < 0.05$) after 28 days of storage, in correlation with the increasing concentration of olive leaf extract. On the other hand, in the study conducted by Licón et al. (2012), the addition of saffron to ewe milk cheese resulted in a darker, less red, and more yellow product. The tendency toward a yellow tone became especially pronounced with increasing concentrations of added saffron.

One of the most significant impacts of PE on the sensory attributes of fortified cheeses is the color change, which most commonly tends to shift slightly or more noticeably toward a green tone (Setyawardani et al., 2023). Such changes were observed in study by Miwada et al. (2023) samples containing up to 4% moringa leaf extract were considered acceptable, showing no significant differences compared to the control, whereas those containing 6% and 8% were rated as undesirable by the sensory panel.

The use of agricultural by-products, which represent an excellent source of bioactive compounds with beneficial effects on human health, plays a significant role in the sustainable development of agriculture when applied in cheese

production (Difonzo et al., 2023). In a study of white soft cheese enriched with varying concentrations of celery leaves (5%, 10%, and 15%), Awda et al. (2019) reported improved flavor and greater overall acceptability for cheeses containing 5% and 10% celery leaves. The addition of *Allium roseum* paste and powder to double cream cheese led to an improvement in sensory properties, particularly in samples containing 6% paste and those with 0.8% powder (Gliguem et al., 2019). Barbaccia et al. (2021) investigated the properties of stretched sheep cheese with the addition of 1% (w/w) grape pomace. The results indicate that, in addition to a high polyphenol content in the cheese, there was a positive impact on sensory attributes, with the biggest impact observed in odour and aroma.

The future of cheese fortification and enrichment with additives of plant origin: how to make a successful and sustainable step forward?

The purpose of this review was to consider the AM and AO activity of natural molecules of plant origin applied in cheese production, as well as their impact on the overall quality of cheese (chemical, nutritional and sensory characteristics of the product, as well as extending the shelf life of cheese). Herbs and spices are multifunctional in the dairy sector and cheese manufacturing as antioxidants, AM agents and flavorings, enhancing the attractiveness of fortified foods. Food production is extremely complex because it affects technological parameters, economic, environmental, and health aspects. Fortification of food makes it healthier/functional, "preventive" or "therapeutic", and address various nutritional deficiencies and disorders. The interaction of the food matrix with the AM mechanism of natural additives requires further knowledge because they can reduce the AM effect. The dose in which PE or other plant-based additives are incorporated into the cheese matrix is also important. The price of natural preservatives is often high, which discourages food manufacturers from using them in the production process, so it must be reasonable compared to synthetic compounds that have the same or stronger effect. Also, the legislation on natural additives is often limited and confusing, and government approvals are required for their use in food. Definitely, additional studies of plant-based ingredients in cheese production processes would be valuable, especially *in vivo* studies, which are otherwise scarce.

Herbs and spices can be used as substitutes for less desirable ingredients, and have the potential to be significant in the dairy sector as partial or complete replacements for salt, one of the main ingredients of various cheeses. Salt adds flavor to cheese, helps in drying the curd, is necessary for the development of a

good rind and has an AM effect. Milk and dairy products are among the most consumed foods by adults in Europe, with a high average cheese consumption of 34.2 g/day. Reducing salt intake to less than 5 g/day (as recommended by the WHO), represents an important challenge to reduce the development of diseases, such as hypertension and cardiovascular diseases, associated with excessive salt consumption. There is no research on partial or complete replacement of salt with natural preservatives, and they should be encouraged.

The results of the research provide valuable information on the strategic potential of using PE and agricultural by-product extracts to enrich a wide range of dairy products. This strategy is a key step in creating innovative, consumer-oriented, dairy products that are in line with the evolving preferences of health-conscious consumers, with environmental benefits through the reduction of by-products (waste) from the agricultural and food industries. The use of by-products from the processing of plant raw materials promotes a circular economy, along with eco-friendly sustainable production of natural food supplements and healthier, fortified foods. Further research into sensory properties is necessary, as their optimization is of paramount importance for consumer acceptance of cheeses and other dairy products.

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Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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PRODUCTION TRAITS OF SIMMENTAL BULL DAMS IN FIRST LACTATION ACROSS DIFFERENT DISTRICTS OF SERBIA

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Abstract: Milk yield and composition are among the most important traits in cattle production. The aim of this study was to analyse the production traits of Simmental bull dams in their first lactation in Serbia and to evaluate the influence of the district (breeding region) on the variability of these traits. The study included 408 Simmental cows selected as bull dams from eight districts: Kolubara, Podunavlje, Braničevo, Šumadija, Zaječar, Rasina, Nišava and Belgrade. Milk production traits in the first lactation were standardised to 305 days and included milk yield (kg), milk fat content (%), milk fat yield (kg), protein content (%) and protein yield (kg). The data were analysed using one-way analysis of variance (ANOVA) with the district as a fixed factor. The average milk yield in the first lactation was 6093 kg, with an average milk fat content of 4.00% and protein content of 3.25%, confirming that these cows represent a highly productive segment of the Simmental population in Serbia. Highly statistically significant differences ($p < 0.001$) were found between the districts for all analysed traits. The highest average milk yield was recorded in the Nišava district (6792 kg), the lowest in the Podunavlje district (5735 kg). The highest milk fat content (4.30%) and the highest milk fat yield (288.89 kg) were recorded in the Zaječar district, while the highest protein content (3.35%) was recorded in the Šumadija district. The results presented indicate the need to optimise selection criteria and improve rearing conditions in districts with lower production levels in order to support the sustainable development of milk production in Serbia.

Key words: Simmental cattle, bull dams, milk yield, milk composition, first lactation, breeding regions.

Introduction

Milk and dairy products are an important source of nutrients for the human diet, mainly due to their content of proteins, vitamins, minerals and energy (Siddiqui et al., 2024). The increasing demand for milk and dairy products continues to dictate the main goal of dairy cattle breeding, which is to increase the average milk yield. Milk production depends on various factors such as genetics (breed), the physiological condition of the animal and environmental conditions (feeding, care and housing of the animals) (Bednarski and Kupczynski, 2024). Therefore, improvements in milk production in cows can be achieved by selecting the best animals and through advances in feeding and farm management.

Serbia is one of the European countries where the Simmental cattle breed is well adapted and has been the predominant breed for at least half a century. As a result, the autochthonous cattle populations have been largely replaced by the Simmental breed. The breed spread mainly through the import of heifers, breeding bulls and bull semen from Austria and Germany. Today, the Simmental is the most widespread cattle breed in Serbia and accounts for 75% of the total cattle population (Institute for Animal Husbandry, 2024). Nikšić et al. (2011) reported that the average milk yield of first-calving Simmental cows was 4348 kg, with a milk fat content of 3.93% and a corresponding milk fat yield of 171.1 kg. According to the results of Karamfilov and Nikolov (2019), the average milk yield of primiparous Simmental cows was 5,016 kg with a milk fat content of 4.22 % and a protein content of 3.40 %. The authors also reported that the farm and year of calving had a highly significant influence on all analysed milk production indices ($P < 0.001$). Perišić et al. (2009) stated that according to the research results of domestic and foreign authors obtained in the study of the productive traits of the Simmental breed in various rearing systems, the milk production of the Simmental breed in Serbia is remarkably low compared to the average production of the Simmental breed in most European countries. Buonaiuto et al. (2024) reported that the average milk yield of primiparous Simmental cows was 6,908 kg, with an average fat content of 3.84% and an average protein content of 3.53%.

Bull dams are high-performance cows that are selected for their milk yield, conformation and genetic potential to pass on desirable traits to their offspring. They should generally represent the top 1% of the best, i.e. elite cows, and mating with elite sires should produce the next generation of bulls. Lazarević et al. (2018) analysed the phenotypic expression and variability of milk traits in Holstein-Friesian bull dams and their offspring and came to the conclusion that different housing conditions, nutrition and care contributed to the differences in the expression of milk traits. According to the results of Pantelić et al. (2010), the

regions had a statistically highly significant influence ($P < 0.01$) on all analysed milk traits in Simmental bull dams.

The main objective of this study is to analyse the milk traits of Simmental bull dams in the first lactation and to determine the possible influence of different regions on the variability of these traits.

Materials and Methods

This study included 408 Simmental cows selected as bull dams in Serbia in 2024. The initial selection of cows as bull dams was based on the completion of the first lactation, but also on subsequent lactations. The influence of breeding regions on milk traits in the cows' first lactation was analysed. The milk recording was carried out in accordance with the rules prescribed by the International Committee for Animal Recording (ICAR). The milk traits refer to the first lactation standardized to 305 days and include

- 1) milk yield (kg),
- 2) fat content (%),
- 3) fat yield (kg)
- 4) protein content (%)
- 5) protein yield (kg).

A one-way analysis of variance (ANOVA) was performed to determine whether district had a significant effect on milk production and milk composition traits of Simmental bull dams in first lactation. District was included as a fixed factor and milk yield, milk fat content, milk fat yield, milk protein content and milk protein yield were used as dependent variables. ANOVA results are presented as least squares means $\pm$ standard errors, together with F-values and significance levels. Statistical analyses were performed using STATISTICA (data analysis software), version 7 (StatSoft, Inc., 2004).

The bull dams included in this study come from eight different districts (breeding regions) in Serbia.

The breeding regions and the number of selected bull dams per region are as follows:

- 1) Kolubara – 45,
- 2) Podunavlje – 46,
- 3) Braničevo – 10,
- 4) Šumadija – 19,
- 5) Zaječar – 6,
- 6) Rasina – 188,
- 7) Nišava – 20,
- 8) Belgrade – 74.

Results and Discussion

The descriptive statistics for milk traits of Simmental bull dams in their first lactation are shown in Table 1. The average milk yield was 6093 kg, ranging from 4552 to 9112 kg, with a coefficient of variation (CV) of 10.63%, indicating moderate variability between animals. The mean fat content was 4.00%, with a low CV (2.60%), indicating a relatively uniform fat percentage among cows. The average fat yield was 243.56 kg (CV = 11.01%). Protein content showed slightly higher variability than fat content (mean = 3.25%, CV = 4.59%), while protein yield showed the highest variability among the traits (mean = 197.95 kg, CV = 11.46%).

Table 1. Milk traits of Simmental bull dams in Serbia (1st lactation)

Milk traits	N	Mean	Minimum	Maximum	Std.Dev.	Std.error	CV (%)
Milk yield (kg)	408	6093	4552	9112	647.97	32.08	10.63
Fat content (%)	408	4.00	3.38	4.58	0.10	0.01	2.60
Fat yield (kg)	408	243.56	182.72	361.34	26.82	1.33	11.01
Protein content (%)	408	3.25	2.39	4.11	0.15	0.01	4.59
Protein yield (kg)	408	197.95	145.56	293.00	22.69	1.12	11.46

CV – coefficient of variation

In order to place the results of this study in a broader context and to understand them better, it is necessary to compare them with the results of other authors to gain an insight into how the milk production of Simmental bull dams differs from that reported in other studies.

This study included bull dams, i.e. elite animals in the population, so their production was expectedly higher compared to the results reported by other authors. Similarly, Karamfilov and Nikolov (2019) and Nikšić et al. (2011) reported lower average values for Simmental cows in the first lactation compared to the results presented in this study.

Stanojević et al. (2022) reported an average milk yield of 5,520 kg, a milk fat content of 3.94% and a milk fat yield of 218 kg, which are also lower than the results presented in this study. Pantelić et al. (2005) investigated the phenotypic variability of milk production traits in Simmental bull dams and reported average results of 4,768 kg milk, 3.77% milk fat and 179 kg milk fat per cow in the first lactation. The lower values compared to the results of the present study confirm that the production traits of Simmental bull dams have improved since 2005.

Biçoku et al (2010) found that inadequate feeding throughout lactation affects milk production. In their study, the milk production of Simmental cows in Albania was 3,735 kg in the first lactation, while their dams in Austria produced 5,499 kg in the first lactation.

Compared to the results presented in this study, lower values were also reported by Shevhuzhev et al. (2017) for the Simmental breed in the North Caucasus region of the Russian Federation. The authors reported that the average production of Simmental cows in the first lactation was 5,040 kg, with 4.11% milk fat and 3.29% milk protein.

Janžeković et al. (2004), Čačić et al. (2022) and Bolacali and Ozturk (2018) also reported lower values for the first lactation in the Simmental breed compared to the results presented in this study.

On the other hand, there are studies that report a higher average milk production of the Simmental breed in the first lactation. Küçük Baykan and Özcan (2017) found an average milk yield of 7,693 l for Simmental cows in the first lactation, while Kara (2022) reported values of 6,928 kg for the same trait. Koç and Öner (2023) reported an average milk yield of 8,720 kg for Simmental cows in the first lactation, which is the largest deviation from the results presented in this study.

Table 2 shows the average values of milk traits of Simmental bull dams in the first lactation by districts in Serbia. Significant variability between districts was observed for all analysed traits ($p < 0.001$). The small sample size in the Zaječar region may affect the reliability of the ANOVA results. The highest average milk yield was recorded in Nišava (6792 kg) and Zaječar (6764 kg) districts, while the lowest yield was recorded in Podunavlje district (5735 kg). The highest fat content was recorded in Zaječar (4.30%), while the lowest was in Rasina district (3.97%). Consequently, the fat yield followed a similar pattern, with the highest value in Zaječar (288.89 kg) and the lowest in Podunavlje (228.50 kg). The protein content was less variable and ranged from 2.92% in Nišava to 3.35% in Šumadija. Despite the lower protein content, Nišava achieved a relatively high protein yield (198.44 kg) due to the higher milk yield.

Table 2. Milk traits of Simmental bull dams in the first lactation by districts of Serbia

District	N	Milk yield (kg)	Fat content (%)	Fat yield (kg)	Protein content (%)	Protein yield (kg)
Mean ± Std. error						
Kolubara	45	6152±90.65	4.03±0.01	247.81±3.64	3.30±0.02	203.20±3.25
Podunavlje	46	5735±89.66	3.98±0.01	228.50±3.60	3.21±0.02	184.13±3.22
Braničevo	10	6228±192.31	4.05±0.03	252.20±7.72	3.32±0.04	206.70±6.90
Šumadija	19	6193±139.51	4.01±0.02	248.18±5.60	3.35±0.03	209.35±5.01
Zaječar	6	6764±248.27	4.30±0.04	288.89±9.96	3.26±0.05	220.73±8.91
Rasina	188	6141±44.35	3.97±0.01	243.95±1.78	3.26±0.01	199.72±1.59
Nišava	20	6792±135.98	4.08±0.02	277.61±5.46	2.92±0.03	198.44±4.88
Belgrade	74	5872±70.69	3.99±0.01	234.11±2.84	3.28±0.01	192.80±2.54
F exp.		8.87***	14.6***	13.09***	23.23***	5.68***

*** – statistically highly significant ($p < 0.001$)

Overall, the results indicate that district has a significant influence on milk yield characteristics, with some regions showing clear advantages in production potential, which is consistent with the results reported by other authors (Stanojević et al., 2022; Petrović et al., 2009; Pantelić et al., 2021).

Regional differences in the milk performance of Simmental cows are due to variations in feeding systems, selection intensity, and management practices. In Serbia, as in many other countries with diverse production environments, feeding conditions vary among regions. Areas with more developed dairy production and better access to high-quality forage and concentrates generally achieve higher milk yields and component contents. Differences in selection programmes and breeding goals also contribute to regional variation. In regions where breeding organisations implement more systematic selection of bull dams based on milk performance records, genetic progress tends to be faster. Overall, the observed regional variability highlights the importance of standardising management and selection practices while considering local, environmental and economic conditions. Adapting breeding and feeding strategies to regional resources can improve both the efficiency and sustainability of Simmental bull dam production.

Conclusion

The results of this study provide an overview of the characteristics of milk production in the first lactation of Simmental bull dams in Serbia. The average milk yield of 6093 kg together with the corresponding fat and protein contents show that the selected bull dams represent a high-performing segment of the population in Serbia. Compared to previous studies, especially on Simmental populations in other countries, it can be concluded that there is a significant potential for improving the production traits of this breed in Serbia.

The analysis revealed statistically highly significant differences between the districts ($p < 0.001$) for all analysed traits, which underlines the importance of regional factors such as feeding systems, housing conditions and herd management for production performance. The highest average milk yield and fat yield were recorded in Nišava and Zaječar districts, while the highest protein yield was observed in Zaječar and Šumadija districts, respectively. These results highlight the potential for further genetic and management improvements by selecting better cows and improving production conditions in lower yielding districts.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this study.

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FIVE-YEAR OVERVIEW OF THE SIMMENTAL CATTLE POPULATION IN CENTRAL SERBIA

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Abstract: Serbia has a high potential for livestock production, which is suitable for breeding dual-purpose Simmental cows, both for milk and meat production. High quality animals with strong breeding potential registered in the main herd book are monitored for their production performance, reflecting the national authorities' commitment to the cattle sector. According to the reports of the Institute for Animal Husbandry and the results of the implementation of the main breeding programme in the past five-year period (2024–2029), not only the number of cows, but also the total number of all categories of Simmental cattle in the main herd has decreased in the territory of Central Serbia. Selection process in milk production is carried out through continuous cooperation between farmers-breeders of high-quality breeding cattle - and local breeding organisations, both at local and regional level. In central Serbia, there are over 150 registered local breeding organisations that take on the tasks of recording/registering and selecting the cattle in the herds. In this study, data from the main breeding organisation for livestock farming for the area of central Serbia, the Institute for Animal Husbandry in Zemun, and its annual reports were used. The tabular analysis and the five-year trend of animal numbers are presented according to the number of registered animals, the number of linearly evaluated cows after the first calving and the achieved lactation yield of milk, milk fat and milk protein (standardised to 305 days). Based on the analysed and discussed results of the selection work on Simmental cows, it can be concluded that there has been some progress in milk production, but also a reduction in the number of individuals. It is important to emphasise that these results have an impact on the overall productivity of the cattle population in central Serbia.

Key words: Simmental breed, herd book cows, milk lactation production, Central Serbia

Introduction

Livestock farming is one of the most important branches of agriculture in the Republic of Serbia, alongside arable farming. Since ancient times, livestock farming has had a long tradition in Serbia, most of which was more extensive than it is today. Geographically, the area of central Serbia (the Republic of Serbia excluding the provinces of Vojvodina and Kosovo), characterised by moderate hills, fertile farmland and natural pastures, is suitable for this type of agricultural production. The Republic of Serbia is a country where the Simmental cattle breed is well adapted and has been the predominant breed for over a century. It has been used for crossbreeding with indigenous cattle breeds, which resulted in a large part of the former domestic cattle population being replaced by the Simmental breed. The breed expanded primarily through the importation of heifers, breeding bulls, and semen from Switzerland, Austria, and Germany. Today, it represents the most numerous cattle breed in the Republic of Serbia, comprising over 75% of the total cattle population. The herd book in Serbia was established in 1935 and is currently maintained at the Institute for Animal Husbandry in Zemun, Belgrade (GOP, 2019).

The Law on Animal Husbandry of the Republic of Serbia defines the primary breeding objectives as increasing the productivity of domestic animals, improving the breed composition and preventing a decline in animal numbers. In 2024, the Institute for Animal Husbandry in Zemun published the main breeding programme for cattle, specifically for the Simmental breed, which covers the period 2025–2029. Based on the current situation in Simmental cattle breeding, the organisational, technical and economic capacities of cattle production and in accordance with the basic principles of genetics, the breeding goals for the controlled population of Simmental cattle-with a maximum of 25% genes of dairy breeds (RHF and Mb) in central Serbia are aimed at to establish a Simmental population whose animals are profitable, highly productive, dual-purpose (milk-meat), with high daily gain, good health and fertility and an increase in the size of the main herd (GOP, 2024).

Materials and Methods

The study presents data from the Reports of the main breeding organisation for the area of central Serbia – the Institute for Animal Husbandry in Zemun. The reports for the past five years (2020–2024), which contain the results of the implementation of the Main breeding programme, define the basic selection

measures, procedures and methods of selection and the way they are implemented and controlled for the Simmental breed. The three main selection measures presented in this study are: the number of animals in the selection trial, i.e. the number of animals under control; the number of linearly evaluated first-calving heifers as newly registered cows; and the number of completed lactations per cow per year (Reports, 2020; 2021; 2022; 2023; 2024). In the structural organisation of the Main breeding programme of the Republic of Serbia, a pyramidal system is used for selection work. At the base of this system are the breeders of high-quality breeding cows, followed by the assistants who carry out direct measurements and sampling of the animals in the so-called primary organisations, then the regional organisations at the level of the administrative districts into which Serbia is divided, up to the highest main organisation, which analyses all the results and data obtained from the lower levels of the pyramid system.

The main organisation prepares the final report each year, which is then submitted to the competent Ministry of Agriculture, which is at the top of the pyramid system mentioned above. The methodology for monitoring the number of animals, their productivity and other performance indicators is in accordance with the provisions of the Law on Animal Husbandry of the Republic of Serbia (Law on Animal Husbandry, 2009, 2012, 2016) and the rules of the International Committee for Animal Recording (ICAR). The Main Register – Herd Book (HB) for the population of the Simmental breed contains data on the total number of registered animals controlled by the implementation of the Main breeding programme, including their productivity and known pedigree, as well as the proportion of newly registered versus previously registered animals in relation to the total population. It also includes data on registered bulls used for the widespread breeding of cows. Checking the productivity of cows registered in the main herd book includes the results for milk yield, milk fat and milk protein as well as the linear evaluation of the first-calving Simmental cows.

Results and Discussion

Based on the five-year overview of the number of registered Simmental cows, Table 1 shows the absolute values of cows under the control of the Main breeding programme for the area of central Serbia (the Republic of Serbia without the provinces of Vojvodina and Kosovo). There is a clear downward trend in the number of cows, from a maximum of 169,378 in 2020 to a minimum of 138,432 in 2023.

Table 1. Number of registered animals of Simmental breed in a five-year period (2020-2024)

Breed	Number of registered animals				
	2020	2021	2022	2023	2024
Simmental	169378	163183	151538	138432	143160

A similar trend as in the previous table, namely a decline in the number of newly registered first-calving cows, can also be observed in Table 2. The number of young cows, which are primarily used for herd renewal, declined from 23,269 in 2020 to a minimum of 18,221 in 2023. After 2024, an increase in the number of cows is expected due to more favourable agricultural policy measures and the subsidisation of breeding animals.

Table 2. Number of first calving females of Simmental breed with linear assessment scores in the five-year period (2020-2024)

Breed	Number of first calving females with linear assessment scores				
	2020	2021	2022	2023	2024
Simmental	23269	22430	20310	18221	19321

Considering the production indicators- milk yield, milk fat and milk protein, Table 3 shows the number of completed lactations of first-calving cows, which increased slightly in 2024 compared to the previous year. The trend of increasing lactation milk yield on an annual basis (305-day lactations) is consistent, although the number of cows has not increased. Milk fat yield is developing slightly better than protein yield. Quality, genetic potential and selection progress are therefore evident, even though the number of lactations and cows has decreased. In addition, there is a trend towards farm consolidation in the Republic of Serbia, where small farms with 1–2 cows are being phased out, while farms with several dozen cows are expanding into larger herds. The decline in the number of producers keeping 1–3 dairy cows has also been reported by other authors (Perišić et al., 2012; Pantelić et al., 2021). Nikšić et al. (2011) reported that the average milk yield of Simmental cows was 4,348 kg with 3.93% milk fat. Stanojević et al. (2022) reported an average milk yield of 5,052 kg with 3.92% milk fat. Stepić et al. (2018) found that cows produced an average of 6,659.9 kg of milk with 4.09% milk fat and 3.40% protein in a standard lactation. In their previous study, Mičić et al. (2022) reported an average daily milk fat content of 4.04% and a protein content of 3.23% for the cow population in central Serbia.

Table 3. Production of milk, milk fat and protein of Simmental breed in the five-year period (2020-2024)

Year	Breed	Number of completed lactations	Milk yield (kg)	Milk fat content (%)	Milk fat yield (kg)	Protein content (%)	Protein yield (kg)
2020		99483	4977	3.98	199.77	3.21	158.47
2021		99718	5045	3.99	202.51	3.23	162.16
2022	SIM	94347	5060	3.98	203.64	3.23	158.75
2023		87029	5092	4.01	204.09	3.21	163.64
2024		91772	5159	4.03	207.98	3.22	166.07

Conclusion

The number of registered cows in central Serbia registered in the main herd book increased slightly in 2024 compared to 2023, although the total number of cattle of all categories in the country decreased. Based on the analysis of the results of the selection work over the past five-year period, it can be concluded that some progress has been made in the milk production of the registered Simmental population in terms of yield and milk quality. However, looking at the breeding goal set for the registered Simmental population in central Serbia, these goals have not yet been achieved (an average of over 6,500 kg in 305 days, with 4.10% milk fat and 3.60% milk protein).

Although considerable efforts have been made in selection to improve the genetic potential of the animals, optimal results also require improvements in environmental conditions-microclimate, balanced feeding according to the needs and categories of cows, housing conditions and health care which are not easy to achieve under the current socio-economic conditions of the country. However, as milk production is a strategically important sector of agriculture in the Republic of Serbia, the proper implementation of the defined goals of the breeding programme must continue in the coming period. An important feature of the new breeding goals for 2025–2029 is the inclusion of important non-productive traits such as health, fertility, life expectancy and longevity as well as temperament.

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Conflict of interest

The authors declare no conflicts of interest.

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PERSPECTIVE DUAL-PURPOSE AND BEEF BREEDS ASSOCIATED WITH INCREASED BEEF PRODUCTIVITY IN CATTLE

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Abstract: A large part of the beef in Bulgaria comes from dairy breeds – about 40% of the total amount. Industrial crossbreeding is also used, with different bloodlines and types of crossbreeds being sold on the market. The main patterns in beef production have been identified, and the biological characteristics of cattle as meat producers have been analyzed. Some perspective, combined, and beef breeds are examined that can contribute to increasing beef productivity in the species and categories of cattle and solving the problem with beef in Bulgaria as a whole. A review of the technologies for breeding three dual-purpose and three beef cattle breeds in this regard is provided. Historical background, distribution, morphological and breed characteristics, productive types, weight development and growth, exterior, milk and meat productivity are described. Emphasis is placed on problems in the development of modern beef cattle breeding.

Key words: breeds, meat, combined direction, exterior

Introduction

Meat, mainly muscle mass, is used for food. Most of the meat consumed by humans is produced from farm animals, poultry, fish, game, and shellfish, which are specially raised for this purpose on farms and slaughtered, cut up, and processed in slaughterhouses and slaughtering centers. Different cuisines around the world use meat from different animals. There are significant cultural, religious, and social differences that determine attitudes toward the consumption of different types of meat. Some people do not eat meat at all. Artificial meat also exists and is produced.

A large part of the beef in Bulgaria comes from dairy and dual-purpose breeds – about 40% of the total amount. Industrial crossbreeding is also used, with various types of crossbreeds being sold on the market. There are about 280 beef farms and holdings in Bulgaria, raising 116,196 beef heifers and cows, or 34.8% of the total number of cows in the country.

The inclusion of beef and veal in our diet is primarily related to natural and economic factors and the utilization of feed resources—bulky, coarse, waste feed, and highly productive pastures (Darzhonov, 1987).

Exposition

Breeds are a source of labor in agricultural production. Improving them increases and reduces the cost of cattle production and increases labor productivity in animal husbandry, thereby creating added value. Individual types of farm animals involve a significant number of breeds. For a long time, cattle breeding worldwide was dominated by dual-purpose breeds. These breeds of cattle for milk and meat are specialized in terms of the time it takes to realize their genetic potential. Such breeds are:

Simmental

The breed was developed in the Sime River valley, Bernese Oberland region, Switzerland—a country with wide mountain pastures and rugged terrain. Cattle there are taken out in mid-spring and remain outside until late autumn. They make very good use of pasture and roughage. Cattle of this breed are calm and easy to raise. Between 46 and 60 million Simmental cattle are bred on six continents around the world. The breed is dominant in Austria, Serbia, Croatia, the Czech Republic, Slovenia, Slovakia, Romania, and others. In Austria alone, 1.6 million Simmental cattle are raised. The breed belongs to the red-and-white, broad-headed breeds. The color is red and white with shades ranging from dark red and brown to light beige. The head is always white, which is inherited when crossed with other breeds (Holstein, Black Pied, Brown Cattle, Grey Cattle) (Markov et al., 2021). The nose mirror and mucous membranes are bright pink. The horns are large, lyre-shaped, yellow, and with dark tips. The hooves are yellow and healthy. There are two productive types that are selected: meat-milk and milk-meat. In Europe, the milk-meat type is more popular (Markov et al., 2024).



Figure 1. Simmental cow owned by Rimsa-Troyan (picture by Rimsa – Troyan)

The Simmental breed belongs to the large cattle breeds. Cows have a height at the withers of 138-140 cm, and bulls have a height at the withers of 142-146 cm. Cows weigh 650-700 kg, and bulls weigh 1000-1100 kg. Calves are born with a live weight of 40-45 kg. At 6 months, they reach 180-195 kg. At 12 months, the live weight achieved is 300-340 kg for females and 400-450 kg for males. At 18 months, bulls weigh 480-520 kg. The average daily weight gain is 1380-1420 g, and the slaughter yield is 58-62%.

The meat is lean and crispy and is highly valued by consumers. The fertility of the breed is 92-95%. Milk productivity is 5500-6600 kg of milk, with 3.9-4.4% fat and 3.4-3.9% protein. Some herds achieve 10,000-12,000 kg of milk. The recommended technology is free-range grazing. The breed record holder is the cow GS VOLT, which in 2016 achieved 18,808 kg of milk, with 5.58% milk fat and 3.31% protein. Simmental has long been introduced in Bulgaria and is successfully bred at the Experimental Base of RIMSA– Troyan, producing boutique dairy and meat products with good added value, which are sold locally.

Norman cattle

The Normandy cattle came about in Normandy in the early 19th century as a result of systematic crossbreeding of local dairy breeds: Ogeron, Cotentin, and Caouchaise (now extinct) with the Shorthorn breed (dairy type), which began to be imported from the United Kingdom in 1836. The aim was to improve physique and early maturity. The breed's stud book was established in 1880, and breeding stock

testing began in 1932. The breed was almost wiped out as a result of large-scale military operations in the area where it was distributed and during World War II.

In the 1960s, the breed began to slowly come back and reached 4.5 million. Right now, there are about 3 million Normandy cattle in France. Normandy cattle have been exported to many countries around the world for a long time, and the breed is present on all continents. There are large populations of Normans in Colombia, Brazil, Ecuador, Paraguay, Uruguay, Venezuela, etc. In Brazil, it is successfully used for hybridization with Zebu, and the resulting hybrid is called Normanzu. It is found in the USA, Australia, and Japan. In Europe, it breeds well and has been acclimatized and approved in Belgium, Switzerland, Great Britain, Ireland, Germany, the Czech Republic, Slovakia, Romania, Bosnia, and Herzegovina. Purebred Normans are also found in Senegal, Mali, and Madagascar.

Table 1. Milk yield, milk fat and protein content in milk from Normande cows for 100 and 305 days of lactation

Indicator	Norman cattle	
	Katarino Farm (I group) n=10	Farm ZP V. Vasilev (II group) n=10
For the first 100 days of lactation		
Dairy,kg	2876.48 ± 212.95	2468.92 ± 121.21
Dairy fat,%	4.88 ± 0.21	4.49 ± 0.16*
Dairy butter, kg	140.37±0.09	112.01±0.12*
Dairy protein,%	3.68 ± 0.11*	3.51 ± 0.9
Dairy protein, kg	105.85	-
305 days lactation		
Dairy, kg	5994.16 ± 187.23	5797.25 ± 191.46
Dairy fat,%	4.53 ± 0.12**	4.21 ± 0.10**
Dairy protein,%	3.57 ± 0.10*	3,49 ± 0.11

P<0.05*, P<0.01**

Table 1 shows the results for Normandy cows raised and acclimatized on two farms in the foothills of Bulgaria. The representatives of the Katarino farm showed better results in eight parameters studied.



Figure 2. Norman cattle (picture by Rimsa – Troyan)

Norman cattle are large animals with good musculature. The height at the withers of cows is 140–144 cm, and that of bulls is 150–155 cm. The chest circumference varies from 209 to 245 cm. Cows usually weigh 700–800 kg, and bulls 1000–1100 kg. Calves are born easily, with males weighing 40–45 kg and females 35–40 kg. The head is white, almost always with dark, spotted eyes. The mucoid membranes are dark, gray-black. The neck has no dewlap. The back is broad and straight, the pelvis is broad and deep, and the thighs are muscular and strong. The udder is voluminous, set high between the hind limbs. The teats are symmetrically positioned and of medium length. Their skin is white, and their coat is red-spotted or spotted (patches), which can be black, gray, or brown (tiger stripes). Sixty-nine coat color variations have been described.

The breed has a long life time, with a minimum of 5 lactations. It shows good adaptability to sunlight and extremely high temperatures. The cows adapt well to machine milking. The average milk yield of the Normandy breed is 6000–6500 kg with 4.25% fat and 3.54% protein. Under selection control in France, there are 210,050 cows with an average milk production of 7469 kg, 4.29% fat and 3.61% protein. The record holder for the breed is the cow Champion, which produced 12,619 kg of milk with 3.89% fat and 5.01% protein.

The milk of Normandy cattle is considered the best in Europe for cheese and butter production in terms of its physical and chemical composition, nutritional value, and anti-allergenic properties. It is used to produce famous cheese brands such as Camembert, Livaro, Pont l'Évêque, Neufchâtel, and others. The local

qualities of this promising French breed are also not to be overlooked. The meat of Normandy cattle is high-quality, crispy, and partially marbled, and is used to produce boutique meat products with good added value. The recommended technology is free-range grazing (Markov et al., 2022).

Normandy sheep are currently bred in two locations: at the Katarino company in the village of Slivovitsa, Kresna municipality, and at the farm of agricultural producer Vasil Vasilev in the town of Kaspichan.

Red cattle breeds

Red cattle breeds are concentrated and bred mainly in Scandinavia, the Baltic States, the steppe regions of Eastern Europe, and Central Asia, and are characterized by relatively good milk productivity. These breeds are specific in their genetic similarity, which is the result of heredity, mainly from the Angler breed. Over the years, there has been an opportunity for continuous genetic drift without changing the type of any of them.

These breeds combine high milk yield with high protein and fat content in milk. They are known for their good health, longevity, early maturity, fertility, efficient utilization of roughage, relatively good meat productivity, and high adaptability, which make them still effective in certain regions of Europe and the world.

