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Danubian Animal Genetic Resources

Volume 11, Issue 2 (2026)

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International Association for the Conservation
of Animal Breeds in the Danube Region
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Hungary

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Indigenous cattle breeds of the Carpathians – the basis of scientific and transboundary aspects of the implementation of the „Carpathian Convention"†

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Abstract

In the author's opinion, indigenous cattle breeds of the Carpathians are the basis of scientific and transboundary aspects of the implementation of the "Carpathian Convention" and protocols thereto. These indigenous cattle breeds are as well the national heritage of the country in which these breeds are bred. The publication provides data on the distribution of 19 local breeds in the Carpathian region, the possibility of restoring officially extinct ones. Based on the results of the study and analysis of scientific works, their list was compiled, the number of breeding stock and the average number in one farm are given. A number of differences in territorial distribution and their different genesis have been established; this is due to the long history of development. A limited number of breeding stock has been identified, a clear trend towards decline is observed, which indicates the threat of extinction. The succession of breed composition that is currently taking place in the region is indicated. The results obtained are important for continuing further research based on cooperation between various institutions of the united countries in the Carpathian Euroregion, studying the features of cattle breeding and the impact of the industry on the functioning of landscapes, preserving biodiversity, restoring the transformed meadow ecosystem and sustainable

† This paper V.P. Terpai's modified previous publication in Ukrainian (Аборигенні породи великої рогатої худоби (ВРХ) Карпат – основа наукових та транскордонних аспектів впровадження Карпатської конвенції і протоколів до неї. Еколого-правові, науково-освітні та транскордонні аспекти впровадження карпатської конвенції та протоколів до неї. Матеріали міжнародного круглого столу. Україна, м. Рахів, 3 жовтня 2024 року, 288–295.) in English.

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development of communities in the Carpathians. They indicate the expanded functions of aboriginal cattle breeds. It is emphasized that one of the forms of wildlife protection is the organization of the preservation of indigenous breeds of cattle, which act as a matrix of farm animal populations and is based not only on ecological and biological principles, but also covers the entire spectrum of ideological, economic, socio-cultural, ethical and ethnic problems.

Keywords: biodiversity, ecology, indigenous breeds, sustainable development, transboundary cooperation

Introduction

According to FAO (2015), over the past decades, population growth, intensification of agricultural production has degraded 25% of the world's soils, 50% have been damaged, and a significant part of indigenous breeds of cattle and natural meadow ecosystems have disappeared. In view of this, it is important to study ways to restore local livestock in the Carpathians – scientific and transboundary aspects of the implementation of the "Carpathian Convention" and its protocols, diversification of environmental technologies and innovative products, development and application of political and economic solutions. Accordingly, the foundations of sustainable development of the Carpathian region will be laid, the biodiversity of cultivated breeds of farm animals will be preserved, the fair and equitable distribution of benefits from the use of genetic resources will be established, which is provided for by the provisions and protocols to the “Carpathian Convention”, in particular Art. 6 and Art. 10 – the preservation of traditional animal breeds of the Carpathians, the Action Plan for the 21st century (1998) and the Framework Convention (2004).

In addition, the adopted documents serve as legal mechanisms and provide the basis for international cooperation, especially in the context of globalization and climate change. Therefore, the importance of transboundary and subregional cooperation is growing, ensuring the ecological integrity of the territory with an area of 210 thousand km² and improve the quality of life of more than 18 million people.

It is important to use local resources sparingly and effectively in harmony with nature and the environment, applying an ecosystem approach to managing a significant sector of the economy – cattle breeding. To reorient the industry in a strategic perspective to more profitable innovative management, to fulfill the obligations of the Carpathian states in this area (VORONINA and DEMURA, 2004). EGEN (2010) noted that even before the last century, the Carpathians had better opportunities for

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animal production than the Alps. At the same time, it will be possible to avoid the irreversible loss of a component of cultural and genetic biodiversity – aboriginal cattle, which has lost its dominant role and is a rare element in some countries, or is on the verge of extinction. For example, only in the Transcarpathian region of Ukraine, the Ryzhka, Mokan, and Hutsul cattle have been lost forever.

In the Carpathian region, 19 native breeds are on the verge of extinction. In the hierarchy – production, balanced development, non-production priorities, protection of nature, environment, traditions, climate, quality of life – they are living evidence of uniqueness of Carpathian region, a platform for scientific research.