Danish Red cattle

Danish Red cattle is the second most common breed in Denmark after Holstein. It was developed through complex three-way crossbreeding and intensive selection. It is a dual-purpose breed. Its milk has excellent taste and technological qualities. Due to inbreeding in the past, it was necessary to inject blood from the Brown Swiss breed years ago. The animals are compact with a strong physique. The head is elongated and delicate. The nose mirror is gray in color. The neck is elongated. The legs are of medium length. The back and chest are broad. The udder is cup-shaped with cylindrical teats.

The live weight of cows varies from 550 to 800 kg, and that of bulls from 950 to 1000 kg. In 1988, the average milk yield of the evaluated cows was 6752 kg, with 4.17% fat and 3.45% protein. Nowadays, the breed produces 9000 kg of milk, with 4.1–4.2% fat and 3.2–3.5% protein. The Red Danish breed was used in the formation of the Bulgarian Red cattle breed. The recommended technology is free-range grazing. About 120 animals of various categories are raised on the Argirovi farm in the town of Saedinenie, Plovdiv Province, and boutique dairy and meat products are sold on the market (Markov and Hristov, 2021).

Bulgarian red cattle

Bulgarian red cattle is a breed that was widely bred until 35 years ago in northeastern Bulgaria, in the former districts of Ruse, Targovishte, Shumen, Silistra, Dobrich, and Varna. Prominent Bulgarian scientists such as Prof. Petko Komitski, Prof. Nikola Palagachev, Prof. Ivan Balabanov, Prof. Emilia Ivanova, Prof. Stefan Kostov, and others have worked to consolidate the breed in our country. Prof. Petko Komitski, Prof. Nikola Palagachev, Prof. Ivan Balabanov, Prof. Emilia Ivanova, Prof. Stefan Kostov, and others. Currently, it is bred on a limited basis by enthusiasts who are committed to preserving the breed in the area around the towns of Aksakovo (Varna region) and Veliko Tarnovo.

The Bulgarian Red cattle is covered with a uniform reddish-brown coat with lighter or darker shades in individual hairs. The nose mirror, eyes, anus, and vulva are dark in color. Bulls have more intense coloring. The horns are light with dark tips. The udder is light red. The average height at the withers of cows is 128-130 cm, the live weight of cows is 520-530 kg, and that of bulls is 800-900 kg. Female calves weigh an average of 32-34 kg at birth, 160-170 kg at 6 months, 270-290 kg at 12 months, and 360-370 kg at 18 months. Bull calves subjected to intensive fattening at 12-15 months reach a live weight of 450-475 kg, depositing a lot of fat in the pelvic area and internal organs. Cattle of this breed are of the dairy type with the characteristic body shape and proportions. The head is narrow, long, lean, well-shaped, with skin folds on the forehead and around the eyes. The ears are large, the horns small.

The neck is medium long and narrow. The dewlap is small. The withers are sharp, poorly muscled, and slightly exceed the back line. The back and loins are long, medium wide. The back line is level or slightly arched. The chest is medium deep, flat with sloping ribs and wide intercostal spaces. The belly is voluminous but not pendulous. The croup is well developed, long, and level. The tail is long, thin, ending in a long tuft of black hair. The legs are well placed, with delicate bones, well-formed joints, and healthy hooves. The skin is delicate, mobile, and elastic. It forms numerous delicate folds on the neck. The musculature is poorly developed. The body is dry, angular, with empty thighs, and a clearly defined skeleton. About 25 animals are monitored. The monitored animals show a milk production of 6500 kg and 3.4% fat and 3.3% protein. The recommended technology is free-range grazing. The breed is threatened with extinction (Markov and Hristov, 2021).



Figure 3. Bulgarian red cattle (picture by Rimsa – Troyan)

Specialized beef breeds offer higher meat content per kilogram of live weight and high slaughter yield of around 69-72%. Beef cattle farming requires less initial investment compared to dairy farming. The rise of culinary culture, the popularization of foreign national cuisines, and increased interest in gourmet food have led to growing interest in high-quality beef. For the Eastern European region, a choice must be made between breeds that have already proven themselves worldwide. Numerous experiments show that high-intensity meat breeds have higher daily weight gain and greater slaughter weight. At the same time, medium-intensity breeds are characterized by higher reproduction rates. These are the breeds we present to you (Markov and Hristov, 2021).

Hereford

The Hereford cattle breed was developed in a hilly region with rich vegetation and long periods of grazing. This has contributed to the development of a healthy constitution, excellent adaptability to breeding conditions, disease resistance, high maturity, very good meat qualities, good maternal qualities, and high reproductive capacity. Hereford is a typical representative of the English early maturing specialized beef breeds.

The coat is red, with a white head, neck, throat, and lower chest, lower limbs, and tail tip. This colouring of the Hereford is dominantly inherited in offspring, even when crossed with other breeds.



Figure 4. Hereford cattle (picture by Rimsa – Trojan)

The head is broad, with medium-length, thick horns. A hornless Hereford breed was developed in Canada, from which around 2,000 animals were imported to Bulgaria in the 1980s and acclimatized, including at RIMSA-Troyan. The neck is short, well muscled, and the chest is deep, broad, and barrel-shaped, with a well-developed sternum. The limbs are short, widely spaced, and strong. The skin is thick and loose, covered with long, coarse, sometimes curly hair. The average live weight of cows is 600–750 kg, and that of bulls is 800–1000 kg. The weight of calves at birth is 30–32 kg, and at weaning at 7–8 months of age – 170–200 kg. Young animals have high growth and fattening capacity. At 18 months of age, heifers reach 430–450 kg.

The slaughter profitability is relatively high – 60–65% when fattened to 500 kg live weight. Young animals accumulate a lot of fat, which impairs the quality of the meat. Hereford beef is high quality, pink in color, well marbled with fat, tender, juicy, and tasty. When animals are fattened on pasture, the meat is produced without excessive fat deposition (Markov and Hristov, 2021).

Galloway

The breed originates from Scotland. Selection methods and conditions – natural selection. Breeding status since 2017. The Galloway breed is one of the oldest beef cattle breeds. They are known for their resilience and can live on the most nutritionally poor land and soil. The breed is characterized by high absorption

of grass feed. They have an extremely calm temperament and high resistance to disease and climate change. The animals are relatively small, compact and sturdy, with a harmoniously developed constitution. The body is cylindrical, with rounded shapes. The head is short and broad with a straight profile line, large eyes, and broad noses turned forward and upward, with strongly hairy ears. Galloways are naturally hornless. The neck is of medium length and well muscled. The withers are short, muscular, and slightly distinguishable. The croup is moderately broad, muscular, and straight. The backline is straight. The animals have a strong bone structure, well-developed musculature, strong legs, and hooves. The most visible characteristic of Galloway cattle is their long fur, consisting of two layers. The outer layer has longer and coarser hairs designed to protect the animal from wind and rain, while the second layer provides good thermal and waterproofing. The color of the coat is unusual, uniform—from white, light brown, brown to brown-black, to black. There are three main varieties of Galloway cattle according to colouring (Yarkov, 2022). Bulls weigh 800–950 kg and cows weigh 450–550 kg. Calves are born weighing 27–29 kg. At 7–8 months, they weigh 180–205 kg, and at 15–18 months, 350–400 kg. The average daily weight gain ranges from 850 to 1200 g. Fertility is high at 96.4%. The intercalving period varies from 340 to 390 days. Calving is easy and does not require medical assistance. The slaughter efficiency is 60–65%. The meat is marbled with pronounced fat layers.

Meat quality is one of the most important characteristics of the Galloway breed. Studies show that beef from the Galloway breed is low in total and saturated fat. It has a high content of beneficial Omega 3 and Omega 6 fatty acids, making it significantly healthier than pork and as good as chicken and salmon. Veal is lean but well marbled. The recommended technology is free-range, pasture-raised. Gaul cattle are raised on several farms in Bulgaria, with the largest herd being at the Training Center of Trakia University in Stara Zagora, numbering around 120 animals. Since 2024, six heifers and one bull have been available to scientists from RIMSA-Troyan for scientific experiments related to industrial crossbreeding.

Wagyu

Wagyu means Japanese cattle in Japanese. ("wa" means Japanese and "gyu" means cattle). They are mainly bred in the Kobe area of Hyogo Prefecture. In practice, the breed is often incorrectly referred to as Kobe. For centuries, this breed has been used for pulling power (harness). During the period 1635–1868, the population was practically "closed," with export and import prohibited by order of the shogun.

It is only after 1868 that the Japanese, under foreign influence and despite their Shinto traditions, began to consume beef. At that time, Brown Swiss,

Shorthorn, Devon, Simmental, Ayrshire, Angus, and Korean cattle were imported into the country, which had an impact on the consolidation of the breed. In 1910, crossbreeding was discontinued and purebred breeding was adopted. The breed was recognized in 1944. In 2017, 1.6 million Wagyu cattle were raised in Japan by 5,010 farmers. Since 1997, the breed has been considered a national treasure of Japan. Zenva is a government organization in Japan that strictly monitors the breed registry. The animals are extremely gentle, with a calm (peaceful) temperament and are suitable for pasture breeding (Markov et al., 2022; Markov et al., 2024; Hristov et al., 2024).

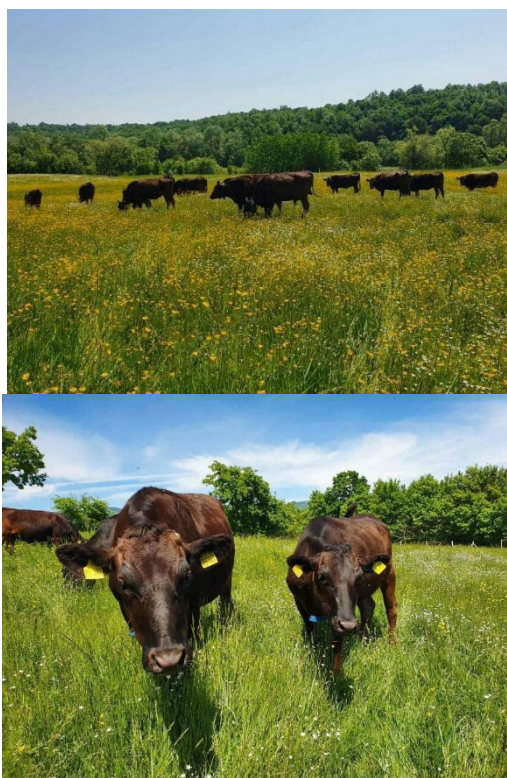


Figure 5. Wagyu cattle (picture by Rimsa – Troyan)

Wagyu show good adaptability. The cattle are medium-sized, well-muscled, with a fine bone structure and a well-developed constitution.

Table 2. Measured exterior body measurements of first-calf heifers, Wagyu in cm, ($X \pm s$) at the farm in the village of Palitsi, Elena municipality

Measurement	n=20	Standard (according Motojama et al.,2016) MAAF,2015, National Beef cattle Advancement Fuund Assotiation
	X $\pm$ s	Standard
Height at withers, cm	128.25 $\pm$ 1.34*	127-129
Height at the crotch, cm	129.6 $\pm$ 0.72**	128-131
Chest depth, cm	66.9 $\pm$ 1.72	65-69
Chest width, cm	45.9 $\pm$ 0.41***	44-47
Chest size, cm	188.1 $\pm$ 1.56	185-192
Athwart body length cm	158.4 $\pm$ 0.52*	155-160
Range of the whistle, cm	14.83 $\pm$ 0.28***	14-16
Width of the croup in the hindquarters,cm	41.5 $\pm$ 0.62*	40-42
Width of the tubercle in the ischial tuberosities, cm	39.8 $\pm$ 0.22***	40-42
Head length, cm	31.5 $\pm$ 0.31*	30-32
Head width, cm	17.6 $\pm$ 0.28	16,5-18
Half-range of the piston, cm	27.4 $\pm$ 1.12	27-28
Live weight, kg	574.8 $\pm$ 3.57	520-600

P<0.05*, P<0.001**, P<0.0001**

The height at the withers of cows is 100–129 cm, and that of bulls is 135–140 cm. The horns are straight, slightly curved forward, and end with a white tip. They have black, red, and brown skin and coat colors and shiny black nostrils. There are four black breeds, which make up 90% of the population. These are the Totori, Tajima, Shimane, and Okaima breeds. There are two red breeds—the Koma and Kumamoto—and one brown breed, which is genetically related to Korean cattle and the Simmental breed. The live weight of cows is 700 kg, and that of bulls is 950 kg. The low birth weight of calves (30 kg for females and 40 kg for males) allows for relatively easy calving. Bulls cover up to 50% more females compared to other breeds. Heifers are inseminated at 12 months. During the period from 11

months to 29–30 months, their diet includes highly concentrated feed, roughage, and beer. Individual massages are also performed to relieve stress and muscle stiffness. They are fattened until 28–30 months of age. At 29.2 months, the fattened male calves reach 576–756 kg, with an average daily weight gain of 0.770 g. Kobe beef is meat produced from male cattle, while Matsuzaka beef is meat produced from female cattle. The slaughter profit is 65%. The carcass of a male Wagyu consists of 47.7% muscle, 41.7% fat, and 10.6% bone. The meat is juicy, tender, marbled, and layered with specific fat. This fat runs through the muscles in thin lines (stripes) and gives the meat its characteristic taste and aroma. The recommended technology is free-range, pasture-raised (Markov et al., 2022).

Wagyu of different categories are raised on a farm in the village of Palitsi, Elena municipality, with about 100 animals, and on a farm in the village of Dalbok Dol, Troyan municipality, with 25 animals.

Conclusion

A significant part of the beef in Bulgaria comes from breeds with a combined purpose – about 40% of the total quantity. Industrial crossbreeding is also used, with various types of crossbreeds being sold on the market.

Currently, there are about 280 beef farms and holdings in Bulgaria, raising 133,100 beef heifers and cows, or 24.05% of the total number of cows in the country. The majority (around 85,000) are purebred cows with pedigrees from beef breeds, mainly English and French, while the rest are high-blooded crossbreeds.

Approximately 80% of farmers engaged in beef cattle farming are members of the two officially registered beef cattle associations. The average area used per beef farm in the country is approximately 2,500 decares.

The essence of the problem is the establishment of beef cattle farms with precisely defined breed zoning, territory, and number of animals, which are able to provide both grazing and forage and succulent fodder during the winter.

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CHESTNUT TANNINS IN LAMB NUTRITION: BALANCING PROTEIN UTILIZATION AND FEED EFFICIENCY IN SUSTAINABLE FATTENING SYSTEMS

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Abstract: Tannins are complex plant-derived polyphenols that are increasingly being investigated in ruminant nutrition due to their protein binding capacity, bioactive properties and potential to support sustainable production goals. They can modulate fermentation in the rumen, reduce protein degradation and alter the dynamics of nutrient absorption dynamics — but their dual role as functional and potentially antinutritive agents requires differentiated evaluation. This paper discusses the broader functional importance of tannins in ruminant diets based on a comprehensive literature review, focussing on their mechanisms of action, influence on digestibility, animal performance and metabolic responses. Within this framework, a practical model of lamb fattening will be analysed to investigate the controlled use of chestnut tannins under different feeding conditions and protein balances. The model serves to illustrate the general challenges of implementing tannin research in commercial feeding systems. The importance of dosage, tannin type, feed matrix and animal-specific factors that determine efficacy and safety is emphasised. Whilst the integration of tannins offers promising routes to nitrogen efficiency and reduced environmental impact, practical implementation requires a clear understanding of the economic trade-offs and variability at farm level. The findings presented will serve as a basis for future research designs and practical implementation strategies for plant additives in small ruminant diets.

Key words: tannins, lamb fattening, protein utilization, feed efficiency, sustainable livestock

Introduction

Tannins are naturally occurring plant secondary metabolites that belong to the broader group of polyphenolic compounds. They occur widely in trees, shrubs and legumes and have traditionally been regarded as antinutritional due to their astringency and protein-binding properties. In the last decades, however, due to their potential to modulate rumen fermentation and improve nitrogen utilization (Patra and Saxena, 2011; Hassanpour et al., 2011), studies emphasise their versatile role in ruminant nutrition for promoting animal health and performance, as well as environmental sustainability (Sharma et al., 2019). Furthermore, tannins can influence protein degradation, rumen microbial ecology and methane production (Decandia et al., 2011)

Among the various tannin types, hydrolyzable tannins (HT) derived from chestnut (*Castanea sativa* Mill.) have shown particularly promising effects when used as addition in rations (Mancini et al., 2019, Sun et al., 2025). Chestnut tannins are particularly rich in hydrolyzable forms, and represent a promising strategy to address protein utilization inefficiencies and mitigate nitrogen-related environmental impacts in ruminants (Hutchings et al., 2006).

Despite these advantages, certain knowledge gaps and several challenges remain unresolved. The dualistic nature of tannins-as both functional enhancers and potential antinutritional agents-raises concerns about their impact on digestibility, microbial balance, and animal health at higher inclusion levels (Barry and McNabb, 1999). The variation in tannin structure, concentration, and bioactivity across sources further complicates standardized application in commercial settings. Additionally, the lack of consistent labeling and formulation guidelines for commercial tannin products limits reproducibility across trials and hampers on-farm adoption.

This review aims to address these uncertainties by integrating experimental findings and literature-based insights into the effects of chestnut tannins in lamb nutrition. It highlights the biochemical nature of tannins, mechanisms of action, effects on fermentation, protein utilization, nutrient digestibility, evaluates their influence on growth performance and health of animals, and explores their role in environmental mitigation strategies. Furthermore, a practical fattening model is presented to illustrate how chestnut tannins perform under protein-deficient conditions. By exploring these themes, the paper provides a foundation for targeted research, precise dosing strategies, and informed implementation of tannin-based feed additives in small ruminant systems.

Classification and tannin properties

Although tannins can be classified in different ways, they are mainly divided into hydrolyzable (HT) and condensed tannins (CT), which are distinguished by their chemical structure and reactivity (Frutos et al., 2004a), as shown in Figure 1.

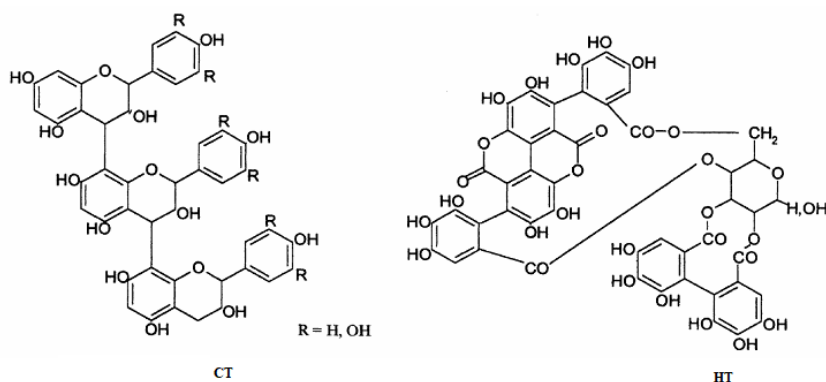


Figure 1. Tannin classification – CT i HT (Hassanpour et al., 2011)

CT, also known as proanthocyanidins, are polymers of flavan-3-ols and are mainly found in forage legumes such as sainfoin, birdsfoot trefoil, sericea lespedeza, sulla and others. HT, on the other hand, are esters of gallic acid or ellagic acid with with a sugar core (glucose) and are mainly found in trees such as oak, chestnut, acacia and others (Frutos et al., 2004a; Furlan et al., 2011). HTs are water-soluble and more susceptible to hydrolysis, while CT are oligomers or polymers of flavonoid units that are resistant to degradation (Furlan et al., 2011). However, in many cases one plant species can contain both groups of tannins (Besharati et al., 2022). Both types can bind proteins, but with varying affinity and stability depending on pH and molecular weight.

As mentioned, chestnut tannins mainly contain HT, notably castalagin, vescalagin, gallic ellagic acid, vescalin, castalin, and others, and often complemented by flavonoids such as rutin and quercetin (Braga et al., 2014; Soares et al., 2019). Their ability to form reversible complexes under rumen pH conditions

underlines their role in modulating protein degradation. Tannins have antioxidant and antimicrobial activity, with certain polyphenols contributing to gut health and potentially influencing rumen microbial populations. The exact composition of chestnut extracts can vary depending on processing, origin and standardization, highlighting the need for reliable characterization methods.

Sources and chestnut tannins extraction

Sweet chestnut (*Castanea sativa* Mill.) serves as both a traditional and industrial source of HT, which is mainly extracted from its bark and heartwood (Comandini et al., 2012). Modern extraction is carried out using aqueous or aqueous-alcoholic solvents to produce commercial formulations, among which are also Farmatan Plus® (Slovenia), Saviotan feed®, Silvafeed (Italy), Tannino50® (USA) and others.

However, the final composition and functionality of tannin extracts is influenced by several factors. These include the age of the plant, the seasonal and environmental conditions during harvest, the part of the plant used (wood vs. bark) and the extraction methods (Spina et al., 2013). Young trees can have different polyphenol profiles than mature ones, and stress and growing conditions (e.g. drought, pathogens) can increase their concentration. The solvent system, temperature and extraction time also influence the yield, reactivity and diversity of compounds (Ebrahim and Negussie, 2020) and as a result, even commercial HT extracts of the same botanical origin may differ in their bioactivity unless they are strictly standardized (Criminna et al., 2024).

This variability requires precise chemical profiling and validation using instruments such as HPLC-DAD-MS/MS, which enable the detection of important molecular constituents such as vescalagin, castalagin, gallic acid, vescalin, castalin and other compounds. Ensuring batch-to-batch reproducibility is critical to achieving predictable biological results in ruminant feeding trials. This consistency is critical for evaluating biological efficacy and determining effective supplementation levels in ruminant feeding systems (Das et al., 2020).

Mode of action in ruminant nutrition

The effect of tannins on ruminant performance is multifaceted and can influence a variety of factors, from feed intake to product quality (Figure 2). The most important nutritional effect of tannins lies in their ability to bind dietary proteins and protect them from microbial degradation in the rumen. This reduces ammonia production (Taha et al., 2022) and increases the proportion of rumen

undegradable protein (RUP) that reaches the small intestine, improving nitrogen utilization efficiency (Makkar, 2003; Barry et al., 2001). Tannins can also interact with cell wall polysaccharides (Hervas et al., 2003), enzymes (Horigome et al., 1988) and microbial membranes (Cannas, 2018) to alter nutrient availability and microbial colonization.

HTs from chestnut generally dissociate in the acidic post ruminal environment due to their reversible protein binding properties, releasing nutrients for enzymatic digestion. This enables better absorption of amino acids in the small intestine and supports improved protein efficiency. In addition, chestnut tannins have been shown to suppress methanogenic archaea and proteolytic bacteria, resulting in lower methane emissions and ammonia concentrations in the rumen (Jeronimo et al., 2016).

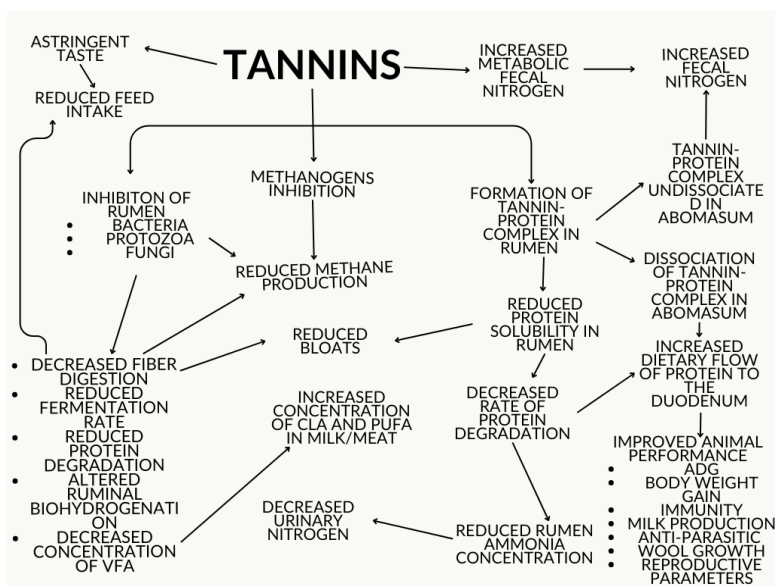


Figure 2. Tannin effects on ruminant performance

Experimental studies also show that chestnut tannins selectively modulate the rumen microbiota (Sun et al., 2025) and reduce the abundance of *Clostridium sticklandii* and other ammonia-producing species (Jayanegara et al., 2012). At the same time, tannins can reduce protozoan populations, which indirectly promotes bacterial protein synthesis by limiting the predation of rumen bacteria (Mueller-Harvey, 2006; Min et al., 2003).

These effects are strongly influenced by the type of tannins, the dosage and the overall context of the diet. However, excessive addition may impair fibre

digestion by inhibiting fibrolytic species such as *Ruminococcus albus*, as demonstrated in vitro and in vivo (Waghorn, 2008).

The efficacy of tannins also seems to depend on the adaptation of the rumen. Some studies report microbial tolerance or adaptation to moderate levels of tannin over time, suggesting that gradual inclusion of feedstuffs and monitoring of rumen parameters are key to optimizing benefits without compromising digestibility (Bueno et al., 2020). Therefore, understanding the nuanced interactions of tannins in the rumen is crucial for their effective and safe use in ruminant diets.

Effects on nutrient digestibility

Chestnut tannins affect digestibility results in a dose- and diet-dependent manner. Moderate use (up to ~20 g/kg DM) in lamb diets has been shown to reduce crude protein degradation in the rumen while increasing nitrogen fixation and overall protein efficiency (Mkhize et al. 2018; Sun et al., 2025). Some reduction in dry matter and organic matter digestibility may occur, especially at higher doses or in rations that already contain a low proportion of fermentable energy (Taghizadeh and Besharati, 2011).

In trials where lambs were fed diets deficient in metabolizable protein, supplementation with chestnut HT improved the apparent digestibility of ether extract and tended to stabilize nutrient utilization (Cekić et al., 2022). Also, fibre digestibility was maintained, supporting the idea that controlled HT levels are compatible with normal rumen function.

Although tannin supplementation may slightly reduce apparent digestibility of dry matter and crude protein, particularly at higher doses, these effects are often offset by gains in nutrient retention and utilization efficiency (Deaville et al., 2010a; Mezzomo et al., 2016).

Effects on protein utilization

Experimental studies show that tannins can improve the efficiency of nitrogen utilization in lambs by lowering rumen ammonia concentrations and improving the flow of undegraded dietary protein (Dentinho et al., 2014). At levels between 10 and 20 g/kg DM in low-protein rations, chestnut tannins increased nitrogen fixation and decreased blood urea concentrations, indicating improved postruminal nitrogen utilization (Deaville et al. 2010b; Ahmed et al., 2025). These results support the hypothesis that moderate tannin supplementation improves the

synchrony between rumen degradable protein and fermentable energy, allowing for greater microbial protein synthesis and more efficient nitrogen utilization.

In conditions of protein deficit, chestnut tannins improve the apparent digestibility of ether extract and may enhance microbial efficiency. The net outcome depends on the balance between protective protein binding and potential inhibitory effects on fibre digestion and enzyme activity (Barbehenn and Constabel, 2011). In a 60-day lamb fattening study, the group receiving 18.87 g/kg DM chestnut tannins had the highest protein efficiency (PER) and nitrogen conversion efficiency, despite lower metabolizable protein levels in the diet (Cekic et al., 2024). These lambs had more efficient incorporation of dietary nitrogen into body tissues, which was reflected in higher average daily gains and serum protein concentrations in the physiological range.

These effects appear to be particularly relevant in practice, as they offer the possibility of lowering total protein levels while maintaining performance. However, the effectiveness of chestnut tannins in improving nitrogen utilization depends on several factors, including the composition of the forage, the energy-to-protein ratio and the form and purity of the tannin source used. In trials with Farmatan Plus®, the bioactive profile of the extract was consistent, highlighting the link between standardization and efficacy in animal performance outcomes (Cekić et al., 2022; Cekic et al., 2024).

The influence of chestnut tannins on nitrogen metabolism is further supported by the reduction of nitrogen excretion in faeces and urine, which is consistent with the formation of tannin-protein complexes that are resistant to microbial degradation but can be dissociated in the acidic post ruminal environment (Deaville et al., 2010a). This mechanism facilitates the shift of nitrogen digestion from the rumen to the post-rumen, improving amino acid absorption in the small intestine and reducing nitrogen excretion.

Several studies have reported positive effects on growth and feed conversion in lambs following the administration of chestnut tannin. In a trial with MIS breed lambs, Farmatan Plus® was added at a dosage of 0, 9.46 and 18.87 g/kg DM to isocaloric and iso-nitrogenous feeds with a deficiency of metabolizable protein. During a 60-day fattening period, the lambs in the group with the highest tannin content showed significantly higher average daily weight gains (228 g/day), improved feed conversion rates (4.4) and increased protein efficiency rates (Cekić et al., 2022).

Influence on animal performance

Chestnut tannins, especially those derived from *Castanea sativa* and formulated in standardized extracts have shown significant potential to improve

growth performance and feed conversion in lambs, especially when diets are deficient in metabolizable protein. When chestnut tannins at 18.87 g/kg DM are added to such protein-limited diets, average daily gain (ADG) increases significantly to 228 g/day, feed conversion ratio (FCR) improves to 4.4 and both protein efficiency (PER) and Kleiber ratio increased, indicating better growth in relation to metabolic body weight (Cekic et al., 2024).

Chestnut tannins, when supplemented within recommended limits, do not impair dry matter intake and can improve growth parameters. In lambs fed rations deficient in metabolizable protein, supplementation with 20 g/kg DM chestnut tannins resulted in significantly higher average daily gain (228 g/day), improved feed conversion ratio (4.4 kg DM/kg BW gain), and elevated Kleiber ratio and protein efficiency ratios (Rivera-Mendez et al., 2017). These improvements are attributed to the improved synchronization of energy and protein supply in the post-ruminal tract and enhanced nutrient absorption.

These improvements are primarily attributed to improved post ruminal protein availability (Qi et al., 2022). The hydrolyzable tannins of chestnut (HT) form reversible complexes with dietary proteins at rumen pH and protect them from extensive microbial degradation (Gao et al., 2024). As these complexes dissociate under the acidic conditions of the abomasum and small intestine, amino acids become available for absorption, leading to more efficient nitrogen utilization and lean tissue build-up. Improved synchronization between available nitrogen and energy after rumination also supports optimal microbial and animal growth (Valenti et al., 2018).

In addition, studies confirm that moderate tannin intake does not affect dry matter intake (DMI), which is a crucial factor for maintaining growth in lambs (Baraza et al., 2005). These results are consistent with previous observations where CT from sainfoin and coltsfoot improved growth performance under grazing conditions (Barry et al., 2001; Min et al., 2003), providing further evidence that moderate levels of tannin can be beneficial in different feeding systems.

The presence of specific compounds such as vescalagin and castalagin— - major components of chestnut tannins— - was associated with improved rumen modulation, antimicrobial effects and better nutrient uptake, supporting the biological plausibility of the observed performance improvements (Furlan et al., 2011; Comandini et al., 2012).

These results support the strategic inclusion of chestnut tannins in lamb fattening systems, especially when nutrients are deficient. They also provide compelling arguments for further on-farm evaluations, particularly with regard to breed, production conditions and long-term results (Mueller-Harvey, 2006; Bueno et al., 2020).

Effects on health and metabolic parameters

Tannins have long been considered antinutritional or even toxic due to their adverse effects on feed intake, protein and mineral digestibility, and their potential to cause gastrointestinal irritation, hepatic and renal lesions, and in extreme cases, death (Decandia et al., 2011; Jeronimo et al., 2016). Their toxicity depends on dose, molecular weight, and adaptation of rumen microbiota, with hydrolyzable tannins being especially harmful if animals are not properly adapted (Chung et al., 1998; Jeronimo et al., 2016). Nevertheless, Sharma et al. (2019) concluded that moderate levels (up to 15,000 mg/kg for ruminants) are generally safe, though effects depend not only on tannin content but also on source, chemical form, interactions, and diet composition. In the numerous studies, inclusion of chestnut tannins in the diet of lambs has been shown to be metabolically safe and functionally beneficial under controlled conditions (Buccioni et al. 2017; Frutos et al., 2004b; Zimmer i Cordesse, 1996; de S. Costa et al., 2020; Min et al., 2019).

These results are critical given the well-documented concerns regarding tannin toxicity at high doses (Reed, 1995). The favourable metabolic profile observed with HT from chestnut supports its use in moderate amounts, especially in low-protein diets. In particular, serum urea concentrations were significantly lower in lambs receiving tannin, suggesting reduced rumen ammonia production and improved nitrogen retention — a result associated with improved protein utilization efficiency.

In addition to maintaining systemic health, chestnut tannins may also contribute to cell protection through their polyphenolic antioxidant effects. Compounds such as gallic acid and ellagic acid, which are abundant in *Castanea sativa*, exhibit strong free radical scavenging properties in vitro, and their inclusion in the diet could help to mitigate oxidative stress in intensively reared lambs (Furlan et al., 2011; Comandini et al., 2012). Although the direct antioxidant effect in vivo in lambs is still poorly understood, the correlation between reduced oxidative markers and tannin supplementation in other livestock species supports this hypothesis.

Furthermore, the antimicrobial and anti-inflammatory properties of chestnut tannins could provide additional benefits for gut health and immune modulation. These effects could be mediated in part by reduced exposure to rumen and intestinal pathogens, as has been observed with other tannin-rich feeds (Mueller-Harvey, 2006; Jeronimo et al., 2016). Although direct evidence in lambs is still limited, the observed improvement in performance indices without signs of metabolic disorders suggests an overall health-promoting effect.

Taken together, these results suggest that chestnut tannins, when used in nutritionally relevant amounts, do not pose a health risk and may provide metabolic

and immunological benefits in small ruminant production. However, further studies are needed to explore the long-term effects, especially under different physiological conditions such as gestation, lactation or disease susceptibility.

Practical applications, sustainability and considerations for implementation

Improved protein efficiency through chestnut tannin supplementation leads to reduced nitrogen losses via urine and faeces, thereby lowering ammonia volatilization and nitrate leaching (Ali et al., 2017). Additionally, these tannins selectively suppress methanogenic archaea in the rumen, resulting in lower methane emissions (Valenti et al., 2020; Jeronimo et al., 2016). Together, these effects enhance the environmental sustainability of lamb production and support circular bioeconomy principles by integrating plant-derived by-products into feed systems (Vasta et al., 2019).

The underlying mechanism involves the formation of reversible tannin-protein complexes in the rumen, which reduce ammonia release and increase amino acid availability in the small intestine. This protein-sparing effect enables reduced dietary crude protein input without compromising animal performance, offering both nutritional and ecological advantages. Chestnut tannins, sourced from wood-processing residues, thus represent a sustainable feed additive aligned with both productivity and environmental goals.

Nonetheless, the efficacy and safety of tannins depend on multiple factors. HT, like those in chestnut, differ from CT in chemical structure and post-ruminal bioavailability. Appropriate dosing—typically within the 10–20 g/kg DM range—is critical, as excessive levels may impair dry matter intake and digestibility. The dietary matrix, along with animal-specific traits such as age, physiological status, and adaptation period, further modulate the response to tannins.

Despite demonstrated benefits, on-farm implementation remains limited. Challenges include inconsistent product labelling, variability in extract composition, and insufficient farmer knowledge regarding tannin-protein interactions. Although tannins may reduce feed protein costs and nitrogen losses, their economic viability depends on the balance between tannin products price and performance outcomes. For successful adoption, clear supplementation guidelines and technical advisory support are essential.

Future research should focus on long-term field trials evaluating reproductive traits, product quality, and environmental impacts. Integrating rumen microbiome and metabolomic analyses will provide deeper insights into host-tannin interactions. Standardizing methods for tannin quantification and bioactivity

will enhance comparability between studies and support the development of precision supplementation tools tailored to specific production conditions.

Conclusion

Chestnut tannins represent a valuable strategy to improve protein utilization, growth performance and nitrogen efficiency in lamb production. Their reversible binding to dietary proteins helps to protect amino acids from degradation in the rumen and promote post-rumen absorption. Experimental evidence confirms improved nitrogen retention and growth on low protein diets when chestnut tannins are added in moderate amounts.

In addition to the nutritional benefits, chestnut tannins contribute to environmental sustainability by reducing urinary nitrogen excretion and methane emissions. Their extraction from wood processing by-products also strengthens their compatibility with circular bioeconomy models.

In practical application, however, careful attention must be paid to dosage, standardization of extracts and feed formulation. Variations in response due to animal factors and feed composition must be taken into account to avoid negative effects on intake or digestibility. When used correctly, chestnut tannins offer a promising route to more resilient and sustainable systems for small ruminants.

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Conflict of interest

The authors declare no conflict of interest.

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CHALLENGES IN BLUP-BASED GENETIC EVALUATION OF PIGS WITH HIGH LEVELS OF IMPORTED GERMPLASM

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Abstract: Accurate estimation of breeding values is essential for effective selection in pig breeding programs. The Best Linear Unbiased Prediction (BLUP) method integrates pedigree, phenotypic, and, when available, genomic information to estimate breeding value. However, in pig populations with a high proportion of imported germplasm, progeny of imported sires often receives underestimated breeding value in the early stages of evaluation. This bias primarily arises from the lack of local performance data and genetic connectedness between imported parents and the domestic reference population. Without domestic phenotypic records, BLUP tends to regress breeding value of such animals toward the population mean, leading to lower selection indices despite potentially high genetic merit. Several approaches can mitigate this underestimation. The use of genetic groups or phantom parent groups can account for differences in genetic level between imported and domestic animals. Genomic evaluations (GBLUP) provide an additional solution by capturing relationships through DNA marker information, increasing connectedness even in the absence of phenotypic records. Rapid accumulation of domestic performance data from progeny also reduces bias over time. International genetic evaluations allow the incorporation of foreign breeding value into national evaluations, aligning genetic bases between countries. Addressing this issue is particularly important in small or developing breeding programs where imported germplasm represents a significant proportion of genetic improvement. Properly accounting for imported genetic material ensures fair evaluation of all candidates, optimizes selection decisions, and accelerates genetic progress.

Key words: pigs, breeding values, accuracy, import

Introduction

Intensive pig breeding is oriented to achieving high performing animals' through strong genetic selection and high input systems. For that reason, breeding programmes in pig production are oriented to intensive genetic progress, often based on import of germplasm from foreign breeding programmes. Genetic improvement in economically important traits, such as growth, fertility or meat quality traits, is the basis for economic success of pig production. For that purpose, import of animals that are considered to be genetically superior has an important role in achieving economic success of production. This can be confirmed by partitioning genetic trends for traits in selection (Škorput et al., 2015), where genetic progress for economically important traits in small populations under selection can significantly be affected by imported germplasm and result in significant genetic improvement.

Moreover, the goal of importing foreign germplasm is to increase genetic variance of domestic population. While genomic selection is increasingly becoming the dominant approach in modern breeding programs, BLUP (*Best Linear Unbiased Prediction*) procedure based on mixed model methodology (*MMM*) continues to play a crucial role in smaller national breeding schemes where resources and reference populations may be more limited. BLUP has numerous advantages over earlier methods: it uses a combination of own results of the individual; data on relatives of the evaluated individuals; and data on environmental factors (Henderson, 1973) and remains a powerful tool in estimation of breeding values in livestock.

However, if included in BLUP genetic evaluation, imported animals and their progeny sometimes surprise with a bad proof (Peškovičová et al., 2004). Such a situation can cause a biased evaluation of selection candidates, and economic loss for breeders who invested into foreign seamen or boars. Moreover, discouraging breeders and associations from occasional import of genetic material might result with impaired genetic diversity in the population and slowed genetic improvement that arises from imported germplasm. This can be especially important for small breeds and populations and breeding programmes without the possibility of genotyping animals and applying genomic selection. Thus, solutions to override biased evaluations via BLUP and ranking evaluated animals is required to ensure genetic progress and genetic variability of the population.

The aim of the paper is to analyse reasons for underestimation of selection candidates and their progeny when BLUP is used as a method of choice for genetic evaluation in pigs. Moreover, the goal is to define relevant approach for solving this problem to avoid underestimation of animals and the economic losses that might occur because of biased evaluation.

2. Systematic Underestimation of Genetic Merit in BLUP Models

Proposed by Henderson (1973), BLUP has been one of the most influential tools in animal breeding. Its widespread adoption stems from its ability to separate genetic effects from environmental influences and to provide unbiased estimates of breeding values under the assumption of complete and well-connected data (Henderson, 1984). However, despite its strengths, a recurring challenge in the application of BLUP is the systematic underestimation of genetic merit, particularly in cases where imported sires or animals with incomplete pedigrees are involved. Imported animals are a base animals with no breeding values till enough progeny has been born and performance tests have been conducted to produce a new breeding value that is significant in the imported country (Van Vleck, 1990). Thus, the analysis of the value of domestic and imported germplasm is not trivial, because foreign data is often not available in sufficient amounts or easy to integrate into domestic evaluations, although methodology for such integrations exists (e.g., Vandenplas and Gengler, 2012).

This problem has been noticed early and discussed by Webb (1976), Hofer et al. (1992) and Peškovičová et al. (2004). According to Misztal et al. (2013), ignoring missing pedigrees, unknown selection processes, or inclusion of several populations can result with biased genetic predictions even when genomic information is included in evaluations. One of the primary causes of underestimation of breeding values lies in the structure of pedigree information. When pedigrees are incomplete or when imported animals lack genetic connectedness to the domestic population, BLUP tends to shrink estimated breeding values towards the population mean (Hofer et al., 1992; Misztal et al., 2013). This shrinkage is mathematically desirable to reduce variance and avoid overestimation, but it simultaneously penalizes animals that are truly superior yet lack adequate progeny or connectedness. As a result, the offspring of imported boars, for instance, may receive lower estimated breeding values than their true genetic potential would justify (Peškovičová et al., 2004; Krüger, 2015).

Another important factor contributing to bias of the estimations is the presence of preferential treatment and environmental differences. Imported sires may be used in herds with management practices that differ from the national average. If such environmental effects are not properly accounted for in the model, BLUP may incorrectly attribute part of the environmental advantage or disadvantage to the genetic component, further distorting accuracy (Arnason, 1999). This problem is particularly relevant in smaller breeding populations, where

management heterogeneity tends to be greater and genetic ties across herds are weaker.

3. Tools for Reducing the Underestimation of Imported Animals in BLUP

Several methodological approaches are available for overriding problem of biased estimation of breeding values for animals from different groups using BLUP (Table 1).

Table 1. Tools and methods to reduce BLUP underestimation

Tool/Method	Principle	Advantage	References
Unknown Parent Groups (UPG)	Group animals with missing pedigrees into genetic groups	Reduces bias for imported sires with incomplete pedigree	Quaas, 1988; Misztal et al., 2013
Metafounders	Generalization of UPG, estimating relatedness of base populations	Accounts for base population differences; reduces bias	Legarra et al., 2015; Legarra et al., 2024
Single-Step Genomic BLUP (ssGBLUP)	Combine genomic, pedigree, and phenotypic data in one step	Improves accuracy and fairness, reduces underestimation	Christensen et al., 2012
Genomic Relationship Matrices (G-matrix)	Use SNP-based relationship matrices instead of pedigree	Captures realized relatedness; works without full pedigree	VanRaden, 2008
Preferential Treatment Adjustments	Model herd × year × season or management group effects	Separates genetic from management/environmental effects	Arnason, 1999
International Genetic Evaluations	Use international evaluation systems to improve connectedness	Enhances genetic connectedness across populations	Schaeffer, 1994
Bayesian Genomic Methods (BayesA, BayesB, BayesC π)	Bayesian methods model marker effects with different prior assumptions	Flexibility, better handling of unequal information	Meuwissen et al., 2001

3.1. Unknown parent groups

Quaas (1988) proposed a solution to handle differences in genetic values of base animals through genetic groups or unknown parent groups (UPG). The method involves assigning animals with unknown or poorly connected parents—such as imported sires—to specific “genetic groups” that account for systematic differences between base populations. For example, imported sires can be placed into separate groups reflecting their country or breeding line of origin. By doing so, the use of genetic groups prevents the underestimation of progeny of imported animals since their genetic merit is not forced towards population mean of domestic herd. Peškovičová et al. (2004) analysed the effect of genetic groups on the efficiency of selection on several traits, such as backfat thickness and average daily gain. Using model with genetic groups showed significant increase of selection efficiency, while using the model without genetic groups significantly reduced selection response. Škorput et al. (2013) used the model with genetic groups to estimate genetic trends on backfat thickness for Landrace and Large White pig and showed that the use of genetic groups is justified when there is a high proportion of imported animals in the breeding programme. Genetic trends for backfat thickness and time on test were partitioned by Škorput et al (2015) using method by Garcia-Cortes et al. (2008) with emphasis on the difference between domestic and imported genetics. Correlations between estimated breeding values from models with or without genetic groups indicated changes in estimated breeding values when genetic groups were included in the model.

Misztal et al. (2013) analysed the use of UPG in single-step genomic BLUP (ssGBLUP). For ssGBLUP, UPG equations also involve contributions from genomic relationships. When those contributions are ignored, UPG solutions and genetic predictions can be biased. The bias can be removed by making appropriate contributions to mixed model equations, by treating UPGs explicitly in the model, by refinements to definitions of UPGs or by adding an additional animal effect to the model. Phocas and Laloe (2004) considered the fitting of genetic groups in BLUP evaluation and concluded that the inclusion of genetic groups should be considered only with many animals per group.

Legarra et al. (2015) proposed the use of metafounders that represent the extension of the UPG concept. Unlike classical UPG, which treat base population groups as unrelated, metafounders explicitly model the genetic relationships among these base groups. This allows evaluations to capture both differences between populations and the degree of connectedness among them. The use of metafounders showed increased the accuracy of evaluation in both pedigree-based and ssGBLUP scenarios. The method was extended by Legarra et al. (2024), new definitions of the concept of meta-founders in terms of cross-product of allele frequencies of

populations with new definitions that can be related to existing concepts of genetic distances, heterozygosity or inbreeding, and can be integrated easily into genomic and pedigree-based predictions.

3.2. Bayesian methods

Bayesian genomic methods such as BayesA, BayesB, and BayesC π have become important alternatives to classical BLUP and genomic BLUP (GBLUP) in animal breeding, particularly when populations are small or include imported animals with limited pedigree connectedness. Traditional BLUP and GBLUP assume that all loci contribute to genetic variance in a similar way and therefore apply uniform shrinkage of estimated marker effects toward the mean (VanRaden, 2008). While this approach is computationally efficient and effective in large, well-connected populations, it can result in systematic underestimation of the genetic merit of imported sires and their progeny. This bias arises because rare alleles, often introduced through importation, are either poorly represented or absent in the domestic reference population, leading to insufficient recognition of their true contribution to performance traits. The relevance of these methods for imported animals lies in their ability to reduce the shrinkage of rare but important alleles. When imported sires introduce novel haplotypes, BLUP tends to undervalue their progeny because the estimation process regresses unknown animals toward the mean of the domestic population (Hofer et al. 1992; Peškovičová et al. 2004). In contrast, Bayesian methods give disproportionate weight to SNPs with larger observed effects, thereby capturing the genetic value of alleles unique to imported lines. This is particularly beneficial in pig breeding, where international exchange of boar semen is common and where imported lines are often carriers of superior growth or carcass traits not well represented in local germplasm (Kruger, 2015).

A relevant methodological development in this context is the work of Garrick and Fernando (2022), who proposed an approach to obtain exact single-step GBLUP results for non-genotyped descendants even when the genomic relationship matrix of ancestors is not available. Their method showed that it is not always necessary to have complete pedigree or genomic information for all ancestors; instead, posterior breeding values and covariance estimates from external evaluations can be incorporated into the local analysis to achieve identical results to a full single-step evaluation. This is particularly relevant for imported animals, where pedigree and genomic data are often incomplete or withheld for proprietary reasons. In such cases, the lack of connectedness leads to systematic underestimation of progeny breeding values under classical BLUP.

3.3. Genomic selection

The integration of genomic selection into animal breeding programs has transformed the way genetic merit is estimated, particularly in cases where pedigree information is incomplete or international genetic exchange plays a central role (Meuwissen et al., 2001). Imported animals, such as boars in pig breeding programs, present a unique challenge in classical BLUP systems, where their breeding values are often systematically underestimated due to weak or missing pedigree connections with the domestic population. Genomic selection provides an effective framework to overcome these limitations and to more accurately evaluate the contribution of imported genetics. Imported animals, which frequently lack full pedigree records in the importing country, are particularly vulnerable to this effect. By contrast, genomic selection employs marker-based relationship matrices that capture realized genetic relatedness among individuals. This enables more accurate estimation of breeding values for imported animals, even in the absence of complete pedigree data (Christensen et al., 2012). Genomic selection plays an important role in maintaining genetic diversity. Imported lines often introduce novel alleles that are underrepresented or absent in the domestic population. Classical pedigree-based BLUP, with its shrinkage effect, may undervalue these lines and discourage their use. Genomic selection, however, ensures that their genetic contributions are recognized and appropriately weighted, thereby promoting genetic diversity and long-term sustainability of breeding programs (Lourenco et al., 2014; Maiorano et al., 2019). Another important advantage of genomic selection is the ability to provide early and accurate evaluations. Imported animals often lack sufficient local phenotypic data, and their progeny may only just be entering performance testing, which significantly reduces the accuracy of BLUP estimates. With genomic information, breeding values can be estimated immediately after genotyping, without waiting for large progeny test records (Mei et al., 2022). This reduces generation interval and prevents valuable genetic lines from being discarded due to initially underestimated evaluations. In addition to accuracy, genomic selection also contributes to reducing environmental bias. Imported sires often originate from production systems with different management practices. If these environmental effects are not accounted for, BLUP may mistakenly attribute part of the variation to genetics. By integrating genomic information, evaluations can more effectively separate true genetic potential from environmental influences (Misztal et al., 2013).

3.4. International genetic evaluation

Unlike in cattle, where breeding values can be compared across herds and countries, international genetic evaluation of pigs is more complex and harder to realise. Although artificial insemination is also widely used, boars tend to be replaced more frequently, and herds are often smaller and more fragmented, resulting in weaker genetic connectedness. Also, there is no international body that unifies data across countries. Instead, evaluations are conducted within proprietary breeding programs managed by large international companies. Each company operates a closed nucleus scheme, and breeding values are calculated independently, making breeding values not directly comparable across herds, companies, or countries. However, the problem becomes more expressed in breeding programmes of small populations without sufficient resources such as in large international companies (Škorput et al., 2017). This lack of standardization in such populations has significant implications for imported animals. When boars are introduced into a new population, their progenies are often systematically underestimated in BLUP evaluations due to incomplete pedigrees and weak genetic connectedness with the local herd base unlike cattle, where international evaluations can adjust for base population differences, pigs lack a harmonized framework to correct for such biases.

4. Conclusion

Reliable genetic evaluation is essential for achieving sustainable genetic progress in pig production. While BLUP has served as the base of modern evaluation methods, its reliance on complete pedigree and strong connectedness makes it vulnerable to bias, particularly in the case of imported germplasm and progeny. Systematic underestimation of genetic merit not only undermines the efficiency of selection but also discourages the use of valuable foreign germplasm, thereby limiting both genetic gain and diversity.

A range of methodological improvements has been developed to address this challenge. Tools such as UPG and metafounders offer corrections for incomplete pedigrees. More importantly, the development of genomic selection and approaches like single-step genomic BLUP (ssGBLUP) have provided robust solutions by incorporating genomic relationships that capture hidden genetic connectedness between populations. These approaches not only improve the accuracy of evaluations for imported sires but also preserve genetic diversity by ensuring that novel alleles introduced from abroad are fairly represented.

In a globalized breeding industry, where international exchange of genetic material is indispensable, overcoming underestimation bias is critical. The integration of genomic information, combined with appropriate statistical and management tools, represents the most effective path forward. However, BLUP still remains a valuable tool, especially in smaller national programs, but its limitations must be recognized and mitigated. By embracing genomic selection and complementary methodologies, breeding programs can ensure more accurate and globally competitive genetic evaluations—ultimately driving faster and more sustainable genetic improvement.

Conflict of interest

The authors declare that they have no conflict of interest.

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GENETIC PARAMETERS AND ESTIMATION OF BREEDING VALUES FOR ECONOMICALLY IMPORTANT LITTER SIZE TRAITS IN PIG PRODUCTION USING ADVANCED STATISTICAL MODELS

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Abstract: The aim of this study was to assess the phenotypic variability and genetic parameters for the economically most important litter size traits in sows, as well as to estimate the breeding values (EBV) of boars for these traits. Fertility data from sows and boars included 18,042 litters farrowed by 6,627 sows on 312 pig farms. Descriptive analysis showed that the Landrace and Large White genotypes expressed a similar average number of live-born piglets (NBA), with somewhat higher variability observed in the Large White population. The heritability coefficient for NBA, estimated using the repeatability model, was $h^2 = 0.098$. The low heritability of this trait confirms that it is a reproductive trait strongly influenced by environmental factors, which highlights the need for particular attention to the accuracy of parameter estimation. The accuracy of genetic parameter estimation is crucial for more reliable EBV, which is of great importance considering the effect of this parameter on EBV reliability and, consequently, on selection efficiency. Although low values were obtained, the inclusion of permanent environmental effects in the model contributed to a more precise estimation of variance components and more reliable EBV. The results emphasize the importance of selection based on EBV for increasing litter size, with a recommendation for continued data collection on progeny to improve estimation accuracy and ensure long-term genetic progress in pig populations.

Key words: pigs, reproductive traits, heritability, reproductive traits, modelling, EBV

Introduction

The implementation of genetic evaluation using advanced statistical methods is applied to improve economically important traits of sows and boars. In pig production, special attention should be given to enhancing reproductive traits of sows and boars, as they directly affect productivity and economic profitability in pork production. The development of statistical models has enabled more accurate estimation of breeding values, thereby accelerating the identification of genetically superior animals and increasing the effectiveness of selection. Among the available approaches, methods based on mixed models (MMM) and BLUP evaluation represent the standard in countries with developed pig production.

Litter size traits are classified as low-heritability traits; however, selection can lead to a positive genetic response (Zaalberg et al., 2023). Estimation of the heritability coefficient provides insight into the potential genetic progress, while selection based on genetic values is crucial for improving economically important traits such as litter size in boars and sows (Holl and Robison, 2003). Numerous studies in pig breeding confirm that increasing reproductive efficiency is most effectively achieved through selection for litter size (Dube et al., 2012; 2013; Costa et al., 2016).

In the past, the application of selection index methods enabled the simultaneous selection of multiple traits, taking into account their correlations and economic value. Although this method contributed to genetic progress in sow reproductive traits, its limitation was the assumption of identical environmental conditions, which is difficult to achieve in practice (Endris, 2020; Wellmann, 2023). With the development of MMM models, combined with pedigree information, modern computing capacities, and large databases, it has become possible to achieve more accurate estimation of genetic parameters and breeding values in pig production (Mrode, 2005). Today, the BLUP animal model represents the standard for genetic evaluation of litter size traits, as confirmed by numerous authors (Peškovičová et al., 2004; Knap et al., 2023).

In practice, depending on the recording system, the number of piglets born alive (NBA) and the number of piglets at weaning (NW) are most commonly used for genetic evaluation. Due to the process of litter equalization and the high genetic correlation of NBA with the number of successfully reared piglets, the NBA trait is most often applied in the creation of selection programs (Tinh et al., 2021).

Materials and Methods

Data on sow and boar fertility included 18,042 litters produced by 6,627 sows across 312 farms. The study encompassed reproductive traits of Landrace and

Large White, the most commonly raised prolific meat-type genotypes on farms in the Republic of Serbia. The simultaneous analysis of phenotypic and genotypic variability across 312 farms was possible due to the existence of genetic connections between animals raised on different farms. There is an exchange of genetic material among the farms included in the analysis.

The trait of litter size at birth was selected because it is strongly and directly related to other indicators of litter size, both at farrowing and at weaning, and it directly determines the overall annual reproductive efficiency of sows as well as the economic profitability of this stage of pig production. It is particularly important to emphasize that the NBA is easily recorded under production conditions, which enables its straightforward inclusion in selection programs.

Data analysis was performed using the GLM procedure in SAS, and the systematic effects for the estimation of genetic parameters were selected by the 'step-by-step' method according to their statistical significance. With this approach, fixed and regression factors were selected and then included in the mixed model for the estimation of genetic parameters.

The repeatability model used for the estimation of variance components and breeding values included the following systematic and random effects:

$$Y_{ijklm} = \mu + S_i + G_j + F_k + OL_l + PR_m + b_{1m}(x_{ijklm} - \bar{x}) + b_{2m}(x_{ijklm} - \bar{x})^2 + p_{ijklm} + a_{ijklm} + e_{ijklm}$$

where,

$y_{ijklmno}$ - observed performance of the trait

S_i - fixed effect of the season of successful mating, presented as the combination of year and month,

G_j - fixed effect of genotype,

F_k - fixed effect of farm

OL_l - fixed effect of litter sire,

PR - fixed effect of parity,

$b_{1m}(x_{ijklmno} - \bar{x})$ - linear term of the quadratic regression effect of sow age at farrowing, nested within parity,

$b_{2m}(x_{ijklmno} - \bar{x})^2$ - quadratic term of the quadratic regression effect of sow age at farrowing (x), nested within parity,

$p_{ijklmno}$ - random effect of the permanent environment associated with sow litters,

$a_{ijklmno}$ - direct additive genetic effect of the animal, i.e., breeding value,

$e_{ijklmno}$ - residual.

Heritability (h^2) of the analyzed traits, expressed as the ratio between the additive genetic variance and the total phenotypic variance, can be mathematically defined by the following formula:

$$h^2 = \frac{\sigma_a^2}{\sigma_{ph}^2}$$

h^2 - heritability of the studied traits

σ_a^2 - additive genetic variance

σ_{ph}^2 - phenotypic variance

In the evaluation of breeding values for the number of piglets born alive (NBA) across successive farrowing's, this trait was treated as repeatable throughout the productive lifetime (repeatability model), which in practice represented the application of a single-trait animal model. Variance components, breeding value estimates, and their reliabilities were obtained using the same model. The software VCE-6 (Groeneveld et al., 2010) was used for the estimation of variance components, while reliability was calculated for all obtained estimates. Breeding values were computed using the PEST program (Groeneveld et al., 1990).

The reliability of estimated breeding values (r^2) was calculated as follows:

$$r^2 = 1 - (PEV / Var_A)$$

where,

PEV is the prediction error variance, and Var_A is the additive genetic variance in the analyzed population (Mrode, 2005).

The standard error of estimated breeding values ($SE(EBV)$) was calculated using the following formula:

$$SE(EBV) = \sqrt{Var_A \times (1 - r^2)}$$

where,

Var_A is the additive genetic variance in the analysed population, and r^2 is the reliability of the estimated breeding values.

Results and Discussion

To assess the phenotypic variability in terms of the number of piglets born alive, Table 1 presents the descriptive statistics for this trait. The analysis shows that in the Landrace (L) genotype, based on 4,108 farrowing, the average number

of piglets born alive was 12.56, with values ranging from 4 to 25 piglets. In the Large White (LW) genotype, on a sample of 13,934 farrowing, a slightly higher average was recorded (12.72 ± 2.42), along with greater variability (ranging from 0 to 28 piglets). These results indicate that both genotypes are similar in terms of average litter size, but that the Large White shows a wider range of values, which may be a consequence of differences in genetic potential, management practices, or environmental conditions on the analysed farms.

Table 1. Descriptive statistics for the trait number of piglets born alive (NBA) on the analyzed farms

Genotype	N	Mean NBA	SD	Min	Max
L	4108	12.5625	2.1817	4.0000	25.0000
VJ	13934	12.7166	2.4199	0.0000	28.0000

N- Number of farrowings, SD- Standard deviation; Min-minimum; Max-maksimum

The application of the repeatability model enabled the estimation of variance components and their proportion in the total phenotypic variance, on the basis of which the heritability coefficient for the number of piglets born alive per litter in sows was calculated (Table 2). The obtained heritability value ($h^2 = 0.098 \pm 0.039$) indicates that this trait has a low level of heritability. This result is consistent with previous studies, where the number of piglets born alive has been recognized as a typical reproductive trait with limited genetic influence, primarily due to the strong impact of environmental factors. At the same time, the very high percentage of residual variance ($e^2 = 0.902$) confirms that the variability of this trait is predominantly influenced by non-genetic factors, such as housing system, feeding, and management. Comparable heritability values for the number of piglets born alive when using the repeatability model have also been reported in studies by Camargo et al., 2020; Stojiljković et al., 2022; Silalahi and Chen, 2023.

Table 2. Estimated variance components and their proportion in the total phenotypic variance for NBA

Var(a)	Var(p)	Var(e)	Var(ph)
0.39765 $\pm$ 0.17799	0.00000 $\pm$ 0.00002	3.67265 $\pm$ 0.03063	4.07030
h^2	p^2	e^2	
0.09769 $\pm$ 0.03946	0.00000 $\pm$ 0.00000	0.90230 $\pm$ 0.03946	

Var(a) – additive genetic variance; Var(p) – variance of the permanent environmental effect of the sow; Var(e) – error variance (residual); Var(ph) – phenotypic variance; h^2 - estimate of heritability; p^2 - estimate of the permanent environmental effect of the sow; e^2 error (residual).

The model also included the permanent environmental effect that the sow transmits to her litters, which enabled a more accurate estimation of variance components. Although the relative contribution of this factor was small, its inclusion proved justified, as it significantly reduced the error in estimating the additive genetic variance. This increased the reliability of the obtained results and allowed a more realistic assessment of the genetic potential for the trait “number of piglets born alive.” From a practical perspective, such an approach contributes to higher accuracy in estimating breeding values, since it allows for a clearer separation of genetic and non-genetic sources of variability. As a consequence, selection decisions based on these estimates will be more reliable, which can contribute to more efficient genetic improvement of the population, as demonstrated in the following studies: 2013; Lopez et al., 2019; Stojiljković et al., 2022; Silalahi and Chen, 2023.

In many breeding programs worldwide, traditional BLUP evaluation has been enhanced with genomic selection, which significantly increases the accuracy of breeding value estimation, especially for low-heritability traits such as litter size (Wang et al., 2024). Genomic selection enables earlier and more reliable identification of genetically superior animals, reduces the generation interval, and accelerates genetic progress. Although genomic selection is not yet widely applied in the analyzed population, its implementation represents an important perspective for the future, particularly if combined with continuous collection of phenotypic and pedigree data. The integration of genomic information into BLUP models (GBLUP) would further improve the reliability of EBV estimates and optimize selection strategies for reproductive traits.

The results presented in Table 3 refer to the estimated breeding values (EBV) for the 10 highest-ranked boars in the analysed population. A clear difference can be observed between the top-ranked boar (ID 48830), whose estimated breeding value for the trait “number of piglets born alive” is the highest (2.31), and the other individuals. In addition to the high EBV, this boar also shows a solid level of reliability of estimation ($r^2 = 0.42$). For the boars presented, the EBV values are positive, indicating their favourable genetic potential for the improvement of the trait.

Table 3. Estimated Breeding Value (EBV) for the 10 Highest-Ranked Boars in the Analyzed Population

ID boars	EBV	PEV	r^2	SE(EBV)
48830	2.3051	0.2937	0.4222	0.4793
42749	1.5398	0.2626	0.5381	0.4286
47057	1.4655	0.3371	0.2389	0.5501
45504	1.1786	0.3537	0.1621	0.5772
48736	1.0921	0.3407	0.2225	0.5560
46069	1.0406	0.3477	0.1903	0.5674
36154	0.7985	0.3410	0.2212	0.5565
49808	0.7910	0.3594	0.1348	0.5865
49810	0.7832	0.3595	0.1344	0.5867

EBV- Estimated Breeding Value; PEV- Prediction Error Variance; r^2 - Reliability of estimated breeding values; SE(EBV) - Standard error of estimated breeding values

The relative reliability of estimation (r^2) ranged from 0.13 to 0.54, indicating that there is already a certain level of confidence in the ranking of boars, while additional progeny data can be expected to further increase the accuracy of the estimates. The standard errors of estimation (SE EBV), which range from 0.43 to 0.59, indicate satisfactory precision of the obtained values and confirm that the applied model provides stable results.

The obtained estimates clearly highlight a group of superior boars that possess a pronounced genetic potential for improving the number of live-born piglets. Their use in the selection program can significantly contribute to further enhancement of reproductive traits in the population. It is particularly important to continue monitoring the performance of their offspring, which will further strengthen the reliability of the estimates and enable even more precise selection for breeding.

Conclusion

The results of this study confirm that the number of piglets born alive is a reproductive trait with low heritability (0,098). Nevertheless, the application of advanced statistical models enables reliable estimation of breeding values (EBV) and the identification of genetically superior boars, which provides a solid basis for effective selection. Although the genetic progress for this trait may be relatively slow due to its low heritability, the inclusion of EBV in selection programs ensures

a systematic and long-term improvement of reproductive efficiency in pig populations. The continued collection of performance data, particularly progeny records, will further increase the accuracy and reliability of genetic parameter estimation, thereby contributing to more precise breeding decisions. In this way, the integration of modern genetic evaluation methods with practical selection strategies represents a key step toward sustainable improvement of reproductive traits and overall productivity in pig production. Future research should consider the implementation of genomic selection (GBLUP) to further increase the reliability of EBV estimates and achieve faster genetic progress for reproductive traits in the population.

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Conflict of interest

The authors declare no conflict of interest.

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LOCAL PIG BREEDS – THE MULTIFUNCTIONALITY ASSETS FOR SUSTAINABLE PORK PRODUCTION

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Abstract: This presentation analyses the role of local pig breeds within sustainable food systems, with a particular focus on their contribution to sustainable pig production. It examines how these breeds respond to evolving societal and consumer expectations regarding intrinsic and extrinsic quality attributes of pig meat and pork products. The analysis addresses critical challenges related to sustainability in livestock systems, highlighting the multifunctionality of local breeds, their environmental impact, socio-cultural significance, consumer perspectives and market potentials of their products. Public funding plays an important role in maintaining local pig breeds; however, a lasting conservation requires their integration into sustainable systems that capitalize on their distinctive qualities.

Key words: breed valorisation, ecosystem services, quality attributes, pork value chain

Introduction

Animal husbandry provides humans with foods that represent an important source of high-quality and easily digestible proteins, minerals, and vitamins, and are part of a healthy and balanced diet. The sector of pig farming holds a significant share in meat production; globally, it accounts for about one-third and in Europe, nearly half of meat consumption. Due to the global increase of human population, land availability and its use (Godfray et al., 2010) represent a limiting factor for agricultural production in particular for the share that can be used for animal production. We can expect an increasing pressure on land use and growing competition between feed and food at a global level, which in the long term leads to the increasing need for the justification for using agricultural land for livestock production (AgriResearch, 2019), and particularly for pig farming, as it delivers mainly one product – meat (meat is the primary output of pig farming, but the

sector also generates a minor share of by-products with high value (e.g. pharmaceutical industry).

The European Commission's Green Deal strategy (EC, 2019) envisions a transition to agroecological food systems, which must be designed more holistically to best balance, both now and in the future, demands for productivity, sustainability, quality, and other societal values (ecosystem services). Pork is an important source of valuable nutrients in the human diet; however, since pigs directly compete with humans for cereals and plant proteins, this sector is particularly vulnerable and increasingly subject to social pressure.

The sector's strength lies in the value of pork for consumers especially in tradition of consumption and processing into highly appreciated products. In the pursuit of sustainable food systems, some studies (Röös et al., 2016; van Zanten et al., 2018) suggest limiting livestock farming to species that can utilize grassland or by-products from the food industry. In this regard, the use of by-products is an important and underutilized feed resource in pig farming. To address this challenge, the pig farming sector will need to respond to future societal demands and integrate the various functions of the system, where the quality of products in its broadest sense will play a central role.

In this context, one of the key attributes for the sustainable development of pork supply chains lies in the tradition of pork consumption, on which pig meat production can build its *raison d'être* (reason for existence); it involves developing products that meet the high expectations of consumers. In this way, pig farmers and the meat industry can take advantage of the consumer trend toward valuing quality over quantity. The idea of "less but better" meat consumption enables producers to increase the price and profitability of their products without expanding production volume (or even reducing it), thereby also contributing to the mitigation of environmental impacts.

Today, most pork and pork products come from intensive production systems that were developed in the past century to improve meat availability/supply and efficiency of pork production. The focus of pig farming on genetic improvement and efficiency has led to certain negative effects on meat quality (technological and sensory characteristics), as well as on the nutritional properties of both fresh and processed pork (Font-i-Furnols and Guerrero, 2014). At the same time, the sector is increasingly confronted with societal demands for healthier products and more sustainable production (e.g., improved animal welfare, lower environmental impact), and the strengthening of circular and local economies (e.g., the use and valorization of by-products and alternative protein sources for animal feed) (Bilatto et al., 2024). A sustainable food system must therefore meet three conditions:

i) economic sustainability –must be cost-effective throughout the entire chain,

- ii) social sustainability – it must provide broad benefits to society, and
- iii) environmental sustainability – it must have a neutral or positive impact on the natural environment (Figure 1).

SUSTAINABLE FOOD SYSTEM



Figure 1. Sustainable food system has three pillars

An analytical review of the literature (Aboah and Lees, 2020) showed that the five most important quality characteristics of meat for consumers are of extrinsic nature, with fat content (an intrinsic trait) ranked only fifth, alongside the organic label and price. For pork, it is also important that it is convenient to prepare, tasty, and produced in line with the ethical values of consumers (Lin-Schilstra et al., 2022).