Despite the above, in some Carpathian countries of the association, there is not enough research, scientific and technical developments. Also funding from budget appropriations does not have a systematic approach, which can cause economic instability in mountainous, depressed areas.

Research of varying degrees of environmental impact of animals that have been bred here for centuries would allow to reorient the industry, using knowledge about their genetic differences, anatomical structure, biology and physiology, to a more profitable, balanced, long-term and integrated way of management, restore degraded Polonian ecosystems to their natural state. Because during the reduction of the cattle population, the reduction, in many cases the cessation of traditional management, the biotopes of meadows, pastures, especially high-mountain ones, have become overgrown with non-native phytocenoses and have displaced plants, including endemic species. In addition, a real threat to the Carpathians and biodiversity is the large-scale production and expansion of highly specialized commercial breeds of European-American origin, uncoordinated actions of countries. For example, in Poland, 96% of the Holstein cow population, all bulls of the named breed have American roots according to pedigrees.

The listed problems will be solved by the development and implementation of joint projects for the sustainable development of mountain areas based on the use of local resources, indigenous cattle breeds, green gold – natural pasture lands. Parallel transition to organic, biological, organobiological, biodynamic and other modifications will include a self-regulating mechanism for restoring ecological connections, ecological conversion (MANIVCHUK, 1996). The task of the scientific community is to promote the implementation and introduction of the "Carpathian Convention" and its protocols based on scientific and transboundary aspects of cooperation and local initiatives. The development and use of environmentally friendly technologies will contribute to the improvement and provision of the diversity of life in the ecosystem (BOYKO, 2002).

The relevance of the article is due to the requirements of the "Carpathian Convention" and its protocols, the presentation of the current state of interaction and the capabilities of individual countries in organizing ecological production, restoration, conservation and management of genetic resources. It is now most necessary to study the impact on the mountain phytocenoses of the Carpathians, to

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publish the maximum amount of information related to indigenous breeds, especially in Ukraine due to martial law, as this will ensure their preservation and development of mountain areas in harmony with nature.

Purpose of the study was to evaluate the impact of indigenous cattle breeds of the Carpathians on the scientific and transboundary aspects of the implementation of the "Carpathian Convention" and its protocols, and on natural meadow ecosystems. To obtain new data about them, to show biological features. Scientific development and practical implementation of joint projects in conditions of sustainable development.

Material and methods

The research was conducted during 2019 – 2023. The study area covers the mountainous territories of the Carpathian region of Slovakia, Romania, Poland, Hungary, the Czech Republic, Serbia and four regions including Transcarpathia of Ukraine. The social, historical and cultural, economic, and resource status were assessed. Local breeds, the main threats to them, the principles and trends of changes in breed composition and the impact of production on the environment were studied. The feasibility of their use in the Carpathians was substantiated as the basis for the scientific and transboundary aspects of the implementation of the "Carpathian Convention" and its protocols.

Indigenous cattle breeds will ensure the transition to the requirements of the provisions of the European Green Deal and the Common Agricultural Policy, and the main Sustainable Development Goals defined in the UN program documents on livestock, "Combating Climate Change", "Conservation of Terrestrial Ecosystems", etc. The implementation of the principles of the Circular Economy, the use of direct product sales channels is presented. The vision is set out for combining the development of the livestock industry, preserving biodiversity, restoring meadow ecosystems, reducing the impact on the state of the environment in the region. The definition and names of cattle breeds were carried out according to the Breeding Resources of Ukraine, 1998 and its summaries.

Results and discussion

The research has established that one of the elements of the basis of scientific and transboundary aspects of cooperation between countries, the implementation of the "Carpathian Convention" and its protocols are indigenous cattle breeds of the Carpathians. The international document clearly defined what an extremely interesting region in the biogeographic sense should be in the 21st century. Implementing its provisions, it is necessary to abandon resource-based approach, protection categoricalness and strict restrictions on economic activity even in

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protected areas, based on the fundamental paradigm of modern ecology, in which the concept of biodiversity is the leading one. Animals and territories should be considered as a single ecological and economic system with signs of integrity.

After all, cattle breeding in the Carpathians has its own history and features determined by economic, soil and climatic conditions, relief, altitude, limited land, traditions, dispersed settlement of people, etc. The industry is based on its own natural and breed, human and financial resources. A significant number of natural hayfields and pastures, a humid climate, contribute to the richness of the floristic composition and high productivity of meadow formations.