Importance of extrinsic cues for consumer

Demand for pork and pork products is increasingly shaped by societal expectations for more sustainable production systems that ensure better animal welfare, biodiversity (e.g., local breeds), reduced use of inputs (particularly veterinary drugs), and lower environmental impact (such as precision feeding, use of local feed sources, and valorisation of food industry by-products). This evolution is also supported at the European level by the Green Deal and the Farm to Fork strategy, aiming for a fair, healthy, and environmentally friendly food system. Consumers are exposed daily to meat of various origins, associating the origin of the meat with food safety (e.g., trust in domestic production), although countries differ in how much consumers trust their food safety systems (Font-i-Furnols and Guerrero, 2014). Consumer attitudes toward animal farming for meat have shifted significantly, with increasing concern about animal welfare and the environmental impacts of livestock production (Liu et al., 2023). Consumer views on pork production vary by consumer segment and country, as well as by the context of

purchase and consumption (Aboah and Lees, 2020). Quality aspects of pork are multi-dimensional and are becoming increasingly important, as a growing number of consumers prefer to eat less meat but of higher quality (Lebret and Čandek-Potokar, 2022).

However, it should be noted that for a significant portion of the population, meat price remains a very important purchasing factor, even in countries with higher consumer purchasing power (Aboah and Lees, 2020; Liu et al., 2023). Furthermore, the views of citizens (on animal welfare and environmentally friendly food production) and those of consumers may differ; for instance, what people support in their role as citizens does not always influence their actual purchasing and consumption decisions regarding pork (Krystallis et al., 2009). The dimensions of quality that influence repeated purchase are linked to demand, trust, and experience (Grunert et al., 2004). Research shows that health, natural attributes, sensory quality, price, and animal welfare are key factors for consumers in various countries. Consumers differ and can be segmented as demanding, average, low-ecological, and indifferent (Lin-Schilstra et al., 2022). Consumers of the modern developed world with adequate purchase power increasingly focus on what they eat. They expect choice and transparency in the supply chain; they want to know where their food comes from and who produces it and how. They are interested in sustainable practices of companies and farms, but often do not want to pay more for products based on sustainable approaches. Regarding pork, it is important to consider whether pork has a future as an accessible and affordable protein source. Expanding sustainable practices and effective communication of sustainability stories, seeking diversity and profitable niches, and especially educating future generations to recognize and appreciate product quality linked to pig farming will be crucial.

Future pig farming systems will need to incorporate and address the complexity of consumer perceptions of meat quality, which, within the context of pig production, means aligning production systems with available natural resources and focusing on delivering high-quality products.

Local pig breeds and their value for sustainable food system

The United Nations organisation has set 17 sustainable development goals focused on human health and environmental sustainability, and the livestock sector must also adapt to these goals (FAO, 2018). These goals address a sustainable food system, which is not sustainable today because food production does not align with or follow healthy human diets (Benton et al., 2018). Therefore, the food system needs to be transformed to provide healthy nutrition and more sustainable and circular food production (reducing food wastes). In this context, European

autochthonous (or local) pig breeds and their specific production systems have great potential due to:

- consumers prefer local food (Holloway et al., 2007),
- consumers prefer food coming from farms with high animal welfare standards (Alonso et al., 2020),
- consumers prefer outdoor extensive farming systems, and environmentally friendly farming (Krystallis et al., 2009).

Meat from local pig breeds is perceived by consumers as better because it possesses desired intrinsic quality traits. These breeds and their (usually extensive) farming systems represent agricultural and biological diversity and are also a prerequisite for unique high-quality regional products. Such products are part of culinary heritage and "traditional" knowledge, often recognized by official quality marks, including geographical indications. Local pig breeds are also associated with extrinsic qualities important to consumers. They are mainly raised in extensive production systems, often outdoors and in organically, meaning their farming ways align better with societal expectations regarding extrinsic quality attributes such as animal welfare and environmental impact. Furthermore, local breeds are mostly fed on local feed resources, are more resilient, and adapted to local agro-climatic conditions. These characteristics make production systems with local pig breeds highly aligned with the concept of sustainable food systems.

There is limited literature data on the environmental footprint of production systems using local pig breeds. Some sources analyzing different farming systems (Dourmad et al., 2013; Dourmad et al., 2014; Espagnol and Demartini, 2014; Lamnatou et al., 2016) indicate that traditional, less intensive farming systems (including organic) generally have a higher potential for global warming per unit of product due to lower efficiency, but environmental impacts related to eutrophication and acidification per hectare of cultivated land are 10–60% lower. Similar findings emerged from research conducted under the H2020 TREASURE project, which analyzed three local breeds—Krškopolje pig, Gascon, and Mora Romagnola (Monteiro et al., 2019). This research found significant differences in environmental footprints between farms, suggesting potential for improvements. The farms with the lowest environmental footprint were those using their own feed resources, with key improvement areas including diet composition and feed origin, feeding efficiency, and adjustment of nutrition to the needs of these breeds.

Conclusion – future perspectives for pig farming

Land availability and its use are recognized as key limiting factors for global food production (Godfray et al., 2010). In a global context, competition for

available land between animal feed and human food will in the long term require limiting animal production to natural capacities and sustainable nutrition (Mottet et al., 2017; Van Zanten et al., 2018). Pork production is particularly challenged in this regard, as pigs provide mainly one food product, meat, and are besides direct competitors with humans for available cereals and plant protein sources. However, local specifics will also be important for sector development. Self-sufficiency in feed resources for pigs will be one of the key factors for sustainability in pig farming systems (Van der Werf and Petit, 2002). A positive aspect is that pigs can be fed by-products, and residues from the food industry that are unsuitable for humans also referred to as low opportunity cost feeds (Schader et al., 2015; Mottet et al., 2017). These feed potentials are still far from fully utilized. Considering the low amount of pork in the reference healthy diet (Willett et al., 2019), which amounts to only 0.6% (15 kcal, i.e., 10–15 g/day), there will likely be very little room in the human diet for pork. Therefore, small-scale but high-quality production is a sustainable long-term approach. The pig farming of the future will have to rely on its own feed resources, provide broader social benefits and ecosystem services, and produce products whose quality will be aligned with consumers' demands for safe, tasty, and socially responsible food (Font-i-Furnols and Guerrero, 2022).

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Conflict of interest

The authors declare no conflict of interest.

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STUDYING HUSBANDRY AND NUTRITION IN KRŠKOPOLJE PIG: IMPLICATIONS FOR FAT DEPOSITION AND MEAT QUALITY WITH INSIGHTS INTO METABOLISM AND GENE EXPRESSION

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Abstract: The Slovenian autochthonous breed Krškopolje pig is receiving a growing interest from both society and consumers, as it represents an important element of agricultural biodiversity, cultural heritage and is valued for its meat quality. The present article summarizes recent studies on Krškopolje pig performance under various rearing and nutritional conditions, with comparisons to lean modern pigs. Investigations covered both intensive indoor and extensive outdoor systems, including assessments of growth, fat deposition, meat quality traits, tissue-specific enzyme activity, and gene expression related to fat metabolism. The impact of dietary protein reduction was also examined in both settings, with emphasis on physiological and molecular responses. These studies provide a comprehensive overview of how Krškopolje pig responds to different production environments and nutritional strategies, highlighting its relevance for sustainable and niche pig production.

Key words: Krškopolje pig, rearing systems, nutrition, fat deposition, gene expression, metabolism.

Introduction

The Krškopolje pig, Slovenia's only autochthonous pig breed (Figure 1), is known for its robust characteristics and good adaptability to diverse rearing conditions, positioning it as a valuable resource for sustainable pork production. Nearly extinct in the past, the breed has experienced a notable revival over the last

decades, with growing interest from breeders and researchers driving an increase in its population. Unlike modern, lean breeds, Krškopolje pig shows moderate growth rate and tends to accumulate more fat, traits that make it well-suited for extensive rearing systems using local feed resources and supporting the production of high value-added meat products (Kastelic and Čandek-Potokar, 2013; Čandek-Potokar and Nieto, 2019). Despite the revival, Krškopolje pig remains relatively understudied compared to modern cosmopolitan breeds. Recent research has begun to address this, examining its performance under different rearing and nutritional regimes and comparing it to leaner genotypes. However, key questions about its specific nutritional requirements, optimal feeding and rearing strategies, and metabolic characteristics remain unanswered, all of which are critical for more efficient and sustainable breeding practices and product quality.

The present article summarizes findings from recent studies conducted at the Agricultural Institute of Slovenia, presenting a more detailed overview of investigations into Krškopolje pig's specific traits, nutritional needs, and production capacity, either by comparing its performance with modern breeds or testing the breed under various rearing systems and/or with different feeding strategies.



Figure 1. Young specimens (app. 50 kg) of Krškopolje pig breed

Krškopolje pig compared to modern breeds

To better characterize Krškopolje pig, it is important to compare it with modern breeds under identical environmental conditions (rearing and feeding systems). However, this is challenging due to the distinct nature of these breeds, particularly their different nutritional requirements. Nevertheless, two studies have addressed this: one conducted under extensive and another under intensive rearing conditions.

The first one (Poklucar et al., 2023) compared Krškopolje pigs (KKP) with modern lean hybrid pigs (MP, crosses of Landrace, Large White and Duroc) raised in an outdoor extensive system (large fenced area with roofed shelter, 2 kg of cereal based commercial feed per pig supplemented with hay and root crops). When fattened to higher weight (165 kg), KKP exhibited superior growth (1.7 fold higher daily gain) and notably greater fat deposition with up to three times thicker backfat and nearly double the intramuscular fat compared to MP, while muscle growth was comparable (Figure 2). This greater growth performance of KKP pigs was rather unexpected, given that both groups had the same feeding regime, suggesting greater resilience and adaptability of KKP breed to outdoor rearing conditions and/or nutritionally suboptimal diets. KKP pigs also displayed higher saturated (SFA) and particularly monounsaturated fatty acid (MUFA, mainly oleic acid) content in subcutaneous fat (Figure 1) which was associated with increased stearoyl-CoA desaturase gene expression. Similar to our results, it has been attested in many local breeds, that their desaturation capacity is generally higher than in modern hybrids, and that they are also less sensitive to non-optimal dietary conditions (Poklucar et al., 2020; Benítez et al., 2017). Furthermore, genes linked to lipogenesis (ACACA, FASN), adipogenesis (PPAR γ) and energy homeostasis (LEP) were overexpressed in KKP (Poklucar et al., 2023) confirming higher adipogenicity of many local breeds (Poklucar et al., 2020). Despite the increased fat deposition, lipogenic enzymes (malic enzyme – ME1, glucose-6-phosphate dehydrogenase – G6PD) in KKP were more than twofold lower than in the modern hybrids. This discrepancy may be explained by differences in physiological maturity at slaughter, as many local breeds, mature earlier and may thus reach peak lipogenic activity earlier in life than modern breeds (Lebret et al., 2014; Mourot et al., 1996).

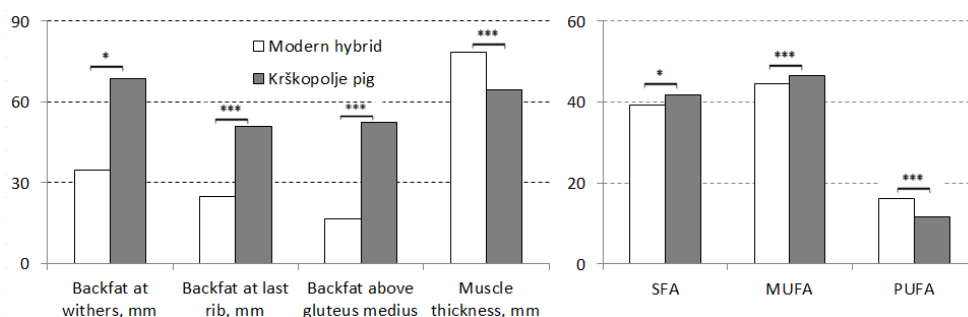


Figure 2. Carcass traits (left) and fatty acid composition of subcutaneous fat in modern hybrids and Krškopolje pigs reared in extensive outdoor conditions (adapted from Poklucar et al., 2023)

In a subsequent experiment, KKP and modern lean hybrids were reared indoor, in intensive conditions, both groups receiving daily equal amounts of the same feed mixture (i.e. 1.6 kg, 2.4 kg and 2.8 kg during three subsequent fattening phases, each lasting 60 days; Škrlep et al., 2024). Contrary to results of extensive rearing, in intensive system KKP exhibited slower growth, with 15% lower daily gain and a 13% lower slaughter weight than modern hybrids. The difference in daily gain (in average approximately 130g/day) remained consistent throughout the trial. This was associated with reduced muscle development (i.e. lower *longissimus* muscle gain resulting in a 1.7-fold smaller loin eye area and thinner muscle at *gluteus medius* level) and greater backfat accumulation throughout the entire fattening period, cumulating in over twofold thicker backfat at the end of the trial. Differences were also found for histomorphological traits, with KKP showing larger adipocyte sizes. Meat quality parameters were largely similar between the genotypes, except for darker colour of KKP meat (Figure 3), and higher myoglobin content, consistent with more oxidative muscle profile typical of local breeds (Park et al., 2007).



Figure 3. Loin cross-section in Krškopolje pig (left) and modern hybrid (right); note the difference in muscle colour and subcutaneous fat thickness

The most notable difference in chemical composition was the intramuscular fat content, which was over 2.5 times higher in KKP than in modern hybrids. As in previous experiment, KKP exhibited higher levels of SFA and MUFA but lower levels of PUFA in both backfat and muscle, though the differences were less pronounced in backfat. Notably, KKP displayed lower n-6/n-3 PUFA ratio compared to MP. While lower ratio is generally considered beneficial for health, the values in this study were still relatively high (above 20, compared to the recommended ratio being below 4; Dougan et al., 2015). Despite the higher proportion of saturated fats, elevated oxidation levels (thiobarbituric acid reactive substances - TBARS) were observed in KKP muscle, potentially due to higher overall IMF content (Fuentes et al., 2014) or increased myoglobin

rearing practice, a comparison between intensive (based on commercial feed) and extensive (cereal based feed supplemented with root crops) was conducted (Tomažin et al., 2017). The study showed that despite *ad libitum* feed access, pigs in the extensive system exhibited significantly lower growth rates after the initial phase (Figure 5), as well as reduced slaughter weight and carcass fat content. This was primarily attributed to a protein deficiency, particularly lysine, as it was estimated that extensive feeding met only 50–60% of the needs for a fatty-type pig (Nieto et al., 2015) and depends on feed availability and nutrient composition. Interestingly, in addition to the negative effects on growth, the meat from extensively reared pigs exhibited a darker colour, indicative of a more oxidative metabolism, as also observed in other local breeds (Lebret et al., 2015).

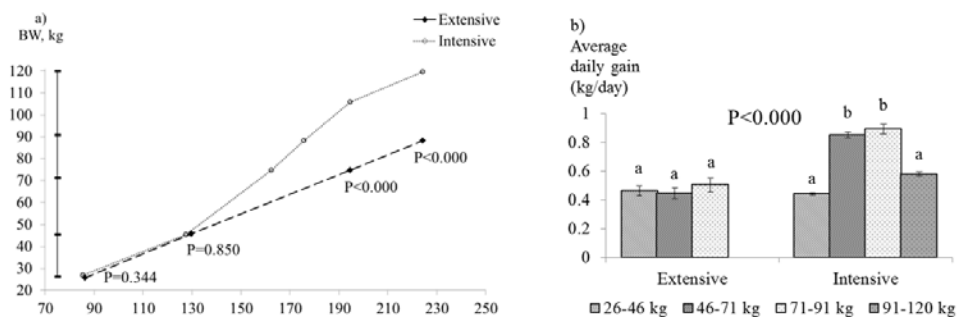


Figure 5. Body weight (left) and average daily gain (right) of Krškopolje pigs reared in intensive or extensive system (source: Tomažin et al., 2017)

A comparative study of conventional (indoor, commercial feed) and organic (outdoor access, supplemented with voluminous feed – alfalfa hay) rearing practices for the KKP (Batorek-Lukač et al., 2016) revealed slightly higher growth rates (13%) in organic system. Higher growth rate in organic system was attributed to significant alfalfa hay intake (estimated at an additional 6.0 ME ME/day) and lower feed wastage in the organic group. Except for dressing (lower in organic group) no major differences between the two rearing systems were observed in either muscle or fat-related carcass characteristics (Tomažin et al., 2019).

The differences between the systems were however more pronounced in meat quality and fatty acid composition (Tomažin et al., 2019). Meat from organic pigs had higher pH 45 minutes post-mortem, likely reflecting reduced stress due to enriched environments or physical activity (Terlouw et al., 2005; Millet et al., 2004), but lower pH 24 hours post-mortem (as to higher glycogene reserves, Bonneau and Lebret, 2010), with higher drip loss and darker meat colour. Intramuscular fat (IMF) content was similar across groups, but organic pigs

exhibited lower saturated fatty acids (SFA) and higher monounsaturated fatty acids (MUFA) in IMF, a fat profile likely driven by alfalfa supplementation and other factors such as increased physical activity (Daza et al., 2009), lower ambient temperatures (Lebret, 2008) or lower dietary lysine (Wang et al., 2018). Additionally, organic pigs showed elevated TBARS, indicating greater lipid oxidation, which, combined with lower vitamin E in backfat, suggests increased oxidation susceptibility that could compromise meat quality during storage. Backfat in organic pigs also had higher polyunsaturated fatty acids (PUFA), reflecting dietary influences from alfalfa. Additional research on products from this meat (Škrlep et al., 2018; 2019) has shown that dry-fermented sausages produced from organic pigs exhibited lower processing losses, softer texture and more intensive oxidation due to lower fat saturation. A further research on the metabolic features associated with both husbandry systems was also conducted on muscle tissue (Fazarinc et al., 2020; Figure 6). It was shown that organic production system influenced the composition of muscle fibre type with a shift towards more oxidative fibres (i.e. more SDH-positive myofibre types, with higher expression of I, IIa and IIx and lower expression of IIb myosine heavy chain isoforms), but with no apparent effect on metabolic phenotype of the muscle including expression of the energy and fat metabolism-related genes (PGC-1 α , PPAR γ , LPL, CPT1B, FASN, GYS1, HK2).

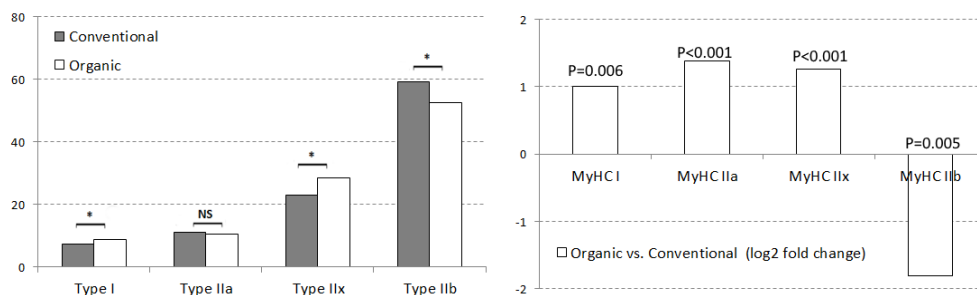


Figure 6. Effect of rearing practice on percentages of myofiber types (left) and myosine heavy chain isoforms gene expression (MyHC, right) in the longissimus dorsi muscle of Krškopolje pigs (adapted from Fazarinc et al., 2020)

Dietary protein reduction

Due to their slower growth, greater fat deposition potential, and higher resilience to feed scarcity, local pig breeds typically exhibit lower protein requirements. However, these requirements are relatively unknown for most local breeds. In addition, there is considerable variation in protein requirements among

local breeds. For instance, the Italian local breed Cinta Senese requires on average as little as 10% crude protein throughout the fattening period (Aquilani et al., 2019). A preliminary study by Brossard et al. (2019) found that KKP, compared to nine other local breeds, have relatively high protein needs, approximately 65% higher than those of Cinta Senese pigs. However, subsequent studies (e.g., Škrlep et al., 2024) suggest that KKP protein requirements are likely much lower. A reduction of approximately 2% points in crude protein (reaching as low as 9.3% in the final fattening stage) did not result in any differences in growth rate, tissue deposition, carcass characteristics, meat quality, biochemical composition, or metabolic response. Furthermore, modelling of amino acid nutritional needs revealed no deficiencies.

In a subsequent study (Škrlep et al., 2025) the effects of dietary protein reduction in two distinct rearing systems (indoor conventional vs. outdoor organic, receiving feeds formulated using the same ingredients but of conventional or organic origin, Figure 7) were investigated to test for the interaction between breed and environment.

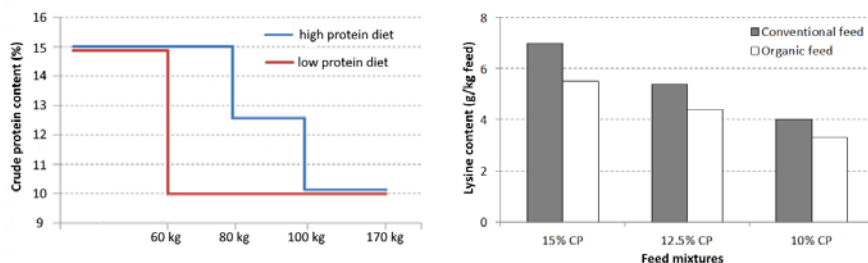


Figure 7. Scheme of the multiphase feeding trial with high and low protein diet in Krškopolje pigs and the lysine content of the feed mixtures with different crude protein (CP) content (adapted from Škrlep et al., 2025)

By modelling growth data (e.g. using InraPorc®), requirements for proteins and essential amino acids can be assessed (Figure 8). The results of modelling showed that, except for lysine in low protein diet, the essential amino acid requirements were covered with control “high protein” diets used throughout the fattening and that lysine deficiency in the case of low protein diet was more pronounced in the outdoor organic system, resulting in different effect of protein reduced diet in conventional and organic rearing system.

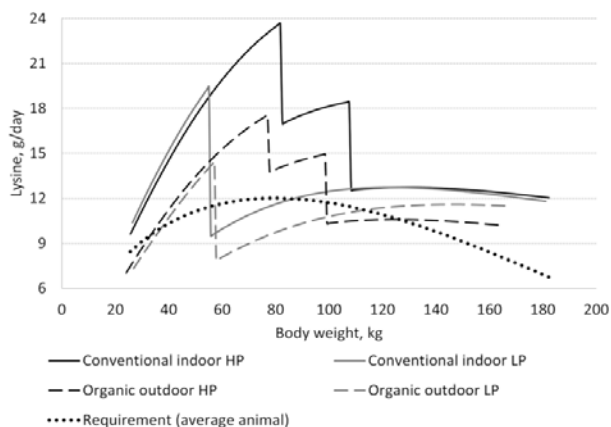


Figure 8. Lysine supply and requirements for Krškopolje pigs reared in outdoor organic or indoor conventional system, fed high (HP) or low (LP) protein diet; lysine requirement was estimated on the profile of conventional indoor HP animals (adapted from Škrlep et al., 2025)

In the outdoor organic system, pigs on low protein diet exhibited slower growth during intermediate phases but achieved compensatory growth in the final fattening stage. Differences in carcass composition and meat quality were minimal, with slightly increased fat deposition and thicker backfat in low protein group. These differences were evident also on biochemical and histological level as higher activity of lipogenic enzymes (Figure 9), increased MUFA content in the backfat and larger adipocytes, while transcriptomic analysis revealed upregulation of genes involved in lipid metabolism, including fatty acid synthesis (ACYL, ACACA, FASN, ACSM5), triglyceride synthesis (MOGAT2), and fatty acid desaturation (SCD, ELOVL6, consistent with higher MUFA), alongside genes related to oxidative stress and mitochondrial function (Poklukar et al., 2025b).

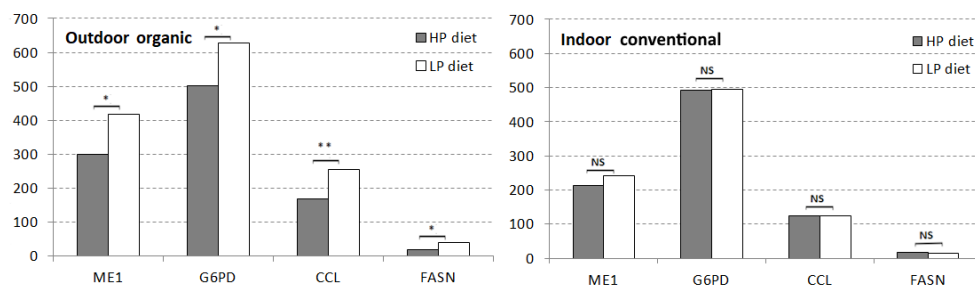


Figure 9. Lipogenic enzyme activities (ME1 – malic enzyme, G6PD – glucose-6-phosphate dehydrogenase, CCL – citrate cleavage enzyme, FASN – fatty acid synthase) in subcutaneous adipose

tissue of Krškopolje pigs from different rearing systems (outdoor organic, indoor conventional) fed high (HP) or low (LP) protein diet (adapted from Poklucar et al., 2025b)

In accordance with smaller amino acid deficit, animals in the indoor intensive system, showed no diet-related differences in growth, tissue deposition, carcass composition, or biochemical properties (Škrlep et al., 2025). Transcriptomic responses nevertheless differed, with upregulation of heat shock proteins (HSPA6, HSP70) and downregulation of genes associated with the innate immune system (MSR1, CSF3R, TREM) in low protein compared to high protein animals (Poklucar et al., 2025b).

Conclusion

Based on the research results, it can be concluded that KKP demonstrated distinct adaptability and fat deposition characteristics either compared to modern breeds or when subjected to different rearing and feeding practices. The effects observed for growth, fat deposition and composition may be utilized to manage breed's aptitude and potential for specialized and sustainable production.

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Conflict of interest

The authors declare no conflict of interest.

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DIETARY CAMELINA AND LINSEED CAKES MODULATE FATTY ACID CLASSES AND SENSORY TRAITS OF YELLOW MEALWORM LARVAE

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Abstract: This study evaluated the effects of including 5% and 10% camelina and linseed cake in the diet of *Tenebrio molitor* L., on the fatty acid (FA) composition and sensory traits of dried larvae. Five isonitrogen and isoenergy diets were tested: a control diet (CON), a diet with 5% camelina cake (CAM 5), 10% camelina cake (CAM 10), 5% linseed cake (LIN 5), and 10% linseed cake (LIN 10). Larvae were fed the diets from the 4th to the 9th week of age, then euthanized by freezing at –60 °C and freeze-dried for FA quantification. A portion of larvae was microwave-dried to conduct consumer sensory evaluation. Visual, olfactory and overall acceptance was assessed by 141 employers or students of the University of Padova. Results showed that incorporating camelina and linseed cakes at both 5% and 10% increased the amount of polyunsaturated fatty acids (PUFA) of larvae ($P = 0.0053$) and reduced their n-6/n-3 ratio ($P < 0.0001$). In terms of visual, olfactory, and overall consumer acceptance, larvae from the CON group received the lowest ratings, while those from the LIN 10 group were rated most favourably by consumers (3.90 vs 5.42, respectively; $P < 0.0001$). In conclusion, this study demonstrated that dietary inclusion of camelina and linseed cakes enhanced the PUFA content and improved sensory acceptance of yellow mealworm larvae. Notably, the improvement in the n-6/n-3 ratio was proportional to the level of oilseed cake supplementation, with the lowest and most favourable ratio of 4.43 achieved in the LIN 10 group.

Key words: oilseed by-products, omega-3 fatty acids, polyunsaturated fatty acids, consumer preference, edible insects.

Introduction

Tenebrio molitor (TM) is one of the main insect species studied and farmed in Europe. This insect is particularly interesting due to its rich nutritional profile, ease of rearing, and lower environmental impact compared to conventional livestock, especially in terms of land and water use (van Huis et al., 2013; Grau et al., 2017; Gkinali et al., 2022). Particularly, TM larvae have been proposed as a valuable alternative to conventional feed ingredients such as soybean meal and fish meal, due to their comparable nutritional profile. TM larvae contain about 50% protein on dry matter (DM) with a good balance of essential amino acids, around 30% lipids rich in beneficial fatty acids, and considerable levels of minerals and vitamins (Nowak et al., 2016; Kröncke et al., 2019; Trukhanova et al., 2022). For these reasons, TM larvae have been included in the diet of different animal species to test how they affect performance and product quality. Inclusion levels up to 30% TM meal have been tested in aquaculture species, showing no negative effects on yield and even improving growth and feed conversion at moderate levels (Belforti et al., 2015; Sánchez-Muros et al., 2016; Gasco et al., 2016; Iaconisi et al., 2017; Piccolo et al., 2017; Mastoraki et al., 2020). In poultry diets, TM meal and oil have been used at levels of 5-10% and below 2.5% respectively, without adverse effects on feed intake, weight gain, or feed efficiency (Ramos-Elorduy et al., 2002; Biasato et al., 2018; Sogari et al., 2019; Hong et al., 2020; Dalmoro et al., 2023). Moreover, including 5% TM meal in laying hen diets has been shown to increase omega-3 levels in eggs and improve egg weight, freshness, and shell strength (Ait-Kaki et al., 2021; Rahmawati et al., 2022).

These findings suggest that TM could serve as a valuable alternative source of protein and lipids in animal feed. However, before this potential can be fully realized, industrial-scale production must be optimized, as several key factors influence productivity (Morales-Ramos et al., 2012; Deruytter et al., 2019; Eberle et al., 2022). One of these factors is the insect diet (substrate), which not only impacts growth performance, fecundity, and survival rate, but also determines the chemical composition of the larvae (Morales-Ramos et al., 2010; Oonincx et al., 2015; Van Broekhoven et al., 2015; Liu et al., 2020). For instance, van Broekhoven et al. (2015) found that including 40% yeast-derived protein in the diet of TM larvae altered their fatty acid profile by increasing the n-6/n-3 ratio, while also promoting faster growth, lower mortality, and greater weight gain. Similarly, Ruschioni et al. (2020) reported that adding 25% olive pomace to the diet increased the protein and amino acid content of the larvae, as well as larval and pupal weight. Rearing mealworms on brewer's spent grain has also been shown to enhance omega-3 and omega-6 fatty acid levels and improve the feed conversion ratio (FCR) (Melis et al., 2019).

In the present study, the potential of supplementing the diet of TM with sources of n-3 FA was investigated. Specifically, camelina (*Camelina sativa* L. Crantz) cake and linseed (*Linum usitatissimum* L.) cake were each included in the diet at levels of 5% and 10%. Camelina is an oilseed crop of the *Brassicaceae* family that has attracted growing interest in recent years due to its favorable attributes, including adaptability to diverse environmental conditions, low input requirements, short growing season and a rich nutritional profile (Murphy et al., 2016). Camelina seeds contain a high oil content (30-49%) with substantial levels of omega-3 and omega-6 fatty acids, along with 24-31% protein and notable amounts of tocopherols, phytosterols, and phenolic compounds (Gesch et al., 2012; Berti et al., 2016; Boyle et al., 2018). Thanks to its nutritional profile, camelina could be used as an alternative to conventionally used soybean. In particular, camelina cake, a by-product of camelina seed pressing, represents an interesting substrate for rearing TM larvae. This by-product contains a high protein content (around 35%), a good amount of essential amino acids, and 10-22% oil with a high proportion of omega-3 fatty acids (Aziza et al., 2014). Similarly, linseed cake, a by-product of linseed oil production, is a valuable substrate for TM, as it provides a high protein content (approximately 36%) and 7-10% residual oil, along with good levels of lignans, antioxidants, and minerals (Poreda, 2017).

Giving these considerations, the present study aimed to evaluate the effect of including camelina and linseed cakes in the diet of TM larvae, assessing their impact on the FA profile (% total fatty acid methyl esters – FAME) on the consumer perception of dried larvae.

Materials and Methods

Experimental design and insects management

The experiment was conducted at the INEF (Insect Novel Ecologic Food) insect farm, (Piombino Dese, Treviso, Italy) and at the Department of Animal Medicine, Production and Health (MAPS) of the University of Padova (Legnaro, Padova, Italy). Four week old TM larvae were randomly assigned to five treatment groups receiving different diets: a control group fed a commercial diet (CON); a group fed a diet with 5% camelina cake inclusion (CAM 5); a group fed a diet with 10% camelina cake inclusion (CAM 10); a group fed a diet with 5% linseed cake inclusion (LIN 5); and a group fed a diet with 10% linseed cake inclusion (LIN 10). All diets were formulated to be isonitrogenous and isoenergy. Each treatment group consisted of 12 replicates.

TM larvae were housed in plastic crates measuring 60 × 60 × 14.5 cm and were provided feed *ad libitum*, along with fresh carrots as a source of water. Ingredients and FA classes of the experimental diets are presented in Table 1.

Larvae were raised for five weeks, and at 9 weeks of age, when they reached the selling size, they were separated from residual feed and frass, then euthanized by freezing at -60°C for one hour using a shock freezer (Challenge 250 TC, Angelantoni, Perugia, Italy). Subsequently, they were transported to the LabCNX laboratory of the MAPS Department (University of Padova) for freeze-drying to perform FA analysis. A portion of larvae from each crate within each experimental group was microwave-dried using a Max Industrial Microwave 30B (Max Industrial Microwave, China) at 103°C (12 kW) for 10 minutes. This method, routinely used by the INEF insect farm, was carried out to prepare samples for sensory analysis.

Table 1. Fatty acid classes (% FAMES) of the experimental diets

	Experimental diets				
	CON	CAM 5	CAM 10	LIN 5	LIN 10
SFA	16.7	15.7	14.8	15.8	15.0
MUFA	25.9	28.3	30.9	26.1	26.2
PUFA	56.0	54.4	53.0	57.4	57.9
n-6	50.9	44.3	38.9	43.5	38.6
n-3	5.12	10.2	14.1	13.9	19.3
n-6/n-3	9.94	4.35	2.77	3.12	2.00

Chemical analyses: FA profile of diets and TM larvae

Lipid extraction to determine the FA profile of the diets and of TM larvae was performed by modified accelerated solvent extraction (M-ASE). For insect samples, extraction was carried out with a chloroform/methanol mixture (1:2 ratio) following the method of Lee et al. (1995), while hexane was used for the diets. The fat content of the samples was determined gravimetrically after vacuum evaporation under a nitrogen stream. Samples were then trans-methylated using a 4% methanolic solution of H_2SO_4 to produce fatty acid methyl esters (FAMES). To achieve biphasic separation, 8 ml of distilled water and 4 ml of n-heptane were added, with these volumes adjusted proportionally due to the high lipid content of the samples. FAMES were quantified by gas chromatography (Shimadzu GC17A). Results were expressed as a percentage of the total detected FAMES, as described by Cullere et al. (2018).

Consumer sensory analysis

For the consumer sensory analysis, a total of 141 employees and students from the University of Padova participated on a voluntary basis. Since a preference test was chosen, participants did not require any prior experience with the specific food matrix, or the sensory method used. Instead, each participant received a

detailed guide explaining the correct procedure for conducting the evaluation. Each participant then evaluated five TM dried larvae samples, representing the different treatments (labelled A, B, C, D, and E), for visual, olfactory, and overall acceptance. Each attribute was rated on a scale from 1 (extremely unacceptable) to 7 (extremely acceptable). The sensory evaluation did not include tasting the samples. TM larvae were subjected to microbiological analysis for the most common pathogens before the sensory test to ensure the safe of the product. However, consumers, were not allowed to touch the samples.

Statistical analysis

Data of FA profile of TM larvae were analysed with a one-way ANOVA following the GLM procedures of the SAS 9.1.3 statistical analysis software for Windows (SAS Institute, 2008), with treatment groups treated as fixed effect. Sensory analysis data were analysed using mixed model ANOVA including diets as fixed effect and effect of consumers as repeated random effect.

Results and Discussion

FA classes of TM larvae

The dietary inclusion of camelina and linseed cakes significantly affected the FA profile of TM larvae (Table 2). The control group showed the highest proportion of saturated fatty acids (SFA) compared to the other groups (27.5% vs 23.8% on average; $P = 0.0001$). Conversely, the control group had the lowest proportion of polyunsaturated fatty acids (PUFA), whereas all supplemented groups showed significantly higher values, except for CAM10 which had an intermediate value (23.6% vs 29.0% and 26.7% on average, respectively; $P = 0.0053$). For monounsaturated fatty acids (MUFA), the CAM10 group exhibited a significantly higher proportion compared to LIN5 group, while the remaining treatments exhibited intermediate values. Supplemented groups also showed an improved n-6/n-3 ratio. The inclusion of camelina and linseed cakes significantly increased the n-3 FA proportion ($P < 0.0001$), thus reducing the n-6/n-3 ratio, with the lowest value observed in the LIN10 group and the highest in the control group (4.43 vs 20.7, respectively; $P < 0.0001$).

These results suggest that feeding TM larvae with PUFA-rich substrates is proportionally reflected in the increased PUFA of the larvae. Notably, TM possesses delta-12 desaturase enzymes and endogenous FA synthesis capabilities. They can synthesize linoleic and α -linolenic acids *de novo*; however, like other terrestrial insects, they are deficient in producing 20:5 n-3 (EPA) and 22:6 n-3

(DHA). Furthermore, they are able to convert n-9 FA to n-6 FA (Brandstetter and Ruther, 2016). Therefore, the diet remains the main factor influencing the proportion of n-3 FA in this species.

Similar to the findings of the present study, Melis et al. (2019) observed that including brewer spent grain, rich in PUFA, in the larvae diet increased their n-3 and n-6 levels.

Consistently, Lawal et al. (2021) demonstrated that supplementing the diet of TM larvae linseed and chia seeds significantly increased the PUFA content and reduced the n-6/n-3 ratio. With an inclusion of 10% of linseed seeds, Lawal et al. (2021) reported a PUFA content of TM larvae of 21.3%, compared to 10.2% found in the present work. Moreover, they reported a n-6/n-3 ratio of 5.92, which was significantly lower than that of the control group, in line with the result of our study. However, it should be noted that in the current experiment, the inclusion of linseed was in the form of linseed cake. This potentially alters the diet's fat content and composition and, as a result, the FA profile of the larvae, explaining the differences in the values between the two studies.

In the present study, the dietary strategy not only increased PUFA levels but also reduced SFA content and the n-6/n-3 ratio, which is a favourable outcome for the potential use of mealworms as an alternative feed and food source. High n-6/n-3 ratios are generally associated with negative health effects, as they can promote inflammation, and increase the risk of cardiovascular diseases. For example, a ratio of 10 has been linked to adverse health outcomes (Simopoulos, 2008; Cao et al., 2024), while a favourable n-6/n-3 ratio is recommended to be around 3:1 to 1:1 (Kim et al., 2007).

Table 2. Effect of camelina and linseed cake dietary inclusion on fatty acid classes (% of Total FAME) of TM larvae

	Experimental groups					RSD	P-value
	CON	CAM 5	CAM 10	LIN 5	LIN 10		
SFA	27.5 ^A	23.7 ^B	23.3 ^B	23.9 ^B	24.2 ^B	1.97	< 0.0001
MUFA	46.6 ^{AB}	46.5 ^{AB}	47.5 ^A	44.8 ^B	46.2 ^{AB}	1.70	0.0165
PUFA	23.6 ^B	28.8 ^A	26.7 ^{AB}	29.8 ^A	28.4 ^A	4.03	0.0053
n-6	22.4	25.8	22.9	25.7	23.1	3.59	0.0729
n-3	1.22 ^D	3.07 ^C	3.85 ^B	4.18 ^B	5.28 ^A	0.55	< 0.0001
n-6/n-3	20.7 ^A	8.39 ^B	5.95 ^{BC}	6.16 ^{BC}	4.43 ^C	2.95	< 0.0001

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

The n-6/n-3 ratio is also a critical factor in animal diets, as it influences growth performance, animal health and quality of the end product. Reducing this ratio, particularly to levels around 4:1 or 2.5:1, through the inclusion of linseed oil or fish oil has been shown to improve the performance and immune response of broilers, enhancing their overall health and resulting in meat enriched with long-

chain n-3 PUFA (Ibrahim et al., 2018). Similarly, decreasing the n-6/n-3 ratio from 30:1 to 5:1 has been shown to reduce the feed conversion ratio (FCR) in broilers (Qi et al., 2010). Furthermore, in Heigai pigs, a lower dietary n-6/n-3 ratio reduced triglyceride and cholesterol levels, while increasing the omega-3 FA content in the *longissimus dorsi* muscle (Nong et al., 2020).

Sensory analysis of TM larvae

Results of consumers sensory analysis are presented in Figure 1. The inclusion of camelina and linseed cakes significantly influenced the sensory attributes of dried TM larvae. For visual, olfactory, and overall acceptance, the CON group received the lowest scores, while the LIN10 group achieved the highest scores (4.10 vs 5.05; 3.76 vs 5.65; 3.90 vs 5.42, respectively; $P < 0.0001$). Intermediate scores were observed for the other treatment groups.

These findings demonstrate that, even though the larvae were not tasted, consumers perceived clear differences in their appearance and olfactory attributes. One possible explanation for these results is the different lipid content observed in larvae. Larvae from the CON group showed a lower ether extract compared to the others, particularly the CAM10 group, which had the highest value. Intermediate ether extract levels were found in the remaining groups (11.5 vs 13.8 vs 12.2 g/100 g, on average, respectively). Lipids play a crucial role in flavour and off-flavour development and depending on the reactions or degradation processes involved, they can impart either pleasant or unpleasant notes to the product. For instance, when exposed to heat, especially at high temperatures, lipid degradation produces aldehydes that can participate in the Maillard reaction and Strecker degradation (Shahidi et al., 2014). The compounds generated by these combined processes contribute to the development of desirable aromas (Kerth et al., 2015; Shahidi et al., 2015). This may explain the higher olfactory acceptance of larvae with a higher lipid content compared to those from the CON group, where the lower lipid content may not have allowed these processes to occur, resulting in less pleasant odours.

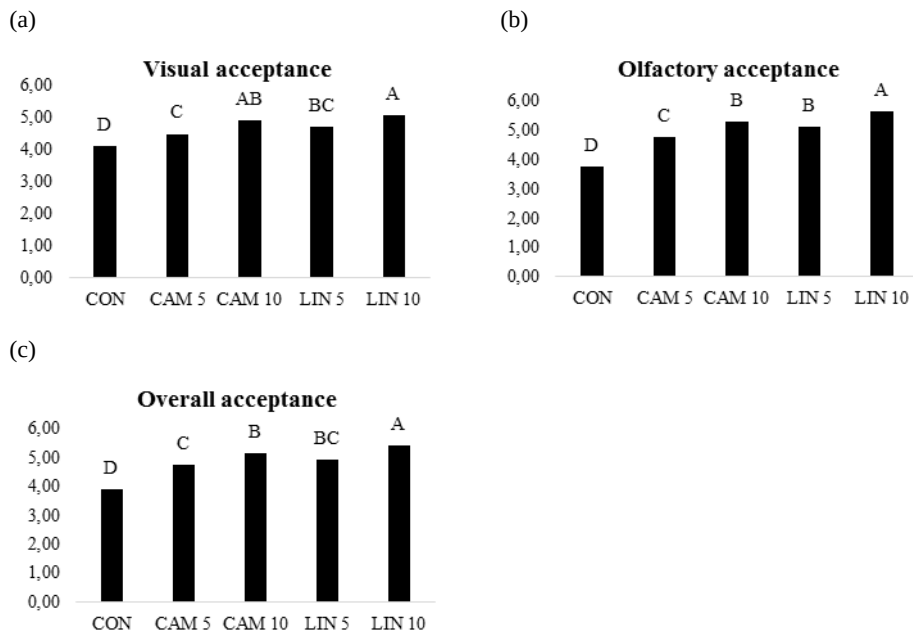


Figure 1. Effect of camelina and linseed cake dietary inclusion on visual **(a)**, olfactory **(b)** and overall **(c)** acceptance of dried TM larvae

value from 1 to 7= 1: extremely unacceptable, 2: very unacceptable, 3: moderately unacceptable, 4: neither unacceptable or acceptable, 5: moderately acceptable, 6: very acceptable, 7: extremely acceptable; A-D Values within a row with different superscripts differ significantly at $P < 0.0001$;

Additionally, although TM larvae were dried using the same method, differences in the final product were possible. Particularly, larvae from LIN10 group showed a lower as compared to CAM5 group, while the other groups showed intermediate values (0.45 vs 0.69 and 0.61 on average, respectively; $P < 0.0125$).

Despite the product was dried, the residual moisture may have influenced the olfactory traits of larvae, resulting in a less appealing odour. Some microorganisms indeed, are able to survive also at low values of a_w , promoting reactions responsible for the formation of odorous compounds. This could have influenced the overall acceptance of the larvae of CAM5 and CON group.

Regarding visual acceptability, no differences in size were observed when comparing larvae across the groups. It is possible that the olfactory traits also influenced the visual and overall evaluation of the larvae.

Conclusion

In conclusion, the present study highlights the potential of incorporating camelina and linseed cakes in the diet of TM larvae, enhancing their FA profile. Specifically, the inclusion of 5% and 10% of camelina and linseed cakes significantly increased the PUFA content of larvae and decreased the n-6/n-3 ratio to more nutritionally desirable levels, particularly at the higher inclusion level of linseed cake. Additionally, dietary treatments significantly improved consumers acceptance of TM dried larvae especially with the 10% inclusion level of linseed cake. Overall, these findings demonstrate that oilseed cakes can be valuable, sustainable feed ingredients to improve the nutritional quality and consumer appeal of edible insects.

Ethics approval

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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COMPARISON OF TIME CONSUMPTION BETWEEN TRADITIONAL AND INNOVATIVE METHODS OF PRODUCING QUEEN CELL CUPS

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Abstract: The production of high-quality honey bee queens is one of the most important beekeeping operations, considering the key role of the queen in the colony. On the other hand, queen rearing is a highly challenging process, so the simplification of certain production segments would represent a significant advantage. In this paper, we examined and compared the traditional method of producing wax queen cell cups using a wooden mold with an innovative method that involves the use of a silicone mold together with a specially designed cell bar. The comparison of efficiency between the tested methods was carried out through the measurement of time consumption for both methods. Our results indicate that the production of queen cell cups using the innovative method is 2.66 times faster compared to the traditional method. In addition, we examined the differences in queen cell cup height obtained by the traditional method, which showed high variability, ranging from 8.1 mm to 15.9 mm, unlike the innovative method in which queen cell cups were uniform and measured 11 mm. On professional apiaries, where there is a high demand for the production of honey bee queens, queen cells, and royal jelly, our innovative method can significantly facilitate production by accelerating the process of producing queen cell cups, while at the same time ensuring the uniformity of the produced cell cups. Also, using a specially designed cell bar allows all queen cells to be added or removed at once from the grafting frame and provides the possibility of simple individual separation of the queen cells.

Key words: queen cup, queen cell, wax, queen production

Introduction

The honey bee queen is crucial for colony survival and functioning due to her high egg-laying capacity and pheromonal regulation of social cohesion (Amiri et al., 2017). Beekeepers have developed techniques for rearing large numbers of young queens, which are used to replace old ones, as young queens enhance productivity and reduce swarming (Tarpy et al., 2000; Wu et al., 2018; Büchler et al., 2013; Akyol et al., 2008; Gatoria et al., 2004a). Most queen rearing methods require queen cell cups as holders for grafted larvae. Cup size affects queen quality parameters such as body weight, thorax size, and number of ovarioles (Wu et al., 2018), and may also influence grafting acceptance (Mattiello et al., 2022). In nature, queen cells are 8–10 mm in diameter, while plastic cups commonly measure about 9.4 mm (Büchler et al., 2013; Shi et al., 2011).

The grafting process and artificial queen rearing have been practiced since the 19th century (Doolittle, 1889). Early systems used wax cups, the basis of today's production (Büchler et al., 2013). In recent decades, synthetic materials such as plastic cups have been introduced (Laidlaw & Eckert, 1962; Warren et al., 1982; Abou-Shaara et al., 2024), along with devices such as Karl Jenter, Cupkit, and Nikot (Gatoria et al., 2004b; Wakijra et al., 2019). While useful, these systems face limitations in intensive production due to their small capacity (up to 150 cups), making large-scale use impractical (Sena et al., 2010; Dhaliwal et al., 2019). In contrast, queen isolation on standard frames provides several hundred to several thousand larvae for grafting into wax cups.

Wax is still commonly used in queen production for making cell cups, as it has shown good results in terms of larval acceptance (Lashari et al., 2022). Even with plastic cups, wax is required for attachment or rim coating to improve acceptance (Ruttner, 1983; Woodward, 2007; Büchler et al., 2013). However, preparing and attaching cups to cell bars is time-consuming and slows down production. Since commercial operations depend on efficiency, high productivity and time saving are essential, although most beekeeping is still carried out by small-scale or hobbyist beekeepers. Commercialization of queen rearing requires mass production of large numbers of high-quality queens (Hatjina et al., 2014; Büchler et al., 2013).

Many studies have examined the effects of queen morphology, nutrition, and rearing method on colony productivity (Skowronek et al., 2004; Akyol et al., 2008; Dolasevic et al., 2020). However, it should be kept in mind that the api-technique and the method of queen rearing are very important in intensive queen production, especially if it affects the speed and quality (improvement) of certain production segments. In this paper, we present an innovative method for producing queen cell cups and compare it with the traditional approach. High productivity and

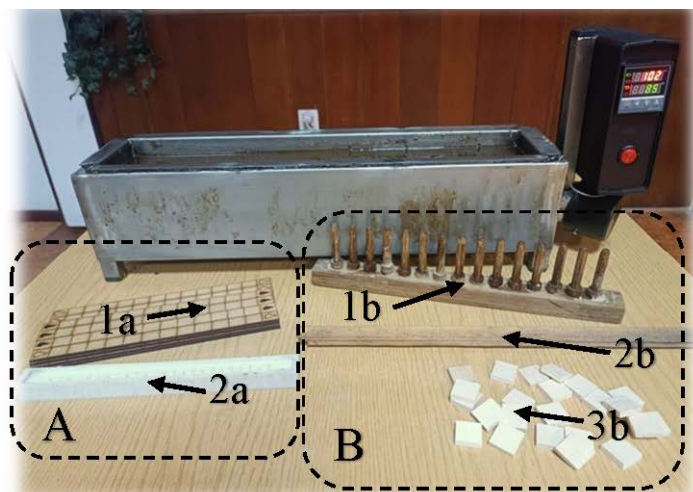
time saving are especially important in large and intensive production. It is important to point out that commercial beekeeping operations usually represent only a minor share of the overall beekeeping sector, as the majority of beekeeping operations are carried out by small-scale or hobbyist beekeepers.

Materials and Methods

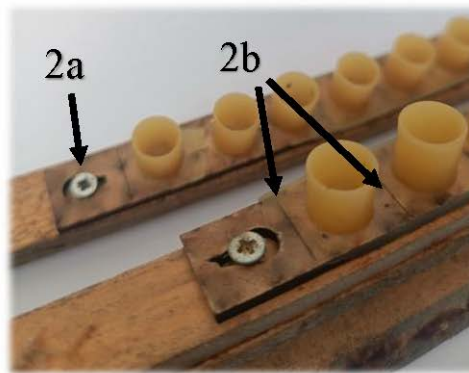
In this study, we examined the time consumption between the traditional and the innovative method of obtaining queen cell cups. During the experiment, we conducted comparative testing of the two technological processes, followed by analysis in order to determine the time differences and potential advantages.

The first technological process, the innovative method, involved the use of a new approach (Picture 1, A) consisting of a silicone mold (Picture 1, 2a) in combination with specially designed cell bars (Picture 1, 1a). The entire process included the simple pouring of melted wax (using a syringe) into the silicone mold. In our case, after pouring the wax into the silicone mold, a specialized cell bar was added to the top of the silicone mold, in the designated position. The placement of the specialized cell bar represents the final step, since all queen cell cups are simultaneously formed and attached to the specialized bar. At the ends of the specialized bar there are two conical openings (Picture 2, 2a) which allow direct attachment to the frame on cell bar. The attachment of the cell bar is ensured with two screws that provide a secure hold. The bar is cut with slots spaced at equal distances (containing segments) (Picture 2, 2b). The specialized bar contains a total of 12 segments designed for wax cell cups.

The second technological process, the traditional method, consisted of using a wooden mold, which is among the most commonly used methods for obtaining wax cups (Picture 1, B). The wooden mold (Picture 1, 1b) must be kept (soaked) in cold water before work begins, after which it is dipped into melted wax. The ideal dipping depth is 10–11 mm, with each subsequent dipping being shallower than the previous one. In our experiment, dipping was performed twice (in wax as well as in water) in three successive dips. The obtained wax cups were attached (glued with wax) with a syringe onto individual wooden holders (Picture 1, 3b), which were then attached to the cell bar (Picture 1, 2b).



Picture 1. Preparation for production of queen cup bases using different methods (A – Innovative method; B – Traditional method)



Picture 2. Principle of attaching the specialized bar to the frame bar in the innovative method

During the process of obtaining and attaching wax cups, we monitored the time consumption for each individual operation (T) in both tested methods.

Traditional method included:

Time required for obtaining (production) wax cups with a wooden mold (T1)

Time required for waxing (sticking) wooden holders to the cell bar (T2)

Time required for waxing (sticking) cups onto wooden holders (T3)

Innovative method included:

Time required for pouring melted wax in the silicone mold and adding the specialized bar (T4)

Time required for removing the specialized cell bar with cups from the silicone mold (T5)

The mounting/attaching of the specialized cell bar with cups was not monitored because the specialized cell bar is mounted instantly — it simply slides onto two screws, allowing all queen cups to be positioned immediately. Time measurement for parameters T1–T5 was conducted after complete preparation (e.g., reaching the proper wax temperature, prior cooling of the wooden mold in water, etc.). In this way, preparatory activities were not taken into account; the efficiency of work was measured from the moment the process began. Time

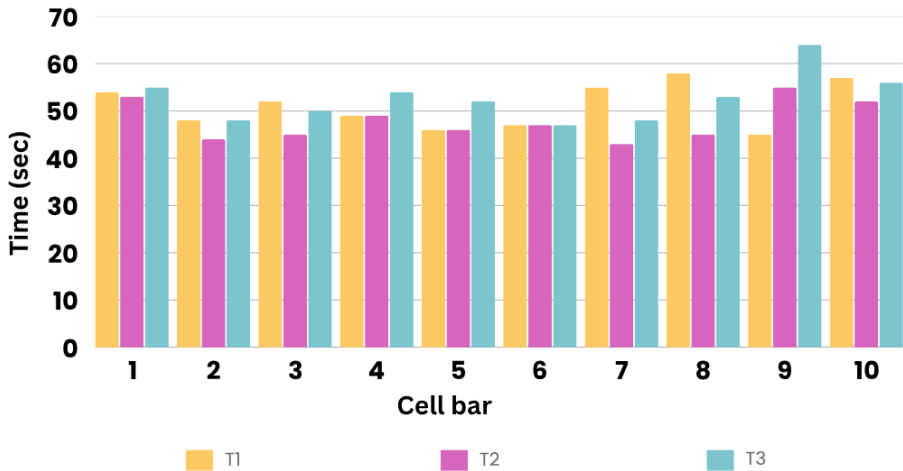
consumption for the listed parameters was calculated per cell bar containing 12 wax cups, expressed in seconds.

We also measured the height of queen cell cups obtained by both tested methods. The measurement of heights was carried out using a digital caliper from the outer side of the queen cup base. The total number of measured cups was 30.

The set temperature for obtaining cups with the wooden mold was 85 °C, while for obtaining cups with the silicone mold the temperature was 105 °C. Wax melting was performed in a special wax melter with digital temperature regulation, used for both methods. During wax heating, the temperature must not exceed 140 °C (Bogdanov, 2016). Sticking of wax cups and wooden holders was carried out with a 20 ml syringe (also at 105 °C). The wax used in the experiment was obtained by re-melting wax from honey cappings, characterized by high quality.

Results and Discussion

In Graph 1, the values of time consumption (T) using the traditional method are presented.

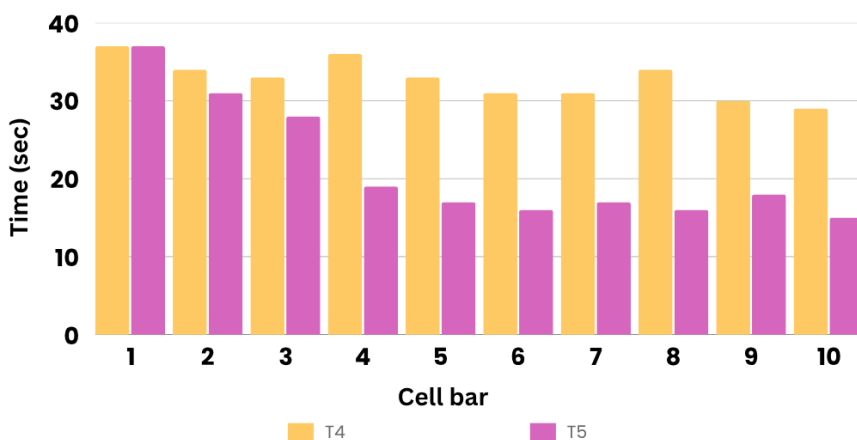


Graph 1. Time consumption expressed (sec) for the traditional method (T1 – time required for making 12 queen cell cups; T2 – time required for sticking wooden holders to the cell bar; T3 – time required for sticking cups onto wooden holders)

The values of time consumption (T1-T4 parameters) were carried out in 10 repetitions, with one cell bar containing 12 wax cell cups representing a single

repetition ($\Sigma = 240$ wax cell cups). The average time consumption for making 12 cell cups using the traditional method was 51.1 seconds, the average time for sticking 12 wooden holders was 47.9 seconds, and the time required for sticking 12 cell cups to the wooden holders was 52.7 seconds. Accordingly, the total time consumption for the traditional method amounted to 151.7 seconds for the complete process per one cell bar.

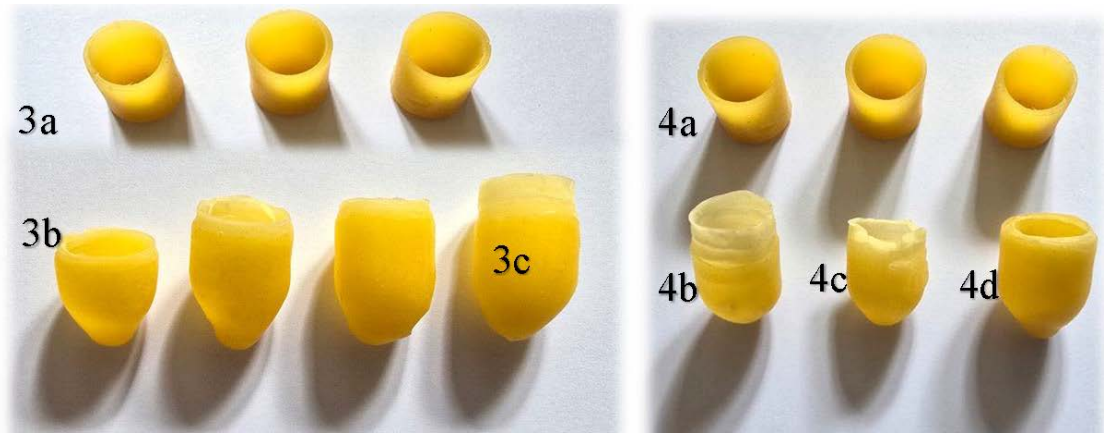
In Graph 2, the values of the time spent (T4 and T5) when using the innovative method are shown. Graph 2 presents the average values of ten independent measurements (i.e. cell bar), which represent the average time spent for 12 cell cups.



Graph 2. Time consumption expressed (sec) for the innovation method (T4 – time required for pouring melted wax in the silicone mold and adding the specialized bar; T5 – time required for removing the specialized cell bar with cups from the silicone mold)

The average time required for filling (pouring) the silicone mold (T4) was 32.8 seconds. The average time required for removing the cell bar with cups from the silicone mold (T5) was 21.4 seconds. The total time consumption with the innovative method was 56.9 seconds for the entire procedure per cell bar.

Furthermore, regarding the uniformity of queen cell cups, the innovative method produces bases that are completely uniform, which is achieved through the use of a silicone mold. Thus, the height of all queen cells obtained by the innovative method is consistent, measuring 11.0 mm (Picture 3, 3a). Contrary, the use of the traditional method primarily results in variation in the height of queen cell cups (Picture 3, 3b, 3c).



Picture 3. Differences in the size of cell cups (3a – innovative method, uniform cell cups; 3b, 3c – traditional method, 3b – smaller cell cup 3c – traditional method, larger queen cell cup).

Picture 4. Differences in the appearance of cell cups (4a – innovative method, uniform queen cells; 4b, 4c, 4d – traditional method, 4b – thin upper part of the queen cell; 4c – damaged edge; 4d – thick wall of the queen cell).

Moreover, with the traditional method, the appearance of queen cells largely depends on the individual producing them, since it is challenging to dip the wooden mold to the exact same depth in the wax melter each time, as the entire process is performed manually. As a result, various abnormalities occur in queen cells obtained by the traditional method, such as differences in wall thickness, damage caused during removal or handling, and other irregularities (Picture 4).

The following table (Table 1) presents the values of the height of the cell cups produced by the traditional method.

Table 1. Differences in the height (mm) of queen cells obtained using the traditional method

Queen cell	Height (mm)	Queen cell	Height (mm)	Queen cell	Height (mm)
1.	9	11.	12	21.	12
2.	13	12.	10	22.	13
3.	14	13.	14	23.	11
4.	11	14.	16	24.	16
5.	11	15.	9	25.	12
6.	14	16.	12	26.	11
7.	12	17.	14	27.	9
8.	13	18.	8	28.	10
9.	15	19.	12	29.	12
10.	9	20.	15	30.	13

The height of queen cell cups produced by the traditional method ranged between 8 mm and 16 mm, with a mean value of 12.0 ± 2.1 mm (SD = 2.17). The high coefficient of variation (17.5%) indicates significant inconsistency in cup height produced by the traditional method. Unequal queen cell height can be inappropriate, as bees are less likely to accept large or very small queen cell cups, resulting in lower acceptance rates. In addition, during the storage and handling of fragile cell bars (with queen cell cups), large cell cups are often damaged, leading to cracking and falling off the cell bar. This highlights that the traditional method should be standardized to improve the quality and efficiency of queen cell production.

By comparing all data of the tested methods, it can be concluded that the time savings with the innovative method is 2.66 times greater compared to the traditional method. This time saving indicates that the innovative method significantly increases productivity, which is very important in a large-scale beekeeping companies where it is often necessary to make tens of thousands of cell cups every year. Our innovative method directly results in greater efficiency in commercial beekeeping production of queen bees, queen cells or royal jelly.

In addition to the above, the presented innovative method has certain technical advantages (such as easier maintenance of the silicone mold compared to the wooden mold, uniformity of all obtained cell cups, and faster production of cell cups), as well as specific beekeeping (apitechnical) advantages (simplified handling during grafting since all queen cells are positioned on a specialized cell bar, one-step placement and removal all cell cups (queen cells) from the specialized cell bar, and the possibility of separating each individual queen cell by simply bending (cracking) the specialized bar). Silicone molds are inexpensive and highly durable, often lasting for many years if used properly. They do not require cleaning from wax residues, as wax does not adhere to silicone surfaces in the same way it does to wooden molds. These characteristics contribute to reduced maintenance time and long-term cost-effectiveness in queen cell production.

It should be mentioned that there are silicone molds (available today) that allow the making of individual wax cups, but individual production of queen cell cups is not a solution for intensive production since the process is very slow. In contrast, the specialized bar in our innovative method serves as a common holder for all queen cups, which eliminates the entire procedure of individual production of cups, their attachment to wooden holders, as well as attaching the wooden holders to the cell bar. In this way, the specialized bar enables one-step addition and removal of queen cell cups or ripe queen cells.

The bar contains slots spaced at equal distances, which allow that, with a simple slight bending, the segments of the specialized bar can be separated,

enabling easy detachment of ripe (individual) queen cells. This structural advantage simplifies further manipulation during the rearing process.

When using the traditional method with a wooden mold, it is important to emphasize that multiple dips are necessary to ensure the required thickness of the cup walls (Gupta et al., 2014). Although the wooden mold we applied contained fifteen “fingers,” only twelve were used in our investigation in order to ensure comparability with the silicone molds.

Finally, the quality of the wax is an important factor influencing the success of obtaining queen cell cups. Wax originating from old comb may contain a significant amount of cocoons, feces, propolis, and pollen (Svečnjak et al., 2019), and therefore should be avoided for the production of wax queen cell cups.

Conclusion

The presented method of using a silicone mold and a specialized cell bar can greatly simplify work and increase the efficiency of queen cell cup production. The method of producing queen cell bases using the standard approach (with a wooden mold) requires considerable time and effort, with the final outcome being non-uniform queen cells obtained through a time-consuming process. For this reason, this type of wooden mold does not meet the requirements of intensive beekeeping operations.

The results presented here are part of a broader study that will also assess additional quality parameters. However, this study primarily focused on the efficiency of the production of queen cell cups themselves. Future research will include the integration of the specialized cell bar into the complete rearing system at the apiary, with the aim of examining its overall impact on production efficiency, the amount of wax needed, and the strength of adhesion between the queen cell cups produced by both examined methods. These parameters will contribute to a more comprehensive understanding of all the advantages of the presented innovative method.

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Conflict of interest

The authors declare no conflicts of interest.

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ANTIPARASITIC EFFECTS OF *Artemisia absinthium* L. IN THE TREATMENT OF HEXAMITIOSIS IN RAINBOW TROUT (*Oncorhynchus mykiss*) CULTURE

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Abstract: In recent years, the inefficacy and adverse effects of synthetic drugs and therapeutic agents used against the increasing prevalence of diseases in aquaculture have highlighted the need for introducing medicinal plants with antimicrobial properties into the sector. The use of plant-based products in the treatment of cultured fish species holds significant potential, particularly in supporting organic aquaculture practices. In this study, the antiparasitic activity of the ethanol extract of *Artemisia absinthium* L. (wormwood) was investigated against Hexamitiosis disease caused by *Spironucleus salmonis* in juvenile rainbow trout. The plant extract was incorporated into fish feed at doses of 1.5, 2.0, and 2.5 g/kg and administered to infected fish with an average body weight of 1.5–2.0 g for both short-term (7 days) and long-term (21 days) treatment periods. The results revealed that the *A. absinthium* extract exhibited antiparasitic efficacy against *S. salmonis* on both the 7th and 21st days. Cumulative mortality rates decreased in all experimental and positive control groups, with the 21-day application proving more effective than the 7-day treatment. The highest antiprotozoal activity was observed with the 2.5 g/kg dose on the 21st day, showing a statistically similar effect to that of the positive control ($p < 0.05$). Based on these findings, it was concluded that ethanol extracts of *A. absinthium* could be used as a natural and alternative antiparasitic agent for controlling *S. salmonis* infections in rainbow trout aquaculture.

Key words: *Artemisia absinthium* L., *Spironucleus salmonis*, rainbow trout (*Oncorhynchus mykiss*), antiparasitic, treatment

Introduction

Spironucleus is a flagellated single-celled parasite that can be found in the intestines of fish. *Spironucleus* infections are becoming serious, especially under high-density fish farming conditions. *Spironucleus* has been found in many other fish species, including salmonids and tilapia and aquarium fish. Infections primarily affect the intestine and tend to be chronic in nature. It has been reported that the disease is transmitted by oral ingestion of cysts and trophozoites of the parasite. Fish population density is effective in the occurrence of the disease (Woo, 1999). The disease plays an important role in cichlid cultivation and is frequently the most important profit-limiting factor in intensive aquaculture. Dimetridazole and metronidazole are commonly administered through feed in the treatment of *Spironucleus* infection. However, due to the adverse effects of metronidazole on DNA and cell structure, its use in aquaculture within European Union countries is prohibited by Directive No. 613/98. Thus, there is an urgent need to systematically evaluate the traditionally used medicinal plants for getting an alternative measure for controlling Spironucleosis in aquaculture (Noor El Deen and Mohamed, 2009; Militz et al., 2013).

The use of phytochemicals as antiparasitic drugs for fish health may help to support sustainable and environmentally acceptable treatment applications (Park et al., 2011; Hao et al., 2012). There are previous studies reviewing investigations that have examined the antiparasitic effects of plants on fish parasites (Wink, 2012; Ramudu and Dash, 2013; Reverter et al., 2014; Syahidah et al., 2015; Lieke et al., 2019). In recent years, many researchers have focused on the use of herbal plants against parasitic species in aquaculture (Chitmanat et al., 2005; Puk and Guz, 2014; Williams et al., 2016; Trasvina-Moreno et al., 2017; Yildiz et al., 2019).

Traditionally, *Artemisia absinthium* L., a plant widely distributed and naturally occurring in Anatolia, particularly in the Mediterranean region, exhibits diverse biological properties such as antihelminthic, antibacterial, antifungal, antioxidant, antimalarial effects, with terpenoids and flavonoids as active ingredients (Kordali et al., 2005; Bora and Sharma, 2010). *A. absinthium* has always been of pharmaceutical and botanical importance and used to manage several disorders including hepatocyte enlargement, hepatitis, gastritis, jaundice, wound healing, splenomegaly, dyspepsia, indigestion, flatulence, gastric pain, anemia, and anorexia.