Favourable geographical location, large capacity of domestic and foreign markets, stable demand for high-quality, healthy, safe, craft products of animal origin determine that it is promising to engage in the production of milk, beef with deep processing in the future. Also, this type of activity, built based on aboriginal breeds, can partially eliminate the problem of employment, provide a significant source of income for hundreds of thousands of personal farms, a significant number of small businesses, replenish local budgets and ensure sustainable and long-term development of communities.

There is a lot of information about the value of local animals in the scientific literature. Realizing this, employees of the University of Life Sciences in Lublin, Poland, through the implementation of a pilot project and perseverance, hard work and connections with farmers, regenerated in 2003 the aboriginal white-spine breed of cattle, which was officially considered extinct in the 60s of the last century. Now the animals are used for grazing in the Roztochanskyi National Park and other parks, which ensures the development of agricultural production in protected and problem areas, its low cost, higher profitability, productive longevity of cows, climate neutrality and enrichment of biodiversity while preserving the originality of landscapes.

There are a weak documentary and information base about the main means of production, aboriginal breeds of cattle in the Carpathians, limited data in scientific sources. Therefore, with the support of the above-mentioned scientific institution, a study of local breeds of cattle in the mountainous areas of the Carpathian countries was conducted and 19 breeds were identified. The gene pool covers 479,425 animals. Breeds were differentiated by the following characteristics: dairy-meat or meat-dairy direction of productivity, the highest level of variability, reproductive ability and longevity of productive use. The colour is mostly uniform, the animals have a strong constitution and hoof horn, a well-developed respiratory system, better reactive properties, resistant to many diseases, genetically determined production of high-quality milk, meat, best adapted to eating grass vegetation of natural meadows and pastures and converting it into milk, beef, etc. Due to the combined direction of productivity and relatively low live weight, they are characterized by an unshakable balance of "genotype – environment", emit 10% less methane into the atmosphere

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(FAGAN, 2016). Individual breeds originate from the domestication of the small Carpathian aurochs.

At the same time, globalization, unification and introduction, have caused structural, quantitative and qualitative changes in the breed composition. There is a significant decline in the breeding stock. Generalized data on breeds in breeding countries are presented in Table 1.

It should be noted that a significant part of breeds is distributed in the zone of anthropogenic landscapes and in protected areas, therefore it requires constant monitoring and conservation in order to optimize the functioning of historically formed ecosystems. Analysis of the data obtained allows us to confirm that the most widespread are the Czech motley, Simmental and Slovak motley breeds. The highest share (35.5%) is made up of genetic resources of Czech motley cattle. Its gene pool is concentrated in 1141 farms. A significant area is covered by Serbia motley cattle. A small number of livestock breeds such as Polish red, Ukrainian gray, Ukrainian white-headed and Lebedynska are in Ukraine; black-white and white-spine breed in Poland.

Table 1: Native cattle breeds of the Carpathian region, total number and average per farm, heads.

Country	Breed	Total no.	Average no. per farm
Czechia	Czech motley	170,000	149
Hungary	Hungarian grey/Magyar	4,965/14,000	24/36
	Romanian brown, Bruno	1,000	18
Poland	Polish red	2,399	9
	black-white	1,575	12
	red-white	3,293	10
	white-spine	653	6
	Simmental	100,000	15
Romania	Romanian brown, Bruno	3,000	3
Slovakia	Slovak motley	105,229	84
	Pinzgau	3,412	107
Serbia	motley cattle	43,000	5
Ukraine	Carpathian brown	24,051	1
	Lebedynska	640	160
	red-steppe	1,609	201
	Polish red	126	126
	Ukrainian grey	173	173
	Ukrainian white-headed	300	300

In the current situation, the balanced and long-term development of the regions where indigenous breeds are bred is lost. The production of ecological milk and the income per year per cow is 2.4 times higher than in the production of conventional

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(SZUMIEC and MUSIAL, 2021). The Figure 1. presents the criteria for measuring the assessment of balanced and long-term development according to the aforementioned authors.