The risks of chemical use for eliminating the parasites include anthelmintic resistance, risk of residue, environmental contamination, and toxicity to hosts. Therefore, this study aims to determine the potential *in vivo* antiparasitic activity of *A. absinthium* L. extract as an alternative for controlling Spironucleosis (Hexamitiasis) in rainbow trout.

Materials and Methods

Infected juvenile rainbow trout samples were obtained from trout farms in the Aksu-Isparta region in January-February. The intestinal tissues of infected fish were examined under a microscope. Spironucleus infestation is easily confirmed by making a fresh-tissue squash preparation of the intestine and examining it with a light microscope at 200 and 400x magnification. The flagellates have a distinctive, ovoid to pyriform (“pear”) shape and rapid, erratic movement. They are most easily seen in the mid to posterior intestine. The infected rainbow trout were transported to Fisheries Faculty Research and Application facility. The experiment was set up for 50 fish each group with an average weight of 1.5-2 g, as triplicate. Fish were placed in 400-liter round fiberglass tanks. The research was carried out over two different periods: short-term (7 days) and long-term (21 days) and a running water system was used.

Preparation of ethanol extract and essential oil of *A. absinthium* L.

A. absinthium L. was defined by Prof. Dr. Hasan ÖZÇELİK from Süleyman Demirel University, Faculty of Sciences, Department of Biology. The chemical structure of *A. absinthium* L. was determined by the Gas chromatography device (using GC/MS apparatus) at the Experimental and Observational Research Laboratory of Süleyman Demirel University. For preparation of plant extract, after keeping 15 g of dry plant sample in 50 ml of ethanol for 2 hours, it was passed through Whatman No1 filter paper for filtration, evaporation was carried out with the help of a water bath, and the resulting extract was stored at -18°C until use. To obtain essential oil, the crude powder samples (200 g) were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus.

Experimental diets

Different concentrations of ethanol extract (1.5, 2.0, 2.5 g/kg) were added to the mixture together with sunflower oil (0.05 mL/kg). Juvenile rainbow trout were fed at 3% of their body weight for 7 days (short term) and 21 days (long term). The negative control group was fed with commercial trout feed at 1% of their body weight. In the positive control group, they were fed trout feed supplemented with 0.5 mg/kg of Metronidazole. All procedures on experimental fish were carried out in accordance with national ethical rules approved by the Süleyman Demirel University Animal Care and Use Committee (Republic of Turkey Süleyman Demirel University Experimental Animal Use Ethics Committee, E-77211729-804.01-18968).

Determination of the therapeutic effect of *A. absinthium* L.

Determination of survival rate

The survival percentage of the fish (RPS = Relative Percent Survival); it was calculated according to the formula (Amend, 1981):

$$RPS = [1 - (\text{Mortality in the } A. \text{ absinthium L. fed group (\%)} / \text{Mortality in the control group (\%)})]$$

Determination of the number of parasites in tissues

Parasite density was determined by counting the parasites in the intestine and feces according to Tojo and Santamarina (1998). For this purpose, after the fish were stunned with phenoxyethanol (0.1-0.5 ml/L), slide coverslip preparations prepared from feces and intestinal contents were examined under a microscope at 400x magnification. In the evaluation; if more than 50 parasites are found in the microscopic area, 4 points (high (+++)), 3 points (medium ++) between 10-50), 2 points (low (+) between 1-10), 1 point (if there was 1 piece, it was interpreted as minimal (+/-)), if no interference was found, it was interpreted as zero score (-).

Data Analysis

The data obtained in the experiment were evaluated with the ANOVA test (Duncan multiple comparison test) in the SPSS 16.0 package program (SPSS Inc, Chicago, IL, USA). When comparing the importance levels of various parameters examined in the experiment, the results are given as mean value and standard deviation. The significance level was chosen as $p < 0.05$.

Results

***A. absinthium* L. GC-MS analysis**

The composition of *A. absinthium* L. was analyzed by GC-MS and the major components were sabinol (42.22%), chrysanthenylacetate (14.73%), epoxyocimene (4.97%), thujone (2.19%), d-isothujone (3.97%).

Result of relative survival rate (RPS) and cumulative mortality

According to research findings, it was determined that cumulative mortality (%) decreased and survival rates increased in fish fed with *A. absinthium* L. extract, similar to the positive control, for 21th day (Table 1).

Table 1. Effect of *A. absinthium* L. extract on relative survival rate (RPS) and cumulative mortality in infected rainbow trout

	Cumulative Mortality (%)		RPS (%)	
	7th day	21th day	7th day	21th day
1.5 g/kg	8.67±0.67 ^{ab}	14.00± 0.67 ^{bc}	12.50±1.25 ^a	29.91±1.34 ^a
2.0 g/kg	8.67±2.00 ^{ab}	10.00±2.00 ^{ab}	25.90±1.16 ^a	49.11±1.34 ^{ab}
2.5 g/kg	8.00±0.00 ^{ab}	4.00±1.00 ^a	19.65±5.35 ^a	78.57±2.14 ^b
Positive control	9.34±1.34 ^{ab}	14.00±2.00 ^{bc}	19.65± 5.35 ^a	30.36±5.33 ^a
Negative control	10.00±0.67 ^{ab}	20.00±1.33 ^c	-	-

*Different letters in the same column are statistically significant (p<0.05)

Effect of *A. absinthium* L. extract on the number of parasites in the digestive canal of rainbow trout

It was determined that *A. absinthium* L. extract was not effective on the 7th day, but on the 21st day, the group treated with 2.5 g/kg of *A. absinthium* L. was found to be more effective in terms of the intensity and prevalence of infection than positive control and other groups (p<0.05) (Table 2).

Table 2. Effect of *A. absinthium* L. extract on infection intensity and infection rate (prevalence) in infected rainbow trout

	Intensity of infection		Prevalence (%)	
	7th day	21th day	7th day	21th day
1.5 g/kg	++	+	83.21±1.06 ^b	54.26±1.40 ^c
2.0 g/kg	++	+	83.21±2.03 ^b	29.62±1.46 ^d
2.5 g/kg	+	-	86.95±0.12 ^a	3.47±0.04 ^f
Positive control	++	-	55.14±1.48 ^d	1.55±0.08 ^f
Negative control	+++	+++	88.14±0.79 ^a	100.00±0.00 ^a

*Different letters in the same column are statistically significant (p<0.05)

Discussion

The flagellated protozoan from the genus *Spironucleus* (Kolisko et al., 2005) causes significant losses in both food and ornamental fish production (Sterud et al., 1998; Paull and Matthews, 2001; Jørgensen and Sterud, 2006). They often cause disease when the host has low resistance or is adversely affected by

predisposing factors such as low oxygen content or overcrowding (Lom and Dykova, 1992). Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is a 5-nitroimidazole; a heterocyclic compound with a nitro group on the fifth position of an imidazole ring. It is the active compound of nitroimidazole, used in the treatment of infections induced by anaerobic bacteria and protozoa (Guz and Szczepaniak, 2009; Sangmaneedet and Smith, 1999). But has been banned from use in the treatment of food fish in Europe and the USA due to its potential carcinogenic properties, persistence in the environment and toxicity to aquatic organisms (Lanzky and Halling-Sorensen, 1997; Payne et al., 1999; Treves-Brown, 1999). The severe restrictions on the use of metronidazole highlight the need for alternative Spironucleus treatments in food and ornamental fish (Millet et al., 2011).

Medicinal plants have been shown to possess antiprotozoal activity, and their use in traditional medicine has led to the development of new antiparasitic drugs. Unlike conventional chemical treatments, medicinal plants are generally considered safe for the environment and do not pose a risk to human health. Additionally, many medicinal plants are readily available and inexpensive, making them an attractive option for aquaculture producers (Özil, 2023).

Antiprotozoal activity of medicinal plants is attributed to their chemical composition, such as monoterpenes (1,8-cineole, alfa pinene, camphor, linalool, sabinene, and thujone), sesquiterpenes (e-nerolidol, germacrene B, and cadalene), isoprenoides, and terpenoids that include aldehydes, ethers, alcohols (carvacrol, thymol, eugenol, and borneol), and sulfur- or nitrogen-containing compounds (diallyl disulfide and indole) (Morsy, 2017).

A. absinthium and its extracts have antiprotozoal activities. The extracts of the plant exhibited antiprotozoal action on several apicomplexan parasites (e.g., *Eimeria*, *Plasmodium*, *Toxoplasma*, *Babesia*, and *Theileria*) and other protozoan parasites (e.g., *Trypanosoma cruzi*, *T. brucei*, and *Leishmania infantum*) (Fernández-Calienes Valdés et al., 2008; Nozari et al., 2016; Batiha et al., 2019). Coccidiosis is one of the most important infections in livestock caused by *Eimeria* species and of greater economic importance due to high morbidity rates. Anticoccidial activity of *A. absinthium* extracts has been reported in ruminants as well as in poultry; however, the activity depends on the number of oocysts and the type of *Eimeria*. Aqueous *A. absinthium* extract at a dose of 3 mg/kg of feed per day induced a substantial reduction in the number of oocysts in broiler infected with *Eimeria tenella* and can be used as prophylactic treatment for moderate coccidiosis (Kostadinovic et al., 2012; Habibi et al., 2016).

In a study, *in vitro* the antiprotozoal properties of aqueous and alcoholic fractions of *A. hippocastanum*, *A. sativum*, *A. rusticana*, *O. vulgare* and *T. vulgare* was examined against *Spironucleus vortens* and concluded that extracts of these

plants are suitable candidates for antiprotozoal drug discovery for control of spironucleosis (Puk and Guz, 2014).

In a another study was to determine the *in vivo* antiparasitic activity of the *Artemisia campestris* (L) ethanol extracts for control of Spironucleosis (Hexamitiasis) in rainbow trout (*Oncorhynchus mykiss*). Treated rainbow trout with an average weight of 1.5-2.0 g, were fed with 7 and 21-day periods with the addition of plant extracts of 1.0, 1.5, 2.0, 2.5 and 3.0 g/kg. The composition of ethanol extract of *A. campestris* (L) was analyzed by GC-MS and the major components were acenaphthylene 1,2-dihydro (62.70%), n-octadecane (4.66%), capillin (4.95%), curcumene (4.45%), 2,4-pentadiynylbenzene (7.28%), benzaldehyde (2.53%), methacrolein (1.35%). Their results of study showed that cumulative mortalities were decreased in all treatment groups and positive control, compared to the negative control (Diler et al., 2018).

In this study, 58 different components were obtained in the chemical composition of the extract of *A. absinthium* and they are rich in monoterpene components which, sabinol (42.22%), thujone (2.19%), d- isothujone (3.97%). Terpenes interacts with the lipids in the cell wall, increase the permeability of the membrane. Naturally, disruption of the physicochemical structure causes in proton movement and electron flow and therefore transport in the cell and coagulation of the cell content (Erdoğan and Everest, 2013). In this research, *A. absinthium* was effective on *Spironucleus*. It has been understood that *Artemisia* species are an alternative for the protection of the aquatic ecosystem and sustainable aquaculture production and can contribute to organic production. It has been determined that higher doses are more effective than lower doses. It has been observed that *Artemisia* group herbal products and has no harmful effects on fish. In our research, the effective doses of the ethanol extract of *A. absinthium* L., was found for the treatment of fish pathogenic *Spironucleus* infections and it was determined that the long period (21 days) was more effective than the short period (7 days) in the treatment against the parasite ($p < 0.05$).

Conclusion

In future studies, should be conducted on sectoral field practices for the treatment of *Spironucleus* infections and disease prevention strategies should be developed by giving importance to prophylactic studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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THE INFLUENCE OF SEASONAL FACTORS ON DAILY MILK PRODUCTION AND CHEMICAL COMPOSITION OF MILK IN DAIRY COWS

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Abstract: The production characteristics of dairy cows are subject to the influence of numerous environmental and management factors, among which seasonal changes play an important role. The aim of this study was to analyze the influence of the seasons on the amount of milk produced daily and the amount and proportion of milk fat and protein in the milk. Data collected during regular milk control were used, and statistical analysis was performed at the level of more than one million records for each characteristic. The results showed a pronounced seasonal variability of production parameters. The highest daily milk yields were recorded in spring and winter (23.50 kg and 23.00 kg respectively), while these values were lower in summer and autumn. The milk fat and protein content also varied depending on the season - the highest fat and protein contents were recorded in the colder months, while the lowest were recorded during summer, which can be associated with heat stress and reduced feed intake. Seasonal variability in production indicates the need for seasonally adapted herd management strategies, including ration optimization, thermal comfort, and technological adaptation. The research provides an important contribution to understanding the dynamics of production traits throughout the year and provides a basis for further modelling of effective management systems that would enable more sustainable and stable dairy farming under climate change.

Key words: dairy cows, seasonal effect, daily milk yield, milk fat, milk proteins

Introduction

Dairy farming is one of the most important branches of livestock production, with an emphasis on producing high-quality milk while preserving animal and environmental health. Modern trends in agriculture, including the growth of the world population and changes in eating habits, are putting pressure on dairy producers to increase production while reducing negative impacts on the environment (FAO, 2009; Dopelt et al., 2019). Sustainable intensification of milk production is becoming a necessity, and in this context, optimizing the production characteristics of dairy cows is of particular importance.

One of the key factors affecting milk production and its composition is climatic and seasonal conditions. The season in which a cow is during lactation can significantly affect the daily amount of milk produced, the proportion of milk fat and protein, due to changes in ambient temperature, the availability and quality of feed, and the physiological responses of the animal to environmental stresses (Gantner and Barać, 2016; Domaćinović et al., 2015). Heat stress during the summer months reduces feed intake, resulting in lower milk production and changes in its chemical composition (Kirchgessner et al., 1986; Eicher, 2004). In contrast, during colder seasons, production stabilizes and milk quality is often improved.

Milk yield monitoring, as a system for collecting and analyzing data on the milk production parameters of cows, provides valuable information for assessing the impact of various factors on milk production.

In Croatia, it is carried out in accordance with ICAR standards, using AT4/BT4 methods, and the data is processed at the Central Milk Control Laboratory (HAPIH, 2020; ICAR, 2009).

Thanks to the development of precision dairy cattle breeding, it is now possible to systematically monitor production parameters at the individual animal level, which allows for a better assessment of seasonal influences at the farm level and timely management decisions (Gantner, 2020).

The aim of this work was to analyze seasonal variability of daily milk yield, milk fat content and total milk protein content, in order to identify patterns that can serve as a basis for adjusting feeding and housing technology in different seasons. Special emphasis was placed on identifying differences between seasons and on determining the potential for improving production efficiency under climate change conditions.

Materials and Methods

This study was based on a comprehensive database for monitoring the milk yield of Holstein dairy cows, which was collected as part of the official production control in the Republic of Croatia. The database contained a total of 5,691,083 records, collected on the control day in the period from 1 January 2005 to 31 December 2022, thus covering a large period of 18 years. To analyse the seasonal variability of production traits, the part of the database relating to daily milk yield (DMY), daily milk fat yield (DMF), daily protein yield (DMP) and milk fat content (DAMF) and protein content (DAP) was used. All data were collected using methods in accordance with the recommendations of the International Organization for the Control of Livestock Production (ICAR), with the AT4/BT4 method used in Croatia. Milk quantity measurement and sampling were carried out by authorized controllers of the Croatian Agency for Agriculture and Food (HAPIH), and milk composition analysis was performed at the Central Laboratory for Milk Quality Control located in Križevci. In the aforementioned laboratory, analyzes are performed in accordance with accredited laboratory methods, i.e., infrared spectrophotometry is used to determine the proportion of milk fat, protein, lactose and urea, and the fluoro-opto-electronic method is used to count somatic cells. Testing of milk samples in the laboratory is carried out with modern analytical equipment. The chemical quality of milk is tested on four MilkoScan analyzers, two MilkoScan FT 6000 and two MilkoScan 7 RM, which are located in a combi system together with Fossomatic analyzers for determining the number of somatic cells. The results of the quality control analyses of milk samples were downloaded in electronic form. The downloaded data were statistically analyzed at the Faculty of Agrobiotechnical Sciences in Osijek. The data were classified by season: spring, summer, autumn and winter, according to the date of control. Descriptive statistical parameters were calculated for each seasonal period and each observed characteristic: mean value (Mean), standard deviation (SD), coefficient of variability (CV), as well as minimum and maximum value. Statistical processing was performed using the SAS/STAT software package (SAS Institute Inc., 2019), which was used to perform univariate analyses and compare seasonal groups.

Results and Discussion

Due to the sample size, length of the time frame and geographical range of production (different regions and housing conditions), the results have a high level of reliability and representativeness for assessing seasonal variability of production indicators in dairy cattle breeding.

Table 1 shows basic descriptive statistics for the main production traits of Holstein dairy cows, obtained from the official milk control database for the period 2005–2022.

Table 1. Basic statistical parameters of analysed traits

Variable	N	Mean	SD	CV	Min	Max
DMY	5411650	22.76	9.76	42.88	3.00	99.90
DMF	5544603	4.17	0.98	23.44	1.50	9.00
DMP	5579436	3.45	0.47	13.74	1.01	7.00
DAMF	5287610	0.94	0.44	47.24	0.05	7.94
DAP	5319298	0.77	0.31	40.39	0.06	4.97

*DMY – daily milk yield (kg); DMF – daily milk fat (%); DMP – daily milk protein (%); DAMF – daily amount of milk fat (kg); DAP – daily amount of protein (kg); N = absolute number of cows; SD = standard deviation; CV = coefficient of variability; Min = minimum, Max = maximum

The average daily milk yield (DMY) was 22.76 kg, with high variability (CV = 42.88%), with a range of values from 3.00 to as much as 99.90 kg. The milk fat content (DMF) had an average value of 4.17%, while the protein content (DMP) was 3.45%. The absolute amount of milk fat produced (DAMF) was on average 0.94 kg, and the protein (DAP) was 0.77 kg per day. The coefficients of variability for DAMF and DAP were high (47.24% and 40.39%), indicating marked differences between animals and production conditions.

The results of the analysis of the variability of production characteristics, daily milk yield and composition of milk (daily content and daily quantity of milk fat and protein) of dairy cows are divided into 4 tables according to the seasons: spring, summer, autumn and winter and are presented below in Tables 2-5.

Table 2. Basic statistical parameters of daily milk yield characteristics in spring

SPRING						
Variable	N	Mean	SD	CV	Min	Max
DMY	1423248	23.50	10.03	42.66	3.00	99.90
DMF	1471608	4.13	0.96	23.22	1.50	9.00
DMP	1481268	3.42	0.46	13.38	1.06	7.00
DAMF	1396825	0.96	0.45	47.26	0.05	6.47
DAP	1405304	0.79	0.32	40.28	0.07	4.97

*DMY – daily milk yield (kg); DMF – daily milk fat (%); DMP – daily milk protein (%); DAMF – daily amount of milk fat (kg); DAP – daily amount of protein (kg); N = absolute number of cows; SD = standard deviation; CV = coefficient of variability; Min = minimum, Max = maximum

Table 3. Basic statistical parameters of daily milk yield characteristics in summer

SUMMER						
Variable	N	Mean	SD	CV	Min	Max
DMY	1230201	22.64	9.30	41.07	3.00	99.40
DMF	1264659	3.98	0.92	23.04	1.50	9.00
DMP	1273050	3.34	0.44	13.29	1.01	7.00
DAMF	1196292	0.89	0.41	46.08	0.05	7.16
DAP	1203996	0.75	0.29	39.08	0.06	4.44

*DMY – daily milk yield (kg); DMF – daily milk fat (%); DMP – daily milk protein (%); DAMF – daily amount of milk fat (kg); DAP – daily amount of protein (kg); N = absolute number of cows; SD = standard deviation; CV = coefficient of variability; Min = minimum, Max = maximum

Table 4. Basic statistical parameters of daily milk yield characteristics in autumn

AUTUMN						
Variable	N	Mean	SD	CV	Min	Max
DMY	1379755	21.85	9.47	43.33	3.00	99.30
DMF	1406383	4.24	0.99	23.44	1.50	9.00
DMP	1415213	3.52	0.49	13.92	1.03	7.00
DAMF	1349517	0.91	0.43	46.87	0.05	7.94
DAP	1357721	0.76	0.31	40.41	0.06	4.33

*DMY – daily milk yield (kg); DMF – daily milk fat (%); DMP – daily milk protein (%); DAMF – daily amount of milk fat (kg); DAP – daily amount of protein (kg); N = absolute number of cows; SD = standard deviation; CV = coefficient of variability; Min = minimum, Max = maximum

Table 5. Basic statistical parameters of daily milk yield characteristics in winter

WINTER						
Variable	N	Mean	SD	CV	Min	Max
DMY	1378446	23.00	10.09	43.86	3.00	99.50
DMF	1401953	4.32	1.00	23.21	1.50	9.00
DMP	1409905	3.51	0.48	13.63	1.02	7.00
DAMF	1344976	0.98	0.47	47.79	0.05	6.76
DAP	1352277	0.79	0.33	41.09	0.07	4.87

*DMY – daily milk yield (kg); DMF – daily milk fat (%); DMP – daily milk protein (%); DAMF – daily amount of milk fat (kg); DAP – daily amount of protein (kg); N = absolute number of cows; SD = standard deviation; CV = coefficient of variability; Min = minimum, Max = maximum

The results of this study, covering a period of 18 years and more than 5.6 million records, clearly confirm the existence of significant seasonal variability in the production traits of Holstein dairy cows. This long-term time frame allowed a reliable assessment of the effect of seasonal changes on daily milk production and milk chemical composition, independent of annual fluctuations in herd management and environmental conditions.

Daily milk yield (DMY) was highest during spring (23.50 kg) and winter (23.00 kg), while lower values were recorded in summer (21.93 kg) and autumn (21.85 kg). This pattern confirms the findings of previous studies that established a negative effect of heat stress and reduced dry matter intake in the summer months on milk production (Kirchgessner et al., 1986; Eicher et al., 1999; Gantner and Barać, 2016). High temperatures during the summer disrupt the thermoregulation of cows, reduce appetite and nutrient intake, which ultimately results in lower milk yield and changes in milk composition (Domaćinović et al., 2015).

Milk fat content (DMF) and protein content (DMP) also showed a clear seasonal dependence. The highest fat content was recorded in winter (4.32%), while the lowest was in summer (3.98%). Protein content varied in a smaller range (3.34%–3.52%), but was also lowest in the summer period. Similar patterns have been described in the literature, where it is stated that colder seasons result in a higher dry matter content in the ration (due to better feed consumption), which has a positive effect on the synthesis of milk fat and protein (Gross and Bruckmaier, 2019; Gantner, 2020).

It is important to emphasize that the absolute amounts of milk fat (DAMF) and protein (DAP) were highly variable throughout the year (CV about 47%), while the relative values (DMF and DMP) were quite stable (CV about 23% and 13% respectively) (Table 2-5). This confirms that the amount of milk produced has the greatest influence on the absolute amounts of nutrients, while the proportions are more related to the physiological state of the animal, nutritional status and microclimatic conditions (Swenson and Reece, 1993; Gantner and Barać, 2016; Cappellozza, 2024).

Seasonal variability clearly indicates the need to adapt feeding strategies and housing technology throughout the year. During the summer, it is recommended to introduce more energy-rich and digestible rations and ensure thermal comfort in the barns through ventilation, water sprays or shading (FAO, 2009; Gantner, 2020). On the other hand, the winter months can be used to maximize production, with careful monitoring of urea levels and optimization of the protein component of the diet.

Long-term analysis of seasonal production patterns provides the basis for the development of predictive models that can be used for production planning, cost optimization and environmental footprint reduction. Furthermore,

understanding seasonal dynamics is useful for interpreting milk yield monitoring results and for spotting deviations from expected values that may signal problems in feeding or herd management.

Conclusion

The results of this study, conducted on an extensive database collected during 18 years of official control of Holstein cows' milk yield, indicate a pronounced seasonal variability in production traits. The highest daily milk yield, as well as higher milk fat and protein contents, were recorded during the colder months (spring and winter), while the lowest values were determined in summer, confirming the negative effect of heat stress on production results.

These findings clearly indicate the need for a seasonally adapted approach to herd management, with an emphasis on optimizing feeding, improving microclimatic conditions and increased use of precision dairy farming technologies. Such an approach enables more stable and efficient milk production throughout the year and represents an important step towards more economically and environmentally sustainable dairy farming.

The data obtained and the identified patterns can be used as a basis for further modelling and development of practical recommendations for production management in the context of seasonal challenges and climate change.

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ARTIFICIAL NEURAL NETWORK AND REGRESSION MODELS TO PREDICT BW OF HOLSTEIN AND BROWN SWISS CATTLE USING REAL-TIME BODY MEASUREMENTS

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Abstract: In this study, it was aimed to evaluate the possible use of some morphometric body measurements to predict body weight (BW) of Holstein (H) and Brown Swiss (BS) cattle grown in a 12-month Intensive beef production system. Associations between bodyweight (BW) and some body measurements such as heart girth (HG), wither height (WH), body length (BL), body depth (BD), hip width (HW), hip height (HH) and pin bone length (PL) were examined for prediction ability, using the data with 1068 observations for each trait. The linear, quadratic and cubic regression models (REGR) were performed to predict BW for both breeds and since there were no statistically significant differences in body measurements between breeds. The data of these breeds were combined and found that BL and HG would be the most advisable traits in estimating BW ($R^2=95.8\%$ and 95.7% respectively) among the other measurements. Regression analysis showed that addition of quadratic and cubic terms provided little contribution in BW prediction. Therefore, linear terms of all variables were considered for analysis and they were significant and R^2 values for other body measurements WH, HW, BD, HH and PL were approximately 93.2, 79.9, 87.1, 90.9 and 79.2% respectively. The best predictors were the same to estimate BW by Artificial Neural Networks (ANN). The best predictive variables were HG and BL by ANN with $R^2= 98.2$ and 98.1% respectively. In conclusion, BW can be estimated precisely by both REGR and ANN using BL and HG alone or both HG and even WH and different models may be needed to predict BW in different feeding and environmental conditions and breeds. However, ANN produced higher accuracy in prediction of live weights than REGR.

Key words: Artificial Neural Networks, body measurements, bodyweight, Brown Swiss and Holstein cattle, prediction, regression model

Introduction

Body measurements of beef cattle are used for a number of reasons, particularly since ration preparations are based on the animal's body weight (BW), which is crucial for predicting BW. Small-scale farming, particularly in developing nations, is characterized by inadequate resources and investments, and decisions about agricultural activities are primarily based on trials and errors at the small farming level (Wilson et al., 1997; Fourie et al., 2002).

Animals are typically marketed between farmers based on visual evaluation, particularly in underdeveloped nations. Based on BW criterion, the majority of veterinary medications are administered. However, the majority of pharmacological prescriptions and dosages are determined by rough approximations. Complex and costly facilities are needed for small farmers to employ BW as a criterion in ration formulation, selection, medical usage, body condition score, and marketing.

A straightforward and sensible method should be taken into consideration while making managerial choices, as scientists recognize the need of precise BW forecast. There is a correlation between BW and certain body measures, according to several research (Peters and Ball, 1995; Nesamvuni et al., 2000).

The most studies have agreed that some factors such as breed, animal type, age, size and physical status of animal can affect the accuracy of predicting BW from the chest circumference or other body measurements in different environmental conditions (Heinrichs et al., 1992; Enevoldsen and Kristensen, 1997).

The use of Artificial Intelligence to agricultural methods is gaining popularity quickly. Nevertheless, there are currently few studies that rank and compare the most widely used supervised learning algorithms. In animal sciences, research is being done to compare different machine learning methods for predicting body weight, genetic qualities, lactation, performance, illness, and hatchability etc. According to all of the research mentioned, Random Forest Trees, an algorithm similar to artificial neural networks, have proven to be highly helpful and, in most situations, superior to the traditional methods because of the vast amount of data.

Some ANN models were also used to predict market value of carcass parts (Hill et al., 2000; Hatem et al., 2003; Adamczyk et al., 2005), mastitis (Yang et al., 2000) and milk, fat and protein yields (Lacroix et al., 1997). Salehi et al. (1998), the milk and fat content of somatic cells in the estimation of the number of somatic cells (Whyte et al., 2000), evaluation of the physiological status of cows (estrus,

calving and health status) (Molenda et al., 2001; Vassileva and Radev, 2001) and developed for use in the analysis of invitro embryo development (Wilkinson et al., 1996).

Therefore, this study aimed to obtain detailed information about the relationship between BW and some real time body measurements of Brown Swiss and Holstein cattle and also determine the value of using single variable entry to the model to predict BW and validate the potential of these methods as means of prediction under small scale farming conditions.

Material and Methods

Animals

The animals used in this study were comprised of 40 BS and H cattle in total, divided into two groups on the basis of weight. The average weight was 132 and 158 kg for BS and H groups respectively. Some body measurements were obtained from the animals grown in university research farm. The experiment lasted for 12 months. A total of 1068 data were collected for each trait measured. The animals were weighed using a mobile weighing scale once fortnightly month. BWs were recorded to the nearest kilogram (kg). All measurements were taken by the same individuals throughout the experimental period. The animals were fed ad-libitum.

Body Measurements

Real time body measurements were taken while animals were standing on weighing scale. A plastic tape marked in centimetres (cm) was used for the measurement of most body traits except WH, which was measured by measuring stick (Hauptner, Germany). BW was measured in kilograms and the body measurements in centimetres.

A typical ANN consists of layers. The first layer is known as inputs layer (sex, age, breed, BW at slaughter etc.) and the last one is called output layer (Model output for predicted values for carcass quality) (Bozkurt et al., 2009). All the other layers were called hidden layers. The ANN training then was necessary for model prediction ability. HG, WH, BL, HW, BD, PL and HH used input variable, bodyweight used output layer. For this purpose, all input and output values were normalized to have a zero mean and a unified standard deviation, and finally outputs were transferred back to the same units as the original outputs (using MATLAB's poststd function). After confirming the input and target of the network, then network optimization is conducted in order to increase prediction accuracy as indicated by Fausett (1994).

Statistical Procedure

The best prediction equations were determined for BW from other traits as independent variables, including HG, BL, WH, HW, BD, HH, PL. Descriptive statistics on a monthly basis and regression analysis of BW on each of the independent variables were performed using the General Linear Model procedure (Minitab, v16).

Correlation coefficients were also obtained between parameters. Polynomial regression analysis of BW on HG, WH, BL, HW and BD was performed.

Linear, quadratic and cubic effects of independent variables on BW were included in the following model:

$$y_i = b_0 + b_1 X_i^1 + b_2 X_i^2 + b_3 X_i^3 + e_i$$

Where

y_i = BW observation of an i th animal,

b_0 = intercept, b_1 , b_2 , b_3 = corresponding linear, quadratic and cubic regression coefficients I,

X_i = body measurement (HG, WH, BL, HW, BD, PL, HH) and

e_i = residual error term

Several different regression analyses were conducted;

1- All seven body measurements, expressed as linear functions, were combined in BW prediction equation

2- Each body measurement was included separately in regression analysis as linear, quadratic and cubic expressions to predict BW; and

3- The linear regression of each other measurement was then also added to the model as described previously.

Artificial Intelligence Procedure

The analysis of acquired data has been conducted using artificial neural networks. A total of 1069 data points were collected and split for the training and testing processes of the artificial neural network model. For this purpose, 80% of the data was used for training the model, and the remaining 20% was utilized for testing. To enable the processing of the available data in the artificial neural network model, the data underwent preprocessing, including standardization, using the StandardScaler function from the sklearn library in the Python language. During the training of the ANN model, a two-layer architecture was employed. The data was provided in batches of four, and the model underwent 50 epochs.

Results

It was observed that in every step of regression, inclusion of BL and HG in the equations increased R^2 greatly. It was also found that when all variables were

included in the regression PL, HW and BD were not significant while the rest gave significant slope values. The table containing the REGR equations with all combinations of all body traits was cumbersome therefore it was not shown in the paper.

There were no statistically significant differences in body measurements between breeds ($P > 0.05$). The data of these breeds were combined for all statistical analysis.

Descriptive statistics of BW and body traits on a monthly basis are shown in Table 1.

Table 1. Descriptive statistics of body weight and body traits by weighing times on 1-12 month intervals

Weighing time (month)	BW (kg)	HG (cm)	WH (cm)	HH (cm)	HW (cm)	BL (cm)	BD (cm)	PL (cm)
1.	144.7	117.8	98.7	105.2	28	102.3	50.1	21.2
12.	507.8	183.5	133.4	137.7	41.3	151.4	69.8	28.2
Difference	363.1	65.7	34.7	32.5	13.3	49.1	19.7	7

The average values for BW increased throughout the experimental period from 144.7 kg to 507.8 kg with 363.1 kg difference. The corresponding ranges for HW, BL, HG, WH, BD, HH and PL were 28 cm to 41.3 cm with 13.3 cm difference, 102.3 cm to 151.4 cm with 49.1 cm difference, 117.8 cm to 183.5 cm with 65.7 cm difference, 98.7 cm to 133.4 cm with 34.7 cm difference and 50.1 cm to 69.8 cm with 19.7 cm difference, 105.2 cm to 137.7 cm with 32.5 cm difference, 21 cm to 28 cm with 7 cm difference, respectively (Table 1).

ANN and REGR equations using individual variables are shown in Table 2. As Table 2 shows ANN Models with one input and REGR Models with one variable together with determination coefficients (R^2).

Table 2. Prediction equations of body weight and the linear effects of other body traits

Models with one variable by REGR	R^2 %	Models with one input by ANN	R^2 %
BW = - 445 + 4.99 HG	95.7	Output $\sim 0.96 * \text{Target(HG)} + 12$	96.3
BW = - 786 + 9.29 WH	93.2	Output $\sim 0.93 * \text{Target(WH)} + 22$	93.6
BW = - 550 + 6.68 BL	95.8	Output $\sim 0.96 * \text{Target(BL)} + 13$	96.4
BW = - 373 + 18.6 HW	79.9	Output $\sim 0.82 * \text{Target(HW)} + 55$	83.6
BW = - 606 + 14.8 BD	87.1	Output $\sim 0.88 * \text{Target(BD)} + 38$	87.1
BW = - 236 + 20.9 PL	79.2	Output $\sim 0.81 * \text{Target(PL)} + 59$	80.1
BW = - 855 + 9.55 HH	90.9	Output $\sim 0.92 * \text{Target(HH)} + 25$	91.1

It was found that BL and HG would be the best possible traits in predicting BW ($R^2=96.4\%$ and 96.3% respectively by ANN) and ($R^2=95.8\%$ and 95.7% respectively by REGR) among the other measurements. When examining the Table 2, it is observed that the Random Forest algorithm provides higher R^2 values across all parameters compared to the classical regression and artificial neural network models

Figure 1 shows the regression curve of HG, which provides the highest predictive success in single-parameter BW estimation and Figure 2 shows the regression curve of PL.