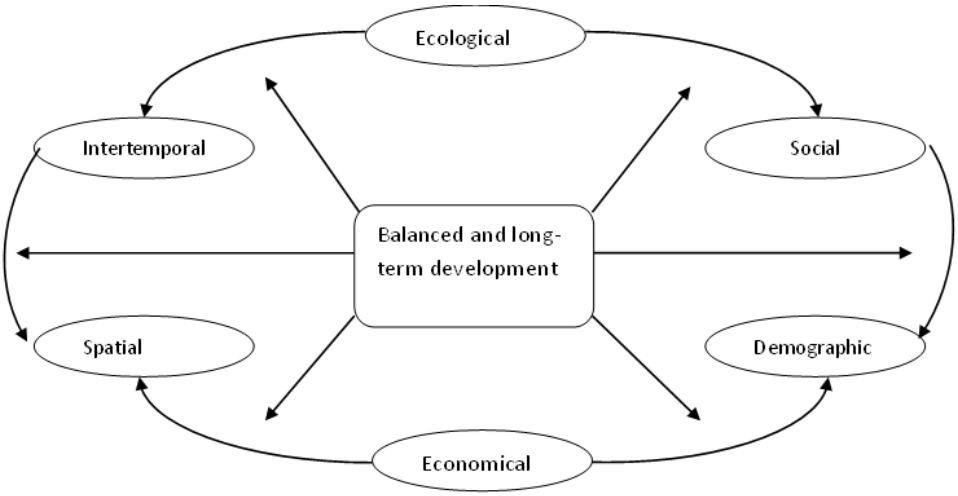


Figure 1: Criteria for measuring the assessment of balanced and long-term development according to SZUMIEC and MUSIAL, 2021

In addition, made from valuable in a biological sense, vitaminized milk obtained in the pasture system on natural meadows, craft, environmentally friendly, organic, butter, hard cheeses also have a bouquet of specific aromas. For example, in Ragusano cheese 27 aromatic compounds have been identified, while in cheese made from milk obtained from cows that consumed a traditional diet only 13 (CARPINO et al., 2004).

Leading the Polonian economy, adhering to the age-old traditions and culture of the Hutsuls, Boykas, Lemkos and other ethnic groups, today they have learned to use the genetic, biological, anatomical, physiological properties of local cattle, to live in harmony with nature and the environment, as required by the "Carpathian Convention" and its protocols. After all, closely connected with the Carpathians is the fate of the ancestors, who began to inhabit this territory in the early Palaeolith – more than a million years ago.

As in ancient times, cows are kept for 8-10 hours a day, and young animals are kept around the clock in the spring-summer-autumn periods on natural meadows and pastures, consuming green, high-quality, highly digestible feed with an optimal ratio of protein-carbohydrates-energy, with partial feeding with concentrates and hay in winter, while fertilizing poor soils. It should be noted that thanks to this technology, animals have good indicators of somatic development and the ability to maximally manifest genetically laid down growth and development tendencies and phenotypic

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parameters that are inherent in breeds, coexist with wild herbivores and birds common in these areas, and pastures reach a yield of 60-70 centners/ha of feed units. The basis of motivation is also the economic component. The level of feed conversion into products increases, the cost of the ration decreases, and its good payment is noted. The calculations in Table 2 confirm the lowest relative cost of green mass. That is, if the mass unit of natural pasture fodder we take as 100 percent, the cost of concentrates will cost 31.6 times more, and in terms of nutritional value 3.92 times more. It is this kind of maintenance and feeding that ensures competitive and profitable production of milk and beef, increases the growth rate of grasses in height, mass of stems and leaves, and stimulates changes in the diversity of botanical composition for the better.

Table 2: Relative cost of green fodder of natural meadows and pastures

Indicators	In % of pasture fodder	
	natural	feed units
Green mass of natural pastures	100	100
Green mass of mowed perennial grasses	230	158
Green mass of corn and annual grasses	400	332
Hay from natural hayfields	608	247
Hay from sown perennial grasses	960	301
Hay from sown annual grasses	1,210	380
Forage root crops	960	1,051
Concentrated feed	3,160	392

In addition to economic and economic significance, pastoral traditions that are passed down from generation to generation contribute to the restoration of anthropogenically transformed meadow ecosystems. The day of entering on the alpine meadows is solemnly celebrated. It is known that now throughout Europe such a system is encouraged, supported by state and EU subsidies, and is consistent with global trends. This means that the importance of cross-border and subregional cooperation in this regard is growing, and the “Carpathian Convention” has opened new opportunities for using the reserves laid down by nature itself.

It is necessary to conduct comprehensive studies of the consequences of economic activity, to reach an agreement on technological processes to be used for balanced, economic management of the livestock industry, elimination of contradictions between production and ecology, preservation of biodiversity and development of local cattle breeds for the benefit of people who live and work here, to activate the formation of environmental awareness among the population.