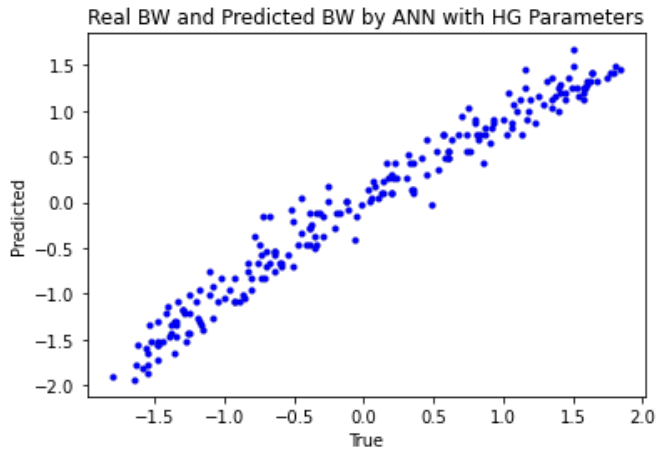


Figure 1. Real BL and Predicted BL by ANN with HG Parameters

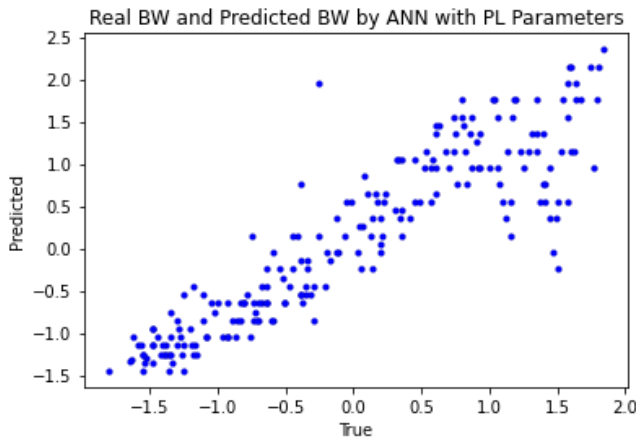


Figure 2. Real BL and Predicted BL by ANN with PL Parameters

Moreover, it was also found that R^2 values for predictors WH, HW, BD, PL and HH were 93.6, 83.6, 87.1, 80.12 and 91.1 % respectively by ANN and 93.2, 79.9, 87.1, 79.2 and 90.9 % respectively by REGR.

Results of regressions of BW on the linear, quadratic and cubic effects of each body measurement are presented in Table 3.

Table 3. Regressions of body weight on the linear, quadratic and cubic effects of each body measurement[#]

Measurements	Intercept	Linear	Quadratic	Cubic	R ² %
Heart Girth (HG)	- 444.7	4.987	-	-	95.7
	27.54	- 1.400	0.02111	-	96.4
	240.8	- 5.802	0.05094	0.000066 ^{ns}	96.4
Body Length (BL)	- 549.5	6.677	-	-	95.8
	- 161.8	0.5859	0.02352	-	96.1
	578.6	- 17.04	0.1619	- 0.000358	96.1
Hip Width (HW)	- 372.8	18.56	-	-	79.9
	- 260.0	12.22	0.08718 ^{ns}	-	80.0
	2364	- 214.2	6.476 ^{ns}	- 0.05904	81.5
Wither Height (WH)	- 786.1	9.294	-	-	93.2
	67.73	- 5.336	0.06203	-	93.7
	5459	- 144.3	1.247	- 0.003349	94.0
Body Depth (BD)	- 605.8	14.79	-	-	87.1
	- 307.9	5.040	0.07863	-	87.3
	5081	- 259.6	4.361	- 0.02285	89.0
Pin Bone Length (PL)	- 236.3	20.91	-	-	79.2
	- 704.7	56.61	- 0.6579	-	81.2
	442.4	- 73.89	4.176	- 0.05838	81.8
Hip Height (HH)	- 854.8	9.550	-	-	90.9
	- 527.4	4.167	0.02194	-	90.9
	9958	- 255.8	2.158	- 0.005815	91.6

[#]only none significant regression coefficients had superscripts (ns), the rest were significant at $P < 0.05$.

It was observed in this study that a 1 cm change in HG resulted in almost 5 kg change in weight. Similarly, a 1 cm change in BL, WH, HH, BD, HW, PL, and resulted in 6.67, 9.29, 9.55, 14.79, 18.56 and 20.91 kg change in weight respectively (Table 3).

Correlation coefficients of the traits are shown in Table 4.

Table 4. Pearson correlations between body weight and body traits

Variables	BW	HG	WH	BL	HW	BD	PL
HG	0.978						
WH	0.965	0.958					
BL	0.979	0.971	0.967				
HW	0.894	0.897	0.892	0.893			
BD	0.933	0.928	0.941	0.934	0.873		
PL	0.890	0.885	0.864	0.882	0.897	0.845	
HH	0.953	0.941	0.974	0.951	0.870	0.933	0.846

BW: Body weight, HG: Heart Girth, WH: Withers Height, BL: Body Length, BD: Body Depth, HW: Hip Width, HH: Hip Height, PL: Pin Bone Length.

All correlation values were statistically significant ($P < 0.05$). Amongst all the body measurements, the highest correlation was found between BL and BW ($r=0.979$). The second highest correlation was between HG and BW ($r=0.978$). In addition, the correlation value between BL and HG ($r=0.971$) was higher than the correlation between the rest of the traits (Table 4). It was expected that BL would provide higher correlation coefficient value than the other body measurements since the R^2 value between BW and BL was also high.

Discussion

The results showed that ANN Models were superior than REGR Models. In REGR Models, the highest R^2 values were obtained from the equation contained all body traits ($R^2=97.5\%$) and the equation that included all body measurements except WH, HW and BD ($R^2=97.5\%$) and those equations that included HG, BL and HH ($R^2=97.4\%$), HG and BL ($R^2 =97.1\%$) and only BL ($R^2=95.8\%$). These results were in line with those of Tuzemen et al. (1993), Ulutas et al. (2001) and Bozkurt (2006) who reported high R^2 value from the equation including all body traits. Bozkurt (2006) found that when considering individual equations with one predictor HW and BD had the lowest R^2 values; 69% and 66.2%, respectively. HG and WH had the highest R^2 values, approximately 90% and 77% respectively. However, in this study, the individual equations with one input and one variable predictor HW and PL had the lowest R^2 values; 83.6% and 80.1% respectively by ANN and 79.9% and 79.2% respectively by REGR. In contrast, BL and HG had the highest R^2 values, approximately 96.4% and 96.3% respectively by ANN and 95.8% and 95.7% respectively by REGR (Table 2).

Higher order polynomial equations were examined. The R^2 values from the regressions indicated that BL and HG to be the most highly related to BW when all linear, quadratic and cubic coefficient terms were considered (Table 3). These results were in line with those of Bozkurt (2006) who reported a 1 cm change in

HG resulted in approximately 4.9 kg change in weight and this value for BL is 6.67 kg change in prediction of weight. Moreover, a 1 cm change in BL, WH, BD and HW resulted in 6.54, 9.33, 11.8 and 14.8 kg change in weight respectively. It was evident that a 1 cm change in HG resulted in lesser weight change compared with the rest of body traits indicating it was a best predictor.

For all body traits, addition of the equation with the cubic term increased the R^2 slightly. In this study BL and HG contributed 95.8% and 95.7% of variation respectively. However, while the equation with all linear, quadratic and cubic terms of HG and BL were significant ($P < 0.05$), HW has not significant quadratic term ($P > 0.05$). However, Heinrichs et al. (1992) pointed out that none-significant cubic term for HG and significant term for WH. The quadratic term of BL was not significant ($P > 0.05$). Contrarily, Heinrichs et al. (1992) reported that quadratic term of BL was significant. Both quadratic and cubic coefficients term of BD were not significant either. However, all linear terms of all body measurements were significant ($P < 0.05$). These results were in line with Heinrichs et al. (1992), Wilson et al. (1997), Ulutas et al. (2001) and Bozkurt (2006).

It can be emphasised that for accuracy of BW predictions additional body measurements (e.g. HW, BD, BL and even WH) in the REGR equations provided a little increase except HG alone. The same trend was observed for ANN Models. However, all body measurement parameters used in both models performed better in ANN than REGR as indicated by higher determination and correlation coefficients.

Conclusion

As the most of previous studies showed, this study also indicated that HG and BL can be used with great accuracy in predicting the BW for BS and H male cattle grown under small-scale farming condition. HG and BL exhibited the highest correlation to bodyweight of the traits studied. The results in this study also showed that linear, quadratic and cubic expressions of both HG and BL are the most useful predictors, and support the findings of Wilson et al. (1997) and Bozkurt (2006). When any of the other seven measurements were used in the models that contained linear, quadratic and cubic terms, HG generally made the most important contribution compared with other body dimensions. BL can be considered the second-best predictor.

Among body measurements, the use of HG and BL provided a simple way of predicting BW confidently in REGR and ANN models which is the overall purpose applying the technique in the practice. However, there is always a need for further studies for the breeds in this study and other breeds as well to determine and

develop different models to predict bodyweight in different management and environmental conditions. It is also important to pay a great attention when measuring body dimensions to reduce the experimental errors.

In conclusion, both approaches are useful resources in the data scientist's toolbox, whether it's the ease of use of linear regression or the strength of neural networks. Machine Learning and ANN applications will certainly open up new dimensions in performance predictions in animal production practices.

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EVALUATING AND FORECASTING LIVESTOCK PRODUCTION TRENDS IN CROATIA: A SECTORAL ANALYSIS BASED ON ARIMA MODELING

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Abstract: The primary objective of this study was to develop a predictive model for the supply of live livestock in the Republic of Croatia for the period 2024–2029, based on the analysis of historical data. Livestock production has long been a strategically important sector in Croatia, supported by a strong tradition and a notable presence of indigenous breeds. Nevertheless, despite these advantages, the supply of live livestock per production unit has demonstrated persistent negative trends. The study applies the Autoregressive Integrated Moving Average (ARIMA) model to analyze time series data from 2019 to 2023 in order to identify structural patterns and forecast future supply dynamics. Supplementary statistical and econometric methods are employed to examine variation, autocorrelation, and the significance of fluctuations within the series. The analysis also highlights that the cost of production, as a key non-price determinant, plays a decisive role in shaping livestock supply trends. Findings indicate a regressive trend across most livestock sectors, emphasizing the need for targeted policy measures to stabilize and enhance future production.

Key words: Livestock supply, ARIMA model, time series analysis, production forecasting, agricultural economics

Introduction

Land represents the fundamental resource of agricultural production and a key economic asset. Economic goods derive their value from scarcity relative to human needs, meaning they are both desirable and limited in availability. Economics, by definition, concerns the most efficient use of available resources

and the realization of optimal decisions in their use. In doing so, it addresses two essential issues: the scarcity of resources and the need for choice (Krugman, 2009).

The problem of scarcity arises from the persistent gap between satisfied and unsatisfied human needs. Although this gap can be reduced through economic progress, it can never be completely eliminated. The scarcity of goods and services that fulfill diverse human needs originates from the limited availability of resources required for their production. Consequently, the treatment of scarce resources and the necessity to choose among their alternative uses establish economics as a science of rational choice, one that seeks to identify and enhance the principles guiding individuals in their economic decision-making (Olson et al., 2009). Making rational economic choices involves comparing benefits and costs, revenues and expenditures, and ultimately striving for a net benefit that justifies the decision.

The selection of a specific type of production is largely influenced by the availability of natural resources and the associated costs (Bašić et al., 2002). Labor, capital, and technology costs determine the most feasible combination of inputs. Through their combination, livestock is raised and produced, and subsequently processed into food and other products that meet consumer needs (Grahovac, 2005). These activities are framed within the concept of economic efficiency, which refers to the production of output units at the lowest possible cost (Bošnjak et al., 2003). The degree of efficiency depends on the prices of production inputs. A key point in understanding economic efficiency is recognizing that it occurs when the cost of producing a given quantity of product is minimized (Arsenović et al., 2002).

In simplified terms, supply refers to the quantity of goods a producer is willing to offer and sell in a specific market, within a given period, and at various price levels. There is a direct relationship between market price and the quantity supplied. When the market price of a good increases, the quantity supplied also increases, assuming other factors remain constant, and vice versa. In the Croatian market, the most commonly raised and marketed livestock species include pigs, cattle, poultry, goats, and sheep. The total supply of a product by all producers in a market at a specific time is referred to as aggregate or market supply. Geometrically, the market supply curve is obtained by horizontally summing the quantities offered by each producer at all alternative price levels.

In addition to price, several other determinants influence supply. Among the most significant are production costs. An increase in input costs leads to higher marginal costs at every level of output, reducing the quantity that producers are willing to supply at a given price (Blanchard, 2005). Conversely, a decrease in costs has the opposite effect, increasing the quantity supplied. Technological progress also contributes to an increase in supply by enabling producers to generate the same output with fewer inputs or greater output with the same input

consumption, thereby making them more willing to offer additional products at the same market price.

Other important factors include changes in the prices of related goods. When the price of a substitute product rises, producers may increase the supply of other goods, shifting the supply curve to the right for products whose prices remain unchanged. Government policies also exert a considerable influence on supply. Through fiscal and monetary policy, environmental regulations, product standardization, customs policy, and related measures, the state directly or indirectly shapes the structure and level of production costs (Krueger, 2009). Non-economic factors, such as weather conditions, can also significantly impact agricultural supply. Moreover, the structure of the market and expectations regarding future prices are additional factors influencing supply decisions.

In certain circumstances, a negative relationship may arise between the quantity supplied and the market price of a good. In such cases, the supply curve takes on a negative slope and is referred to as a regressive supply curve. This phenomenon occurs most frequently in agricultural production and is often driven by motivational factors. For example, when the market price of agricultural products increases, producers may maintain the same income level by reducing their output volume, thereby achieving equivalent personal satisfaction with less labor input (Defilippis, 2005). This behavior is especially common among Croatian producers of pigs, cattle, poultry, goats, and sheep, the main livestock categories on the national market.

Based on this theoretical framework, the aim of this paper was to explore the causes of regressive livestock supply, despite the Republic of Croatia possessing comparative advantages in agricultural production relative to its market competitors.

Material and Methods

There are significant regional disparities in livestock production within the Republic of Croatia. Livestock production is closely integrated with crop farming, and the high level of self-sufficiency enables surplus production to be directed toward export markets, making livestock one of the country's most important export commodities. As the global economy continues to develop, research indicates a growing market demand for cattle, pigs, poultry, goats, and sheep, as well as for processed products derived from these species. In light of favorable market trends, it is essential to systematically improve production capacities and strengthen the overall supply. For the purposes of this research, data were obtained from the Croatian Bureau of Statistics and the Ministry of Agriculture. The data are expressed in absolute numbers (head of livestock).

Table 1. Comparative overview of livestock production volumes from 2019 to 2023

Year	Cattle	Pigs	Goats	Sheep	Chickens
2018	450,727	1,121,032	76,771	636,808	11,412,805
2019	414,125	1,049,123	80,064	636,294	12,746,691
2020	420,239	1,022,350	81,540	657,197	13,056,718
2021	422,881	1,033,048	86,258	661,992	12,096,168
2022	427,587	971,307	85,783	654,339	10,916,570
2023	421,844	944,495	81,581	642,808	10,744,878
Total	2,557,403	6,141,355	491,997	3,889,438	70,973,830

Source: Compiled by the author based on data from the Croatian Bureau of Statistics and the Ministry of Agriculture of the Republic of Croatia.

According to the data presented in Table 1, variations in the volume of livestock production are evident; however, they do not exceed 15% of the multi-year average. Furthermore, the significant increase in input prices during 2021 and 2022 led to a noticeable decline in production volumes, particularly in the years 2022 and 2023. Among the livestock sectors, the most pronounced reductions were observed in the production of pigs, chickens, and sheep.

For the purpose of modeling the supply of crop products, the ARIMA model was employed. This time series model, specifically the AutoRegressive Integrated Moving Average model, expresses the current value of a time series Y_t as a function of its previous values at time lags $t-1$, $t-2$, etc. ARIMA is a structured, multi-stage modeling methodology used for the identification, estimation, and validation of models that combine both autoregressive (AR) and moving average (MA) components (Hillmer et al., 1982), with the aim of achieving the best fit to historical data and producing reliable forecasts. The general form of the ARMA(p,q) model is:

$$y_{t1} = \frac{\mu + \phi_1 \cdot y_{t-1} + \phi_2 \cdot y_{t-2} + \dots + \phi_p \cdot y_{t-p}}{AR(p)} + \frac{\varepsilon_t - \theta_1 \cdot \varepsilon_{t-1} - \theta_2 \cdot \varepsilon_{t-2} - \dots - \theta_q \cdot \varepsilon_{t-q}}{MA(q)}$$

In this model, μ , ϕ_1 , ϕ_2 , ϕ_p , represent the model parameters estimated based on sample data (i.e., the time series), while ε_t denotes a random variable

following a white noise process, also referred to as the innovation term. The parameter μ represents the average level of the process, whereas $\phi_1, \phi_2, \dots, \phi_p$ are the parameters associated with lagged variables. Here, p denotes the non-seasonal AR (autoregressive) order, and q denotes the non-seasonal MA (moving average) order.

The ARIMA (p, d, q) model is an extension of the ARMA (p, q) model, where the parameter d indicates the degree of differencing applied to the time series to achieve stationarity (Fischer et al., 1998). This form is considered standard as it encompasses, in a single expression, the AR(p) model, the MA(q) model, and the ARMA (p, q) model. The ARIMA (p, d, q) model is as follows:

$$\Delta^d y_{t1} = \frac{\phi_1 \cdot \Delta^d y_{t-1} + \phi_2 \cdot \Delta^d y_{t-2} + \dots + \phi_p \cdot \Delta^d y_{t-p}}{AR(p)} + \frac{\varepsilon_t - \phi_1 \cdot \varepsilon_{t-1} - \phi_2 \cdot \varepsilon_{t-2} - \dots - \phi_q \cdot \varepsilon_{t-q}}{MA(q)}$$

Where:

d – Non-seasonal differencing order

In the case where $p = 1$, the model is defined as a first-order autoregressive model, denoted as AR (1). In the AR (1) model, the dependent variable is regressed on its own lagged value (i.e., y_{t-1}), indicating that the current value of the dependent variable depends on its own previous (lagged) value and its average level over time.

Autocorrelation was applied as a method for analyzing time series data. It serves to reveal the relationship between the current value of the variable and its previous values. Autocorrelation represents the degree of similarity of a variable across two-time intervals. The results obtained through this method will be used for modelling and forecasting future values in the time series.

Results and Discussion

Figure 1 illustrates the variation in livestock production volumes across different animal production sectors for the period under analysis. The most notable fluctuations are observed in poultry production, followed by pig and cattle production. In contrast, production levels of goats and sheep exhibit relatively minor deviations, indicating a more stable output in these sectors over time.

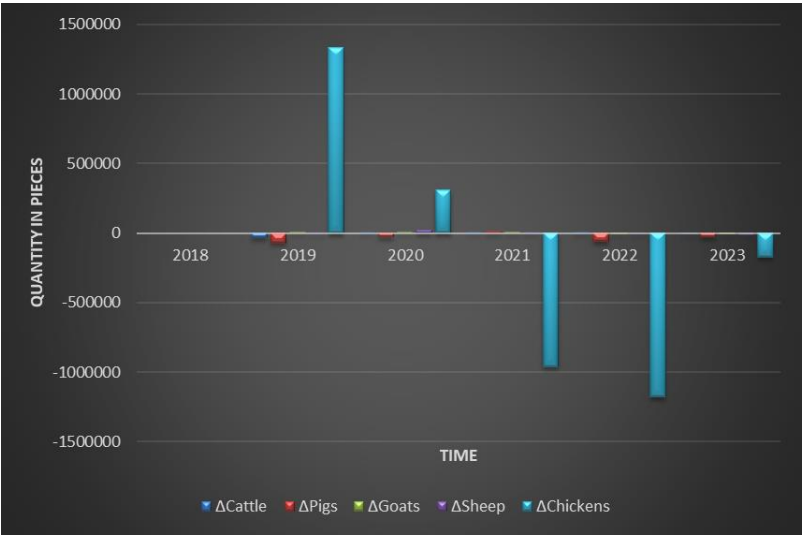


Figure 1. Differences in production volumes by sector

In order to construct the ARIMA model, it is necessary to calculate the standard deviation, variance, mean value, correlation, covariance, and autocorrelation for the analyzed six-year period. The results are presented in Table 2.

Table 2. Comparative overview of statistical results

Species	n	Standard Deviation	Variance	Mean Value	Covariance	Significance Level	Correlation	Autocorrelation
Cattle	5	816,290,612.8	136,048,435.5	426,238.83	30,104,123.5	0.6715	0.5862	0.1222
Pigs	5	19,227,394,227	3,204,565,704	1,023,559.17	-1,158,948,638	0.8611	0.6206	0.0292
Goats	5	63,919,349.5	10,653,224.92	81,999.50	-1,111,272.17	0.1980	0.1092	0.1464
Sheep	5	60,944,731.3	101,574,552.9	648,239.67	94,280,100.33	0.3249	0.8598	0.4705
Chickens	5	460,189,125.78	766,982,116	11,828,971.67	-475,088,458.41	0.0036	0.6007	0.3641

The mean production volume for cattle is 426,238.83 heads. The standard deviation indicates that the data points show considerable dispersion around the mean value. Variance further confirms that the data set is widely spread around the average. The positive covariance reflects the degree to which the average sum of products deviates from the product of their means. A positive correlation value indicates that an increase in one variable is associated with an increase in the other, and likewise, a decrease in one variable corresponds with a decrease in the other; in other words, the two variables move in the same direction. Similarly, the autocorrelation is positive, confirming consistent dependence within the analyzed series. The significance level demonstrates that the time series interval is reliable.

For pigs, the mean production volume is 1,023,559.17 heads. The standard deviation shows significant variation of data points from the mean range. Variance indicates a wide spread of data around the mean, while covariance is negative, reflecting that the average sum of product deviations is below the product of their means. Despite this, the positive correlation suggests that positive production trends can be expected in the future. The positive autocorrelation indicates that the variables move in tandem, following similar patterns. The significance level suggests that the time series interval is relatively reliable.

In the case of goats, the mean production volume is 81,999.50 heads. The standard deviation reveals significant dispersion around the mean. Variance confirms that the data are spread around the average, while covariance is negative, indicating that the average sum of product deviations is less than the product of their means. The positive correlation shows that the variables increase and decrease simultaneously, moving in the same direction. Autocorrelation is also positive, supporting this interpretation for the analyzed series. The significance level confirms the reliability of the time series interval.

For sheep, the mean production volume is 648,239.67 heads. The standard deviation indicates notable variability in the data points. Variance shows that the data are dispersed around the mean, while positive covariance reflects how much the average sum of products deviates from the product of their means. The positive correlation reveals that the variables tend to move together, increasing or decreasing in parallel. Autocorrelation also has a positive value, indicating consistent behavior in the time series. The significance level suggests that the interval of the time series is reliable.

Regarding chickens, the mean production volume is 11,828,971.67 heads. The standard deviation indicates considerable variability in the data. Variance confirms dispersion around the mean, while covariance is negative, showing that the average sum of product deviations is less than the product of their means. A positive correlation indicates that increases and decreases in the variables coincide, moving synchronously. Similarly, the autocorrelation exhibits a positive value,

confirming this pattern within the analyzed series. The significance level indicates that the time series interval is reliable. Based on the statistical results obtained using the ARIMA model, Table 3 presents a six-year forecast of livestock supply.

Table 3. Forecast of livestock supply for the upcoming period

Year	Cattle	Pigs	Goats	Sheep	Chickens
2024	416,101	917,683	77,379	631,277	10,573,186
2025	410,358	920,871	73,177	619,746	10,401,494
2026	404,615	914,059	68,975	608,215	10,229,802
2027	398,872	914,247	64,773	596,684	10,058,110
2028	396,872	818,427	61,382	591,215	9,926,446
2029	393,129	810,435	60,571	585,153	9,886,418
Total	2,023,075	4,320,295	344,875	3,041,075	51,149,010

The values presented in the table 3 indicate that a decrease in livestock supply is expected across all sectors except for pig farming in the upcoming period. This observation is supported by the statistical results shown in Table 2. Specifically, the supply of pigs is projected to fluctuate, with a slight increase anticipated in 2025 and 2027, while negative trends are expected in the other years. The difference in supply between the analyzed historical period and the projected future period is illustrated in the following Figure 2.

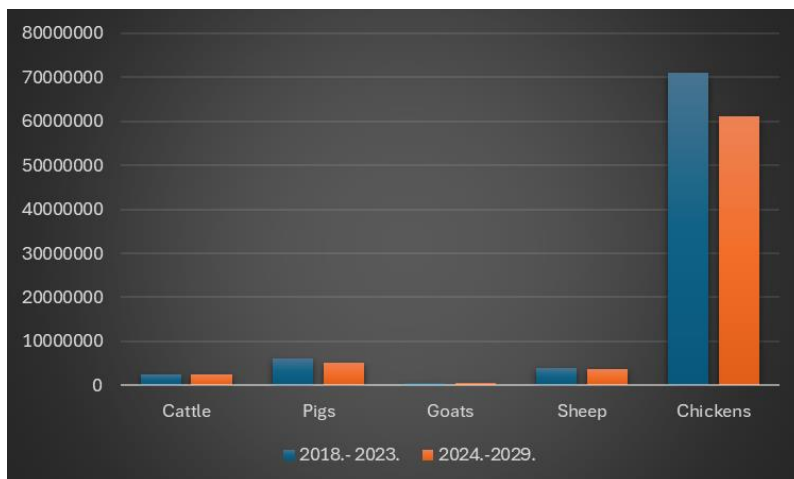


Figure 2. Comparative representation of pig supply trends for the past and future periods

The data presented in Chart 2 demonstrate that the livestock supply during the analyzed historical period exceeds that of the projected future period. A decline in supply is anticipated across all sectors, with average reductions estimated at 5.37% for cattle, 16.32% for pigs, 17.43% for goats, 6.61% for sheep, and 13.94% for chickens. These trends suggest significant shifts in production capacity and market dynamics, which may be influenced by various economic, environmental, and management factors affecting the livestock sector in the forecasted timeframe. Such projections are critical for strategic planning and policy development aimed at sustaining livestock production and meeting future market demands.

Conclusion

The objective of this study was to analyze livestock supply dynamics within the sectors of cattle, pigs, goats, sheep, and poultry over the five-year period from 2018 to 2023 and to project anticipated trends in livestock supply for the subsequent period. The findings indicate that despite the strategic significance of the livestock sector for the Republic of Croatia, forecasts generated using the ARIMA model predict a decline in supply across most sectors in the forthcoming years. Statistical evaluation of the 2018–2023 data series revealed that the standard deviation signifies considerable variability of data points around the mean. Variance analysis further confirmed the dispersion of the dataset around the central tendency, while covariance exhibited a negative average deviation from the mean product values. Notably, correlation and autocorrelation coefficients were

predominantly negative in all sectors except for the swine sector, reflecting inverse relationships within the time series data. Autocorrelation analysis corroborated these findings by demonstrating negative deviation patterns for the evaluated series. The significance levels obtained affirm the reliability and robustness of the time series data intervals. Consequently, based on the rigorous statistical assessment and model application, forecasts of future livestock supply were developed, with correlation and autocorrelation measures substantiating the linkage between historical and projected supply values. In summary, the study's aims were met through the systematic application of time series modeling and statistical inference, providing a scientifically grounded forecast of livestock supply trends.

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EFFECT OF PRECISE SYSTEM FOR NUTRITION MANAGEMENT IMPLEMENTED IN DAIRY FARM

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Abstract: The aim of the present study was to evaluate the effect of implementing precision system for nutrition monitoring and management in a dairy farm. The study was carried out in dairy farm with 200 cows, located in North Bulgaria. It has been established that after the introduction of precise technology for monitoring and managing nutrition in the dairy cattle farm, an increase in milk productivity was observed, as well as a decrease in the amount of bulky and concentrated feed used, and fuel consumed on the farm. Evidence shows that the integration of digital technologies in dairy farming can lead to more efficient use of resources, increase productivity and reduce some negative environmental impacts. Although the optimization process is likely to be slow, the data demonstrate the potential of digital technologies to transform modern dairy farming and its role in achieving sustainable development in the sector.

Key words: precision dairy farming, nutrition management, dairy farm, dairy cows

Introduction

Dairy farming provides a daily source of raw materials for the dairy industry and income, creating employment opportunities not only on farms but also in the transport, milk processing and agricultural supply and service sectors. Due to the increasingly high requirements for the production of quality milk, decision-making in modern dairy farming is becoming more and more complex and difficult (Mourits, 2000). Slavova et al. (2022), noted the ever-increasing demands of society to mitigate the negative effects on the environment, as a result of the breeding of dairy cows. The crises of various nature in recent years have also had a serious negative impact, leading to a significant increase in production costs - fodder, electricity, energy carriers, etc. According to many authors, the introduction of systems for precision dairy farming (PDF) contributes to increasing production

results and recently they are increasingly used (Borchers and Bewley, 2015; Rutten et al., 2018; Halachmi et al., 2019).

Precision animal husbandry involves the use of digital technologies and aims to improve production and reproduction, animal welfare and facilitate the targeted use of resources to reduce the impact on the environment and human health through the precise control of processes (Popova et al., 2024). It contributes to the proper management of large groups of animals, improving profitability, farm efficiency and minimizing environmental impact (Kaur et al., 2024). The efficiency of a dairy cow farm is related to many factors. The desire of every owner is to simultaneously improve them and increase profitability exponentially, which is difficult to achieve. A number of researchers (Dijkhuizen et al., 1995; Tauer and Mishra, 2006; Stankov, 2020) conclude that, for complex reasons, determining the financial value of the technical effects of one or more changes in input factors that occur simultaneously is a very complex task. Sova et al. (2014) and Souza et al. (2022) specify that in order to feed animals more efficiently and increase their profitability, the use of a precise ration formulation and tracking system for accurate and mixing is necessary. According to Bach et al. (2020), in order to optimize the economic return by increasing the average daily milk yield on the farm, even small variations in the amount of feed used in the preparation of the complete ration mixture should be avoided. In long term, this leads to the provision of excess nutrients and their inefficient utilization. Rossow and Aly (2013) found that a change in the ratio of bulk and concentrate forages led to an increase or decrease in the amount of lignin in the complete ration mixture, which affected milk yield.

Hristov and Martins (2022) describe precision feeding as practices that aim to deliver nutrients without exceeding or failing to meet animal nutritional requirements. According to them, in feeding dairy cows, precision would be about controlling the uniformity of nutrient intake to meet the animal's requirements. Despite the potential benefits, PDF systems are not yet widely implemented (Russell and Bewley, 2013; Chavas and Nauges, 2020). In recent years, part of the country's dairy cattle farms have implemented systems for precision dairy management, which monitor and manage nutrition and track the quantities of input raw materials, errors made and deviations from the set ration, as well as report the amount of complete ration mixture distributed by groups.

The aim of the present study was to evaluate the effect of precise system for nutrition management implemented in dairy farm.

Material and Methods

The study was conducted in a dairy cattle farm located in North Bulgaria with a capacity of 200 dairy cows. At the end of December 2020 precision system (Intouch, Alltech) for nutrition monitoring and management has been introduced on the farm. The system works by combining a control device, a software platform and a mobile device to provide a holistic system for tracking and managing nutrition in dairy farms. The controller tracks the weighting, mixing of ingredients, and provides real-time feedback. Main software components are represented by software for nutrition management and monitoring, analytical tools for data processing and recommendation generation, mobile and web applications for remote user access. A mobile device allows users to access and interact with the system through a mobile application that enables remote monitoring and control of processes. The feed control and management system has been developed in collaboration with Intel and its purpose is to ensure that optimal efficiency is achieved in the use and management of feeds during their feeding to animals.

For the purposes of this study, the following indicators are taken into account:

- average daily milk yield based on 305 days of lactation, in liters;
- amount of concentrated and roughage per lactating cow per day;
- spent fuel per lactating cow per day;
- number of doses per inseminated cow.

The data for quantitative indicators were obtained from the primary accounting records of the farm for the period 2020-2024. Primary accounting includes the collection and recording of all financial transactions and farm operating data, such as income and expense journals, records of production, material and labor costs, and other related documents. To process this data, standard accounting methods were used, such as systematization of income and expenses by category, compilation of journals and turnover sheet. The data were processed using the software product IBM SPSS Statistics 19, and the MS Excel software package was used to prepare the graphs.

Results and Discussion

Fig.1 presented the change by year of the quantitative indicators of average daily milk yield, roughage and concentrated feed for the period 2020-2024. The average daily milk yield shows a gradual increase. In 2020 (before the introduction of the precision system) it was 24.48 l, which is a baseline value for the analysis. In the following year, the value of this indicator increases to 26.24 l, which is an increase of 1.76 l or approximately 7.2% compared to 2020. In 2022 values

continued to rise, reaching 26.64 l, an increase of 0.40 l or about 1.5% from 2021. In 2023 average daily milk yield reached 26.74 l, which is an increase of another 0.10 l or approximately 0.4% compared to the previous year. For 2024 the value is slightly lower than that of 2023 - 26.69 l, which means a slight decrease of 0.05 l or about 0.2%. Roughage used in 2020 are in the amount of 35.37 kg. In the following years, a continuous decrease was observed: to 32.14 kg in 2021 (down 9.07%), to 30.72 kg in 2022 (by another 4.27%), and up to 29.79 kg in 2023 (by 2.98%).

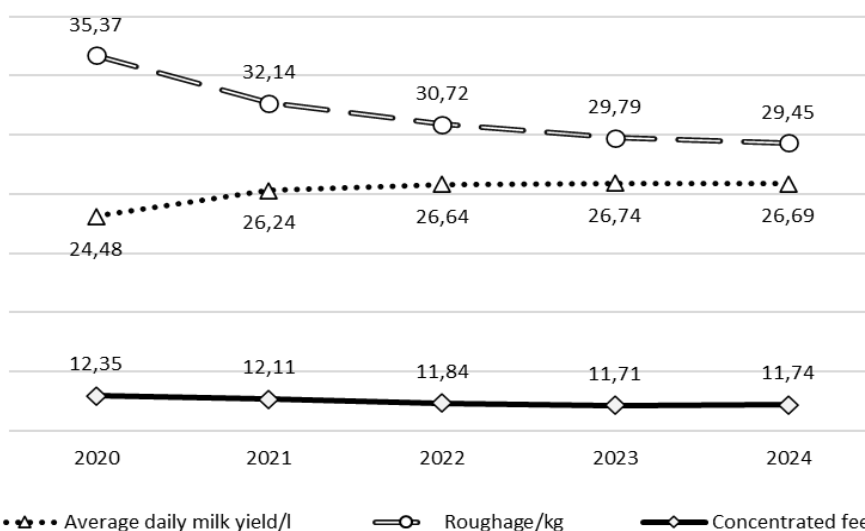


Figure 1. Changes in average daily milk yield and amounts of roughage and concentrated feed during the study period.