Despite partial positive developments after the adoption of the “Carpathian Convention” and its protocols, the establishment of cooperation between various authorities of the Carpathian Euroregion countries, the issues of forming the local market for milk and beef, solving the shortage of animal protein, preserving the environment, fauna, flora, genotypes of breeds of small populations of the food

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subcomplex, and studying the advantages of local breeds over commercial ones remain problematic. The main reason is the systematic reduction of active herds of aboriginal breeds. Most of them have reached their limit minimum, the force of influence of which can really threaten their disappearance, the sustainability of not only cattle breeding, but also the crop industry and production shortage in mountainous areas. To prevent negative trends in the future and for a detailed assessment of such a phenomenon, an in-depth study and analysis of the situation in a specific region of the Carpathians, Transcarpathian region, Ukraine was conducted. Based on the results of our own research, it was found that the peak of the development of the main Brown Carpathian breed (BCB) falls on the mid-90s of the last century, when the number of BCB animals was 113.4 thousand. The dynamics of the livestock are given in Table 3.

Table 3: Absolute and relative indicators of the Brown Carpathian breed and all cows total, production and demand for livestock products in Transcarpathia in 1986; 2004 and 2023

Years	No. of BCB cows, in thousands	No. of BCB cattle, in thousands	Milk production		Beef production	
			yield, kg in thousands	deviation from need	live weight, kg in thousands	deviation from need
1986	113,4	141,7	356	-119	45	-15
2004	100,9	140,2	438	-37	34	-26
2023	36,1	42,0	283	-192	27	-33
change 1986-2023	-77,3	-99,7	-73		- 18	-
% change 1986-2023	-314.1	-337.4	-21		- 40	-

Note: milk demand – 475 thousand tons; meat demand – 60 thousand tons

The results obtained clearly confirm the negative dynamics, a significant decrease in the number of cows during the analysed period in both absolute and relative indicators. Thus, in absolute figures, the number of BCB cows decreased by 77.3 thousand heads, the total decrease – by 99.7 thousand heads. Thus, having considered the above changes and retrospective sources, it can be stated that the brown Carpathian cattle has proven its viability even in different socio-political conditions since 1911. Despite this, its population is steadily decreasing and reaching a critical limit. Per 100 hectares of agricultural land, there was also a significant decline in number of cows (from 30.9 to 9.2) between 1986 and 2023, the condition is assessed as very low. The situation is similar if it the number of cows is assessed per 100 inhabitants; this decreased from 11.3 to 3.3 during the period. More animals are kept in mountain areas, but the above-mentioned patterns are observed, production has partially moved to the lowlands.

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At the same time, gross milk production, after growing by 18.7% in 2004, fell by 35% last year, and beef production recorded declines of 24.4% and 20.7%. As a result, the need for valuable food products has become consistently negative and in 2023 it is provided by 59.6% and 45.0%, respectively. This problem leads to a violation of the basic indicators of good nutrition of the local people, primarily by protein of animal origin. In the structure of the caloric content of the diet, products of animal origin account for 30%, at an optimal level of 55% with a predominance of poultry meat.

It should be noted that the decline of traditional farming on high-mountain pastures has changed the harmonious landscapes typical to the Carpathian territories and worsened the botanical composition of the herbage, impoverished the biodiversity of phytocenoses, which provoked the uniformity of the plant cover of natural meadows and pastures, the role of which in nature is equal to forests. Recent surveys indicate invasions of common juniper, green alder, etc., which displace grass cenoses.

The coefficient of use of available solar energy, which worsened the potential indicators of photosynthesis and, accordingly, the recreational potential, is one of the causes of soil degradation. Such a problem is also observed in Transcarpathia, Ukraine. Currently tussocks, low-value weeds, shrubs and trees are widespread (Figure 2). Of the total land fund of the mountainous part, 571.6 thousand hectares (53.5%) have been declared an area of extreme ecological situation.



Figure 2: Modern appearance of the former natural pasture, photo by the author, 2024

The prolonged disparity in prices for livestock products, goods and services of other sectors of the national economy, not in favour of farmers, the liquidation of the public sector of the economy, breeding farms, processing enterprises during the transition

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period to a market economy accelerated the regression of the BCB population in 2011 to 75 thousand cows or by 52.9%. At the same time, the fact that the coefficient of added value of sold livestock products is 3.2% in the chains of “raw materials – consumption – by-products and raw materials for the light and pharmaceutical industries" is undeniable.