In 2024 the volume of feed reaches 29.45 kg, the decrease compared to 2023 is another 1.18%. Concentrated feed also follows a gradual decreasing trend, from 12.35 kg in 2020 to 11.71 kg in 2023, as in 2024 the value slightly increases to 11.74 kg. The percentage change for the period is as follows: from 2020 to 2021 there is a decrease of 1.83%, from 2021 to 2022. – by 2.07%, from 2022 to 2023. – by 0.33%. In 2024 value increases by 0.26% compared to 2023. The graph shows that in the period 2020–2023 an increase in the average daily milk yield was observed, accompanied by a decrease in the amount of roughage and concentrated feeds. This corresponds to innovative approaches in the feeding of dairy cattle breeding, among which precision feeding systems occupy an increasingly important place. According to Boscoa et al. (2021), these systems have the potential to significantly improve milk yield by optimizing nutrient intake and increasing feed utilization efficiency. van Empel et al. (2016) point out that

precision nutrition allows to precisely meet the needs of animals, thereby increasing their productivity and reducing environmental pollution. Hunt (2024) adds that precision feeding involves accurately measuring and delivering the required amount of feed, minimizing residues and increases milk yield. On the other hand, according to Fischer et al. (2020), precise feed restriction is an effective strategy to reduce inter-cow variation in feed efficiency. This does not disturb the lactation or the growth of the animals, on the contrary, it leads to a more efficient use of resources and improves profitability in dairy farming. As a result, farmers achieve lower feed costs, increased productivity and increased income. This integrated strategy for nutrition optimization and resource management offers prospects for sustainable development of the sector.

Fig. 2 shows the change by year of the consumed fuel (l/cow/day) and the average daily milk yield (l/cow/day), in the period before and after the implementation of the system.

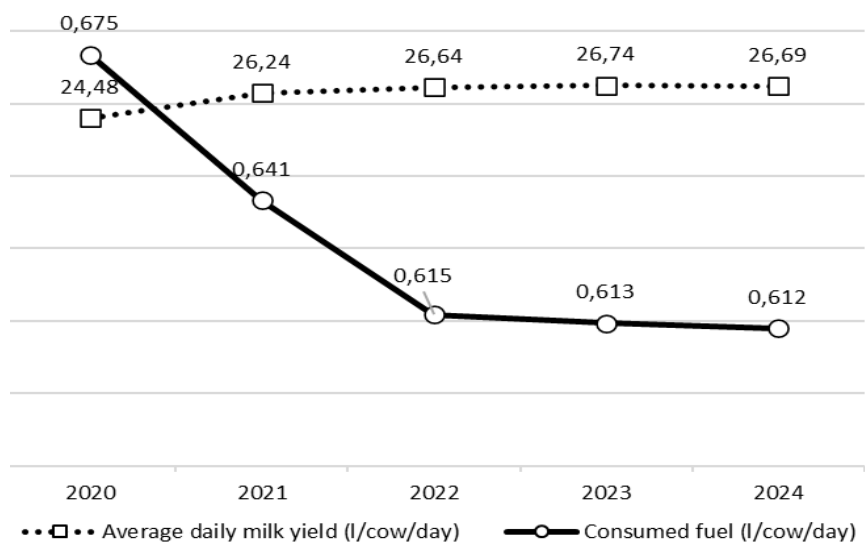


Figure 2. Changes in fuel used (l/cow/day) and average daily milk yield (l/cow/day)

When considering the data for the period 2020-2024 for the fuel consumed, a stable tendency to decrease the values of the indicator measured in liters per cow (l/cow/day) is observed. The values of this indicator decrease from 0.675 l/cow/day in 2020 to 0.612 l/cow/day in 2024, which represents a total decrease of 9.33% for the considered period. In the period 2020 and 2021, the most significant decrease is reported - by about 5.19%. From 2022 to 2024, the values stabilize with a minimal

change, and the final decrease in this period is about 0.16%. This trend corresponds with the introduction of precision technology in 2020, which is aimed at optimizing animal feeding processes, which leads to more efficient use of energy carriers.

The figures show that the introduction of the precision management technology resulted in a significant reduction in fuel costs by 9.33% and an improvement in milk productivity by around 9% over the period. Precision feeding systems on dairy farms play an important role in reducing fuel costs by improving feed efficiency. A similar conclusion was reached by Buckmaster (2009) that by optimizing the performance of the mixer, the fuel used is reduced due to the accurate and efficient observance of the order of mixing the ingredients. In addition, precision systems contribute to the reduction of feed waste and reduce the negative impact on the environment (Pavlova et al., 2025). This is achieved by providing optimal nutrients to the cows, which increases the overall profitability and sustainability of the farm. As a result, less fuel and feed is used to produce the same amount of milk (Lawrence et al., 2015). According to research by Chase and Fortina (2023), the optimization of the feed management contribute to a significant reduction in the carbon footprint of dairy farms, making them more environmentally friendly and sustainable in the long term.

Fig. 3 presents data on the average number of doses per insemination and average daily milk yield. During the reporting period, a gradual decrease was observed in the number of doses of seed material required for the fertilization of one animal.

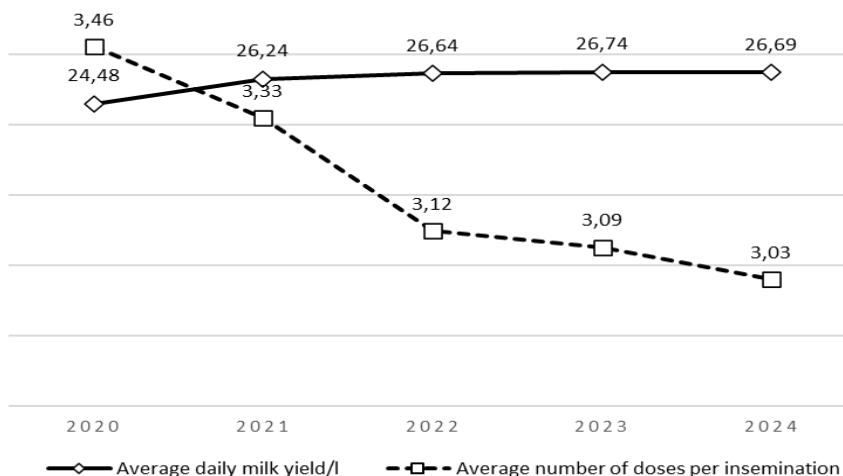


Figure 3. Changes in average number of doses per insemination and average daily milk yield

In 2020, this indicator was 3.46 doses, which in the following years decreased to 3.33 in 2021, 3.12 in 2022, 3.09 in 2023 and 3.03 in 2024. In numerical terms, this represents a decrease of about 0.43 doses or approximately 12.4% compared to 2020. On the basis of the data provided, it can be concluded that precise feeding of animals optimizes metabolic processes and reproductive function, which leads to a reduction in the required number of doses per insemination and an increase in milk productivity. Several studies have found a negative relationship between milk yield and fertility (Grimard et al., 2006; Cutullic et al., 2012). This is partly explained by a negative genetic correlation between milk yield and fertility (Pryce et al., 2004). The positive relationship between high productive and reproductive performance, according to Lucy (2001), can be related to more precise feeding of the animals. Precision nutrition also has a significant impact on body condition assessment and energy balance (Maltz, 2013) and is a major factor associated with improving reproductive performance (López-Gatius et al., 2003; Santos et al., 2004; Grimard et al., 2006). Schmidt (2022) specifies that precision nutrition plays an important role in improving fertility by providing the necessary amount of energy, proteins, vitamins and minerals that are included in the ration.

Because the study was based on the number of doses used per insemination and did not account for the multiple factors responsible for reproductive performance, the results should not be used as definitive proof of any particular theory. Instead, these results can be used as a rationale for further studies on the critical topic of the effects of nutrition on reproduction in lactating dairy cows.

Conclusions

Based on the obtained results, it can be concluded that after the introduction of the precise technology for monitoring and management of nutrition in the dairy cattle farm, an increase in milk productivity is observed, as well as a decrease in the amount of used roughage and concentrated feed, fuel consumed in the farm. Evidence shows that the integration of digital technologies in dairy farming can lead to more efficient use of resources, increased productivity and reduced some negative environmental impacts. Although the optimization process is likely to be slow, the data demonstrate the potential of digital technologies to transform modern dairy farming and its role in achieving sustainable development in the sector.

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STORAGE OF BUCKWHEAT PRODUCTS AS AN FACTOR AFFECTING COLOUR CONDITION AND IMPLICATIONS ON MEAT PRODUCTS

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Abstract: Buckwheat is one of the traditionally high-quality crops recognized for its high nutritional and functional value. Buckwheat products (grain and flour) stored for 0, 3, 6, and 9 months and emulsion-type chicken sausages modified by adding those buckwheat flours (FB0, FB3, FB6, and FB9) were analyzed for instrumental colour parameters (L^* , a^* , b^* , C^* , h , S , ΔE). One- ($P < 0.05$) and two-way ($P < 0.001$) ANOVA tests were used to analyze the data, followed by Tukey's HSD post-hoc test ($P < 0.05$). In terms of storage, a slight decrease in hue angle was observed in grains, with the lowest value of 57.48 (6 months). In flour, trends of decreasing L^* and increasing a^* , b^* , C^* , and S values were observed to the end of storage. The values of hue angle and total colour difference decreased, with the lowest point at 73.92 for h and the largest significant change at 4.73 for ΔE in 6 months. In terms of product type, compared to grains, flour was lighter and yellowish on average 2.3 (L^*) and 1.3 (h) times. Grains consistently demonstrated significantly higher a^* , C^* , and S values. In composite products, added flour slightly affects L^* , and significantly a^* and b^* , where FB0 was lighter, redder and yellower than the other combined products. A significant decrease in C^* and increase in h values caused lower chromatic purity and a more yellow nuance in FB9 (20.66, 45.38). A decrease in S resulted in the greatest attenuation in overall colour perception at FB6, and FB9. An increase trend of ΔE indicating the differentiation of sausages with respect to effect on total colour ($\Delta E \leq 5.10$). The results could indicate the potential utilization of long-term stored buckwheat grains (flour) as a functional ingredient and valuable plant-based raw material in the production of enriched, composite meat products.

Key words: instrumental colour, enriched product, storage, buckwheat, meat products

Introduction

In the growing food industry, especially in the meat industry, the development of functional products incorporating plant-based raw materials (such as grains, flour, husks, powders, vegetable oils, pollen, and extracts) holds a special place. These composite products are characterized by altered and effectively improved chemical, nutritional, technological, functional, and sensory properties (Lee et al., 2018; Safar et al., 2022; Yessengaziyeva et al., 2023). The intake of nutritionally justified and health-beneficial functional products promotes anti-obesity, anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-microbial, and anti-oxidative processes (Wójtowicz et al., 2013; Salejda et al., 2022; Dzyundzha et al., 2023). In general, due to the high lipid and cholesterol content, meat and meat products are considered problematic and unhealthy. However, the inclusion of plant-based raw materials and products in well-known conventional meat products enables the development of improved functional meat foods. In some studies, composite products similar to frankfurter-type sausages, semi-smoked sausages, dried fermented sausages, pork meatballs, horsemeat patties, and chicken patties were examined (Muchekeza et al., 2021; Salejda et al., 2022; Dzyundzha et al., 2023).

One of the traditional high-quality crops recognized for its high nutritional and functional value is buckwheat, as well as its products. The main characteristics are high contents of phenolic acids (dihydrocaffeic, phloretic, chlorogenic, *p*-coumaric, ferulic), bioflavonoids (rutin, quercetin, vitexin, orientin), minerals (K, B, Fe, Cu, Cr, Zn, Co, Ni, Ca, Mg, P, Na), vitamins (B, E), as well as dietary fiber, essential amino acids (lysine, tryptophan, methionine, cysteine), and n-3/n-6 polyunsaturated fatty acids (Vollmannová et al., 2021; Salejda et al., 2022; Pisinov et al., 2024). As an important food in the human diet, buckwheat products (grains) are generally stored for a prolonged period due to strategic reasons.

Some studies have shown that up to 80% of consumer decisions are influenced by colour, and 93% of customers reported that colour (visual appearance) is a primary aspect of their purchasing decisions (Khandelwal et al., 2024). In fact, during the display, storage, and thermal processing of raw materials and products, some factors, such as pH, the Maillard reaction, oxidative reactions, and the presence of antioxidants directly and continuously changed visible colour, influencing consumer acceptance (Wagh et al., 2015; Brugiapaglia and Destefanis, 2009; Atambayeva et al., 2023; Yessengaziyeva et al., 2023; Rakić et al., 2024). Consumers prefer meat with high lightness and a relatively high yellowness. In

general, myoglobin (muscle chromoprotein) is purplish. Oxymyoglobin is formed when myoglobin reacts with oxygen, creating a bright red colour of meat that people often associate with freshness and wholesomeness. In contrast, brown (metmyoglobin) or dark and pale brown (lack of oxygen) colours are considered undesirable (Brugiapaglia and Destefanis, 2009). Buckwheat-enriched meat products, besides a favourable nutritional profile, may exhibit altered sensory features, specifically colour, that reduce the quality attributes of overall acceptability for consumers (Pietrzak et al., 2022). These colour changes can be even more pronounced when green-colored buckwheat flour is used in the production of thermally processed emulsion-type sausages (Wagh et al., 2015; Lee et al., 2018; Ahmad et al., 2020; Atambayeva et al., 2023; Yessengazyeva et al., 2023).

In this research of stored buckwheat products (0, 3, 6, and 9 months) and enriched meat products, the objectives were to investigate their instrumental colour (L^* , a^* , b^* , C^* , h , S , and ΔE) and to consider the possibility of using buckwheat in the production of colour acceptable composite end-products for consumers.

Materials and Methods

Plant material

In this study, the “Novosadska” buckwheat variety was used, which was harvested at the moment of technological maturity of the seeds. The ISO 24333 (2009) method was applied for sampling, and 2 kg of raw material was stored, as previously reported by Ping-Ping et al. (2019). In brief, grains were equally divided into eight samples and placed in plastic containers. Six samples were stored for 3, 6, and 9 months in a drying oven (Digitheat-TFT, J.P Selecta, Barcelona, Spain). The conditions were set at a temperature of 40 ± 2 °C with controlled thermoregulation and a relative humidity of 50%. A sub-sample (roughly 0.5 kg) was formed from two samples at the end of each testing period and then divided into grain and flour sub-samples. The flour was obtained by crushing and grinding at a speed of 20.000 rpm in a laboratory mill A10 (IKA Works Inc., NC, Wilmington, USA). Buckwheat grain and wholegrain flour (particle size 1 mm) materials were prepared in triplicate and used for analysis.

Emulsion-type chicken sausage preparation

Emulsion-type chicken sausages were prepared in a meat processing plant “Milan” located in Dobanovci, Belgrade, which specializes in the processing and sale of chicken meat and products. Five treatments of sausages were prepared, each in batches of 2 kg, with the formulations shown in Table 1, according to Lee et al. (2018), with some modifications. The production process was common for

industrial production and identical for all treatments. All formulations contained equal amounts of ingredients, except for the addition of buckwheat flour. The control treatment (CON) was made of mechanically separated chicken meat (MSM) without adding buckwheat flour, while in other treatments, buckwheat flour stored for 0 (FB0), 3 (FB3), 6 (FB6), and 9 (FB9) months was separately added. After preparation, emulsion-type chicken sausages were vacuum-packed (with 99.9% of vacuum level) and stored at 2 ± 2 °C before analysis. Three replications of the experiment were conducted.

Table 1. Recipe formulation of emulsion-type chicken sausages (kg)

Composition (kg)/Group	CON	FB0	FB3	FB6	FB9
MSM	1.13	1.07	1.07	1.07	1.07
Buckwheat flour	/	0.06	0.06	0.06	0.06
Chicken skin	0.38	0.38	0.38	0.38	0.38
Ice	0.38	0.38	0.38	0.38	0.38
Ingredients	0.11	0.11	0.11	0.11	0.11

Note: Ingredients: 2.00% potato starch, 1.80% salt (with 0.5% NaNO₂), 0.85% seasoning, 0.80% beef protein, 0.30% stabilizer - complete, 0.10% carrageenan, and 0.01% colour (E120).

Determination of instrumental colour parameters

On each flour and grain sample, the instrumental colour was measured at five opposite points on a CR-410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Diffuse light D-65 (average daylight, which has a correlated colour temperature of approx. 6 500 K) was applied, with a standard angle of 2 degrees of shelter and a 50 mm aperture of the measuring head.

On each emulsion-type chicken sausage, the instrumental colour was measured at five opposite points of the longitudinal section on chromameter CR-400 (Konica Minolta Sensing Inc., Osaka, Japan). Conditions were corresponded to diffuse light D-65 (average daylight which has a correlated colour temperature of approx. 6 500 K), a standard angle of 2 degrees of shelter and 8 mm aperture of the measuring head as described by Stajić et al. (2014).

The results were expressed in the CIE L*a*b* system (CIE, 1976; CIE, 1986), using psychometer light (L*), psychometer tone (a*), and psychometer chroma (b*). The L* value represents the lightness, in the range from 0 (black) to 100 (white), a* value ranges from “-” (green) to “+” (red), and b* value differs from “-” (blue) to “+” (yellow). The chromameter was calibrated before measurement against a white tile standard (L* = +97.83, a* = -0.43, b* = +1.98).

C* (chroma - % of black, white or gray) and h (hue angle; 0° - red colour, 90° - yellow colour) were calculated using the following equations (1 and 2):

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \text{ (eq. 1)}$$

$$h = \tan^{-1} \left(\frac{b^*}{a^*} \right) \text{ (eq. 2)}$$

S (perceived saturation), defined as the proportion of pure chromatic colour in the total colour sensation, was calculated using the following equation (3):

$$S = \frac{C^*}{\sqrt{C^{*2} + L^{*2}}} \cdot 100\% \text{ (eq. 3)}$$

The total colour difference (ΔE) of tests stored for 3, 6, and 9 months compared to 0 months was calculated according to equation 4 (Pietrzak et al., 2022):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \text{ (eq. 4)}$$

Statistical analysis

The instrumental colour parameters (mean (M) $\pm$ standard deviation (SD)) were analyzed by one-factor analysis of variance (one-way ANOVA, $P < 0.05$) to evaluate the effect of sausage formulations and two-factor analysis of variance (two-way ANOVA, $P < 0.001$) to evaluate the effects of factors (storage time, product type, and their interaction). Tukey's HSD post-hoc test (Honestly Significant Difference, $P < 0.05$) was used to specify significant differences between means. Statistical analysis was performed using Statistica 12.5 software (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

Table 2 shows the values of instrumental colour parameters of buckwheat products (grains and flour) in relation to the storage time factor. It is noticeable that storage time, product type, and their interaction significantly affected the instrumental colour parameters ($P < 0.001$). However, the interaction of factors ($S \times P$) did not significantly influence ($P > 0.001$) colour parameters h and ΔE . Moreover, there was no significant impact of the storage factor (S) on the ΔE colour parameter.

In regards to grains, as the storage time increased, there was no significant difference ($P > 0.05$) between treatments in the values of L^* , a^* , b^* , C^* , S , and ΔE . However, a decreasing trend was observed in h , with the lowest value at 6 months.

Regarding flour, a decreasing trend in L^* was observed with increasing storage duration, with a significant difference evident at 6 months and beyond. In addition, lightness in the 9th month was the highest and differed significantly from all the others. The pattern inconsistency could originate from differences in sample homogeneity, as more dried shells were easier to shred mechanically. With the passage of storage time, values of a^* , b^* , C^* , and S showed a progressive increase. Compared to the beginning of storage, observed redness, chroma, and perceived saturation increased significantly ($P < 0.05$) by the end of storage but without significant changes after 6 months. The yellow tone had a similar tendency but with significant changes until the very end of storage. On the contrary, h decreased during storage, with the lowest value in the sixth month. Similarly, the value of ΔE also decreased. The total color difference (ΔE) showed that the largest significant change occurred in the sample stored for 6 months and the smallest in the sample stored for 9 months.

Table 2. Variations of instrumental parameters of buckwheat colour depending on storage time (S), product type (P) and their interaction (S×P)

n=3 (five points each)		Product type	Months				Level of significance		
Parameters			0 month	3 months	6 months	9 months	S	P	S×P
L^*	grains	32.70±0.741 ^A	31.62±0.677 ^A	31.68±0.357 ^A	32.55±0.753 ^A		***	***	***
	flour	73.86±0.191 ^{bB}	71.97±0.930 ^{bB}	69.19±1.208 ^{aB}	76.19±0.469 ^{cB}				
a^*	grains	5.40±0.102 ^B	5.52±0.175 ^B	5.70±0.268 ^B	5.75±0.220 ^B		***	***	***
	flour	1.98±0.078 ^{aA}	3.00±0.092 ^{cA}	2.63±0.068 ^{bA}	2.42±0.049 ^{bA}				
b^*	grains	9.23±0.291	8.99±0.246 ^A	8.94±0.273	9.48±0.301		***	***	***
	flour	8.82±0.076 ^a	11.27±0.045 ^{cB}	9.11±0.072 ^a	9.71±0.092 ^b				
C^*	grains	10.69±0.266 ^B	10.55±0.298 ^A	10.61±0.370 ^B	11.09±0.370 ^B		***	***	***
	flour	9.04±0.089 ^{aA}	11.67±0.065 ^{cB}	9.48±0.063 ^{abA}	10.00±0.101 ^{bA}				
h	grains	59.68±0.839 ^{bA}	58.45±0.263 ^{abA}	57.48±0.532 ^{aA}	58.76±0.239 ^{abA}		***	***	NS
	flour	77.34±0.406 ^{cB}	75.12±0.394 ^{abB}	73.92±0.464 ^{abB}	76.02±0.148 ^{bB}				
S	grains	31.08±0.102 ^B	31.65±0.233 ^B	31.75±1.312 ^B	32.25±0.302 ^B		***	***	***
	flour	12.15±0.145 ^{aA}	16.00±0.282 ^{cA}	13.58±0.149 ^{abA}	13.02±0.195 ^{abA}				
ΔE	grains	-	1.44±0.870	1.22±0.919 ^A	0.69±0.272		NS	***	NS
	flour	-	3.31±0.378 ^{ab}	4.73±1.040 ^{bB}	2.53±0.332 ^a				

^{a, b, c} Means within the same row with different superscripts differ significantly ($P < 0.05$); ^{A, B} Means within the same column with different superscripts differ significantly ($P < 0.05$); *** $P < 0.001$; NS – not significant

In terms of product type, in all treatments, flour was lighter and yellowish on average 2.3 (L^*) and 1.3 (h) times, compared to grains. Similarly, during the entire storage period, grains consistently demonstrated significantly higher a^* , C^* , and S values ($P < 0.05$). In contrast, the b^* and ΔE values did not differ significantly between the grain and the flour. Exceptions were in flour products in the 3rd (b^*) and 6th (ΔE) months, where a significant increase in yellow tone and total colour difference was observed.

Our results for flour lightness (darkening) are in agreement with the findings of Muneer (2015) and Anberbir et al. (2023), who stated that the impact of various natural processes, especially of the chemical type, collectively contribute to browning. Additionally, Muneer (2015) reported that increasing storage by one day results in a decrease in the colour value of the stored flour. Various processes, including non-enzymatic (chemical) and enzymatic (biochemical) reactions, collectively contribute to browning *via* the Maillard reaction, caramelization, pigment degradation, and enzymatic activity (Purlis, 2010; Lee et al., 2022; Kamalesh et al., 2025). All these processes are significantly amplified by processing, long-term storage, and thermal effects. Compared to our results, the findings of Jara et al. (2022) were the same in terms of all positive a^* and b^* values, but the disagreement was reflected in slightly lower a^* (1.80) and slightly higher b^* (13.00) values of flour. In comparison with the findings of Jara et al. (2022), who reported a chroma value of 13.00, our results were slightly lower. Also, our h values of wholegrain buckwheat flour were lower and inconsistent with the result (82.11) of Jara et al. (2022). All the mentioned discrepancies in the results of the instrumental colour parameters may originate from the difference in the particle size of wholegrain flour (0.21 - 0.25 mm) because Jara et al. (2022) used a sieve with a different mesh size than ours, as well as non-stored flour. Since the S value is derived from the L^* and C^* values, their changes will directly affect the trend of the S value, correlating the same reasons as for L^* and C^* . The same could be said for ΔE , which is derived from L^* , a^* , and b^* values. It is known that a total colour difference <2.0 indicates differences that the human eye cannot detect (Pietrzak et al., 2022).

Table 3 shows the values of instrumental colour parameters in relation to different emulsion-type chicken sausages as a product type factor. Different formulations of the same sausage product had a significant influence on all instrumental colour parameters ($P < 0.05$). Moreover, progressive changes were noted with increasing storage time of buckwheat flour, in all observed instrumental colour parameters.

Compared to the CON product, the storage period of buckwheat incorporated into the products did not affect the L^* values ($P > 0.05$). However, emulsion-type chicken sausage with 0 months stored buckwheat flour (FB0) was significantly lighter in comparison to other composite products.

In relation to the CON product, significant differences in a^* and b^* values were observed in FB3, as well as in FB6 and FB9. By adding buckwheat grains (flour) stored for a longer period, 3-9 months, red and yellow tones of emulsion-type chicken sausages progressively decreased (from 15.85 to 14.52, and from 15.20 to 14.73, respectively).

Table 3. Changes in instrumental colour parameters in relation to the storage period of buckwheat flour added to emulsion-type chicken sausages

Parameters	Product type				
	CON	FB0	FB3	FB6	FB9
$n=3$ (five points each)					
L^*	59.27±0.500 ^{ab}	60.64±0.465 ^b	58.72±0.090 ^a	58.92±0.525 ^a	58.41±0.875 ^a
a^*	19.05±0.318 ^c	18.55±0.191 ^c	15.85±0.090 ^b	15.11±0.363 ^a	14.52±0.172 ^a
b^*	16.50±0.456 ^b	16.23±0.214 ^b	15.20±0.128 ^a	14.94±0.287 ^a	14.73±0.110 ^a
C^*	25.22±0.535 ^c	24.66±0.276 ^c	21.99±0.145 ^b	21.27±0.455 ^{ab}	20.66±0.190 ^a
h	40.81±0.310 ^a	41.16±0.160 ^a	43.80±0.102 ^b	44.76±0.140 ^c	45.38±0.159 ^d
S	39.16±0.825 ^d	37.67±0.305 ^c	35.08±0.183 ^b	33.92±0.648 ^{ab}	33.34±0.260 ^a
ΔE	-	1.52±0.922 ^a	3.55±0.362 ^b	4.31±0.200 ^{bc}	5.10±0.420 ^c

^{a, b, c, d} Means within the same row with different superscripts differ significantly ($P < 0.05$); Emulsion-type chicken sausages: CON: without buckwheat flour; FB0: 3% of buckwheat flour at 0 months of storage; FB3: 3% of buckwheat flour at 3 months of storage; FB6: 3% of buckwheat flour at 6 months of storage; FB9: 3% of buckwheat flour at 9 months of storage.

The significantly lower C^* values ($P < 0.05$) compared to CON were found in sausages with incorporated 3-month stored buckwheat flour (FB3) and more long-term stored buckwheat flour (FB6 and FB9). Increasing the storage time of buckwheat flour included in the formulation resulted in FB9 sausages having lower chromatic purity, showing the smallest distance from lightness compared to other sausages.

Similar to chroma (C^*), hue angle (h) values between CON and FB0 did not differ significantly, but the significance increased with FB3, as well as in FB6 and FB9. The addition of buckwheat grains (flour) stored from 0- to 9-months, led to a significant progressive increase in the proportion of yellow nuance in emulsion-type chicken sausage, with the largest change in value at FB9.

The S values were significantly different ($P < 0.05$) for all emulsion-type chicken sausages compared to CON, which had the highest value. By adding long-term stored buckwheat flour into the formulation, a decreasing trend of saturation was observed, which became more pronounced with longer buckwheat storage. Our results showed that the proportion of pure chromatic colour in overall colour sensation was significantly reduced from CON to FB9. Since the S value is derived from L^* and C^* values, their reduction by incorporating long-stored buckwheat flour into the formulation directly causes a decreasing trend in S value from CON to FB9, for the same reasons as those for L^* and C^* .

Total colour difference (ΔE) values gradually increased with the addition of longer-stored buckwheat flour to the formulation. The lowest ΔE value was observed for FB0, and the highest for FB9, while there was no significant difference between FB3 and FB6 values ($P > 0.05$). Variation in L^* , a^* and b^* colour parameters of emulsion-type chicken sausages made with differently stored buckwheat flour affected the total colour difference parameter (ΔE). This means that compared to CON, the highest degree of colour change was observed for FB9, i.e. emulsion-type chicken sausage whose formulation contains buckwheat flour stored for 9 months.

In our results for emulsion-type chicken sausages, the difference in lightness (darkening) was associated with buckwheat grains (flour) stored for 3-, 6- and 9-months, as development of lipid oxidation and the Maillard reaction products readily occurs during thermally assisted long-term storage and drying (Jambrec et al., 2011; Pietrzak et al., 2022; Pisinov et al., 2024). Similar to our results, Atambayeva et al. (2023) reported lower L^* values after adding buckwheat and its flour to horsemeat and chicken patties, and Salejda et al. (2022) found that the addition of 3% and more buckwheat husks in frankfurter-type sausages resulted in a decrease in L^* values. Both studies showed minimal changes in colour-sensory properties during storage. The same group of authors also stated a 2-week storage period and confirmed slow decreases of L^* values. The reported changes in parameter values indicate the preservation of product colour over long-term storage due to the stabilization of oxymyoglobin and the delay of spoilage caused by phenolic compounds. Generally, the findings of Lee et al. (2018) and Kilincceker and Karahan (2020) were in agreement with our results (although FB0 to CON is not significant), as they found that added buckwheat flour to emulsion-type pork sausages (3%) or fried chicken meatballs caused an increase in L^* values. Later disagreements with our results (darker FB3, FB6, FB9 *cf.* CON) came from the fact that their research was performed with only one, non-stored type of buckwheat flour. Yessengaziyeva et al. (2023) noticed that the addition of 12% and more buckwheat flour in semi-smoked sausages made composite product colour unsatisfactorily desirable and more unattractive.

The decreasing trends of a^* and b^* values could be explained by the loss of phenolic compounds as natural pigments, which is even more pronounced during thermally assisted long-term storage and drying (Kilincceker and Karahan, 2020; Pisinov et al., 2024). Our results were in agreement with the findings of Kilincceker and Karahan (2020), who found that added buckwheat flour caused a decrease in redness and yellowness of fried chicken meatballs. Lee et al. (2018) reported that adding 3% of buckwheat powder in emulsion-type pork sausage resulted in a lower a^* value, which was in accordance with our results, especially for FB3, FB6, and FB9. However, there is disagreement about the b^* value, which could be explained by the source and form of flour added to the product. Similar to our results, Salejda et al. (2022) found that the addition of buckwheat husks (3% and more) in frankfurter-type sausages resulted in a decrease in b^* values. Contrary to our results, Atambayeva et al. (2023) reported slightly higher a^* and b^* values after adding buckwheat and its flour to horsemeat and chicken patties.

Cavalheiro et al. (2013) stated that C^* and h values depend on a^* and b^* values, and changes in these values are caused by the same reasons as those in a^* and b^* . Therefore, the progressive loss of phenolic compounds was more pronounced as storage time of buckwheat flour increases from 0- to 9-months, which led to less saturated sausage products because a^* and b^* decreased, and caused changes from red to yellow as the decrease of a^* was more emphasized than that of b^* . Varga-Visi et al. (2021) came to the same conclusion by examining sausage with paprika, where the changes in colour were attributed to the deterioration of the chromophores in meat and paprika, i.e. the decomposition of those pigments.

It is known that $\Delta E < 2.0$ values indicate a colour difference compared to the control that cannot be detected by the human eye (Pietrzak et al., 2022). Wójtowicz et al. (2013) investigated colour parameters of extruded corn snacks enriched with buckwheat flour up to 50%, and found colour differences between treatments similar to our study. In contrast to our results, Pietrzak et al. (2022) reported that there was no significant difference in ΔE values when testing the effect of buckwheat hull extracts on the quality of chicken meatballs.

Conclusion

The results could indicate the potential utilization of long-term stored buckwheat grains (flour) as a functional ingredient and valuable plant-based raw material in the production of enriched, composite meat products such as vacuum-packed emulsion-type chicken sausages.

From the perspective of buckwheat, storage time, product type, and their interaction significantly affected the instrumental colour parameters. The storage

factor (S) did not significantly influence ΔE , nor the interaction of factors (S×P) h and ΔE . In terms of storage, for grains, a slight decrease of h was observed, with the lowest value at 6 months (57.48), but there was no significant difference between treatments in the values of L^* , a^* , b^* , C^* , S , and ΔE . For flour, trends of decreasing L^* and increasing a^* , b^* , C^* , and S values were observed until the very end of storage. The values of h and ΔE decreased, with the lowest point at 73.92 for h and the largest significant change at 4.73 for ΔE , both at 6 months. In terms of product type, compared to grains, flour was lighter and yellowish on average 2.3 (L^*) and 1.3 (h) times. Grains consistently demonstrated significantly higher a^* , C^* , and S values. Between grain and obtained flour, there were no significant differences in b^* and ΔE values.

The addition of 3% buckwheat flour stored for 0-, 3-, 6- and 9-months in the formulation of emulsion-type chicken sausages slightly decreased lightness (L^*), but significantly lowered a^* and b^* values. Furthermore, instrumental colour analysis showed significantly decreased C^* and S values, while the h value progressively increased. Also, the addition of stored buckwheat flour resulted in differentiation of sausages in terms of the impact on total colour ($\Delta E \leq 5.10$).

The degree of correlation between instrumental colour values and combined products depends on all factors governing lipid oxidation, the Maillard reaction and levels of phenolic compounds. Considering that the instrumental colour represents only a part of the more complex methodology of the product sensory evaluation, it is necessary to conduct more extensive researches of sensory attributes (taste, odour...), textural profile, and the technological properties of the buckwheat-enriched meat emulsion stability. Since colour changes mainly influence consumer acceptance and purchasing decisions, establishing a technological reference of buckwheat products and evaluating their effects on the quality characteristics of composite meat foods is therefore important to prove technologically viable and colour-sensory acceptable end-products for consumers.

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Conflict interest

The authors declare no conflict of interest.

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