In order to stabilize the state, scientific and transboundary aspects of the implementation of the “Carpathian Convention” and its protocols, the sustainable use of natural and genetic resources, and the creation of a new model of industry management, we have developed the “Concept for the Preservation of the Local Brown Carpathian Breed and the Development of Dairy Cattle Breeding in the Transcarpathian Region by 2025”, Ukraine. The concept was approved and recommended for publication by the Academic Council of the Institute of Animal Breeding and Genetics of the NAAS on December 18, 2012, protocol No. 411. The concept combines the interests and real possibilities of the state, the region, and small producers. The establishment of a breed association was taken care of, which was officially registered in 2014. In the same year, on October 16, a cooperation agreement was signed with the Association of the Brown Maramorosh breed – Bruno, Sighet city, Romania. In accordance with the recommendations of the FAO, a gene bank was organized in Mukachevo city, Ukraine, where biological material of different generation periods since 1962, of different bloodlines and belonging to different lines of 10 purebred and 28 crossbred BCB bulls is stored. Biomaterial from four of them was transferred to the National Bank of Animal Genetic Resources at the Institute of Animal Breeding and Genetics named after M.V. Zubets, NAAS. At the meeting of the Technical Council of the Regional Office for Eastern Europe and Central Asia of the Food and Agriculture Organization of the United Nations (FAO), Budapest, Hungary, on the issue of preserving priority local breeds, on April 30-31, 2015, the Chairman of the Board of the Association presented the Brown Carpathian breed and argued the feasibility of paying for genetic research. During the following period, meetings of the working group were held at the National University of Bioresources and Environmental Management, Kyiv, Ukraine. In August 2017, a financing project was approved. Practical work was carried out on the research of 183 heads. On August 30, 2016, the BCB Association was accepted into the Federation of Brown Breeds of Europe, Zug city, Switzerland. Membership in the Federation opened opportunities for involving European genetics, the latest techniques and technologies for preserving aboriginal cattle in the breeding process. To establish research cooperation, an agreement was concluded with the Slovak University of Agriculture in Nitra city on February 24, 2017. The results of joint genetic research on the BCB and Slovak Spotted breed were published by KASARDA et al. (2019). To contribute to the development of practical and scientific cooperation, an agreement was concluded on April 5, 2019, with the University of Life Sciences in Lublin, Poland. As a result, on June 15, 2022, a presentation was made at the international conference at the university on the topic "Engaging the

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capabilities of the Carpathian Euroregion and other institutions for the preservation of cattle biodiversity and sustainable development of local communities in the Carpathian countries". With the financial and advisory support of university employees, indigenous breeds of the Carpathian region countries were studied (TERPAI et al., 2023). Under the agreement dated May 29, 2019, with the Institute of Zootechnics, Krakow – Balice, Poland, within the budget, molecular genetic identification of 83 BCB heads was carried out in 2022 to detect the A1A2 allele in animals and genotyping for A2A2, a database was created. The influence of the A2A2 gene polymorphism on milk productivity and technological qualities of the obtained milk with the content of A2A2 protein and the production of hard cheese from it is being studied. The foundations of cooperation with the association of scientific institutions of the countries of the Danube region were laid in 2019 and the possibility of publishing works in the scientific publication DAGENE was opened. According to the scientific and technical task of the Institute of Animal Breeding and Genetics named after M.V. Zubets of the NAAS, an expeditionary survey of the BCB breeding stock was conducted (TERPAI, 2024).

Thus, the listed studies using zootechnical, economic, organizational measures, with further in-depth cross-border cooperation, will help to achieve the results outlined in the "Carpathian Convention" and its protocols. These studies also will contribute to development of a long-term strategy for livestock farming in the Carpathian region, based on aboriginal cattle breeds, using genomic selection and an ecosystem approach in organizing production. As well as to improve methods for preserving cattle's gene pool and restoring meadow ecosystems. Predict structural changes under the influence of global and regional climate change and the selection of tolerant measures to minimize the negative impact.

Conclusion and recommendation

The implementation of the "Carpathian Convention" and its protocols on the basis of indigenous cattle breeds, scientific and transboundary aspects of cooperation between the countries that have united in the Carpathian Euroregion, will return the integration of science and practice, herds of indigenous cattle breeds to natural meadows and pastures, preserve and enrich the biodiversity of flora and fauna, ensure sustainable development of communities and improve the lives of local residents. The reformatting of monobreeding, preferring only one breed in cattle livestock will become an effective mean of adapting the industry to climate change and prevent disruption of pasture and meadow ecosystems of the Carpathians. It is necessary to continue research on the full identification of indigenous breeds to create a cadastre. To eliminate the information deficit and improve the planning of response measures, it is necessary to create an integrated transboundary network for monitoring and assessing the population of indigenous breeds in the countries of the

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Carpathian Euroregion. It is also necessary to organize an international conference on traditional farming in the Carpathians as well as educational and informational publications.

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New aspects of the history and current status of the Milking Tsigai sheep breed in Hungary

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Abstract

The dairy-type Tsigai represents a breed variant of the Tsigai sheep indigenous to Hungary and Serbia. The origins of the Milking Tsigai can be traced back to the 19th century, when selection for milk yield was emphasized in certain flocks of the historical Vojvodina region. In Hungary, the contemporary Milking Tsigai population emerged during the final decades of the 20th century through modern breeding programs and the importation of Serbian animals. With a breeding population of fewer than 300 ewes, the Milking Tsigai is regarded as critically endangered in Hungary. Current conservation strategies emphasize genetic surveillance and planned mating schemes. Prospective applications also involve exploiting the breed's favourable milk quality for niche dairy products and its suitability for sustainable, low-input agricultural systems.
Earlier assessments of genetic diversity were based on analyses of blood groups and protein polymorphisms, which indicated moderate variability within the population. Subsequent molecular investigations have incorporated the analysis of STR polymorphisms. At present, research efforts are focused on characterizing the maternal lineage through the analysis of mtDNA.

Keywords: breed preservation, milking variant, genetic diversity

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Introduction

The conservation of indigenous livestock breeds has become increasingly important as specialized breeds have progressively replaced locally adapted populations in modern animal production. Indigenous breeds represent more than historical agricultural practices: they retain genetic variation accumulated through long-term natural and artificial selection under specific environmental and management conditions. Their conservation is therefore relevant both to cultural heritage and to the preservation of genetic resources.

The Tsigai sheep is a traditional long-tailed sheep breed associated with the Balkans, Central and Eastern Europe and Asia Minor (BECSKEI el al., 2024). In Hungary, Tsigai sheep became established as a multipurpose population, historically valued for milk, meat and wool. Its wool was valued for its relatively fine characteristics compared to the more widespread Hungarian Sheep (Racka), contributing to its persistence in Hungarian sheep farming despite never becoming one of the country's dominant breeds (GÁSPÁRDY and SÁFÁR, 2014; KUSZA et al., 2011). During the twentieth century, however, changes in production systems and breeding priorities substantially reduced the importance and population size, as multipurpose flocks were increasingly displaced by more productive, single-purpose breeds.

Within the Hungarian Tsigai population, two clearly distinguishable breeds are currently recognized: the Native Tsigai, maintained primarily as a genetic resource without selection for a specific production direction, and the Milking Tsigai, which has undergone directional selection for milk production. The latter is now treated as a distinct breed because of its different phenotypic and production characteristics (MJKSZ, 2024).

The present article summarizes the history, breed characteristics, reproductive performance and population status of the Milking Tsigai in Hungary and discusses its potential role in future conservation-oriented and low-input production systems.

Breed history

The Tsigai sheep is traditionally reported to have reached the territory of Hungary from the Balkans at the end of the eighteenth century. RODICZKY (1904) subsequently reported 1792 as the earliest documented date associated with its arrival, when the demand of Hungarian woollen mills and the developing wool trade encouraged farmers in Transylvania and neighbouring regions to replace coarser-woolled sheep with Tsigai animals producing finer wool (GÁSPÁRDY et al., 2006). The breed subsequently became established particularly in Transylvania and later spread to other parts of historical Hungary.

The breed was initially valued primarily for its wool, but its multipurpose character was recognized relatively early. By the beginning of the twentieth century, Tsigai

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sheep were considered better milk producers than Hungarian Sheep and were particularly useful in smallholder farms on the Great Hungarian Plain (KOVÁCSY, 1926). At the same time, in the Balkans the breed was also valued as a meat-producing sheep (NASTIC et al., 2020; SAVIĆ et al., 2000). The breed never became dominant in Hungary, mainly because the Merino was introduced during approximately the same time, with more specialized production, and the Hungarian Sheep also remained more influential (GÁSPÁRDY et al., 2022).

The emergence of the Milking Tsigai represents a later stage: by the turn of the twentieth century, different Tsigai populations had acquired distinctive characteristics associated with local breeding objectives and conditions. Among these were larger-bodied populations in the Great Hungarian Plain, flocks selected for wool production, and populations with particularly good milk yield (KOVÁCSY, 1926).

The historical development of the Milking Tsigai is closely associated with Tsigai populations of the Zombor region and with Pivnica (Pivnička) sheep in present-day Vojvodina, Serbia (BECSKEI el al., 2024), with some speculating other, possibly including Bergamasca or Karakul influence (GÁSPÁRDY and SÁFÁR, 2014). Importantly, the Kisszállás flock, established from animals originating from the Zombor region, already showed characteristics associated with milk-oriented production in the early twentieth century, including relatively large body size, black head and legs, polledness, good milk yield and the ability to utilize relatively poor sandy pastures (KOVÁCSY, 1926). However, the animals did not yet display all of the morphological characteristics subsequently associated with the Milking Tsigai.

The political and economic changes surrounding and following the Second World War contributed to a substantial decline in population size. The animals survived in the region between the Duna and Tisza rivers, on small family farms, with the aim of increasingly prioritizing milk production (KOVÁCSY, 1926; GÁSPÁRDY et al., 2022).

Following the reorganization of Hungarian sheep breeding organizations in the early 1990s, the Tsigai and Milking Tsigai were maintained as separate breeds by the Hungarian Sheep and Goat Breeders' Association (MJKSZ), while official recognition of the Milking Tsigai took place on 18 April 2000 (GÁSPÁRDY et al., 2022).

Breed characteristics and production

Morphological characteristics

The Tsigai is traditionally considered a finer-bodied breed compared to other indigenous sheep. The Milking Tsigai is a comparatively large-framed sheep with a characteristic long head, convex nasal profile and long, pendulous ears. The forehead is free of wool, and the frontal wool tuft characteristic of the indigenous type is

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absent (GÁSPÁRDY et al., 2022). The official breed description characterizes the Milking Tsigai as a medium-to-large, hardy and robust sheep (MJKSZ, 2024). Ewes are hornless, whereas rams may be either horned or polled, with horned rams being more common. The head and legs are black or brown, the skin is pigmented, and the fleece is predominantly white and curly, although black fibres may occur within it (DUNKA, 1997; VERESS, 1997). Milking Tsigai have comparatively coarser, greasier wool, with longer fibres (GÁSPÁRDY et al., 2006).
Compared to the Native Tsigai, they differ markedly in body size and conformation. Comparative measurements demonstrate statistically significant differences in several traits (Table 1).

Table 1: Comparison of body measurements of adult Native and Milking Tsigai ewes, after GÁSPÁRDY et al. (2001)

Parameter	Native Tsigai	Milking Tsigai
Live weight, kg	53.4	76.0
Height at wither, cm	67.5	73.8
Body length, cm	75.0	79.6
Chest depth, cm	35.1	34.8
Chest width, cm	24.3	25.0
Heart girth, cm	91.4	104.6
Rump width, cm	24.4	28.7
Cannon girth, cm	8.5	10.1
Head length, cm	22.1	25.6
Ear length, cm	13.9	20.4

Another qualitative distinction is the colour of newborn lambs: Milking Tsigai lambs are born black, whereas Native Tsigai lambs show different darker or brownish shades that subsequently become white (MJKSZ, 2024).

Milk production

Milk yield is the principal distinguishing production trait of the breed. Historical Hungarian flocks already demonstrated substantial milk potential. In the Kisszállás flock, ewes produced approximately 3 dl of milk per day after a two-month suckling period, with recorded milking yields of 107 litres over 230 milking days or 51 litres over 150 days, depending on the production system (PÓCZOS, 1934). Later descriptions of the Milking Tsigai report approximately 1.1–1.25 litres of milk per day, around 130 litres per lactation and a milking period of approximately 100 days (KUKOVICS et al., 2006; CSANÁDI et al., 2007). Other documented flocks reached substantially higher values, and the breed has been described as capable of

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approaching 200 litres when milk consumed by the lamb is also considered (KESZTHELYI et al., 1998). The composition of the milk is also of practical interest. Reported values for Milking Tsigai milk include approximately 6.0–7.0% fat, 5.8–6.4% protein and 4.27–5.15% sugar (CSANÁDI, 2005; BAJÚSZ, 2008). Such composition is particularly relevant for cheese and fermented dairy products because high concentrations of milk solids increase processing yield. Historically, Tsigai milk was used for a range of products, demonstrating the breed's economic and culinary significance (KUKOVICS and NAGY, 2000; BAJÚSZ, 2009).

Reproductive traits

Reproductive performance is central to the conservation of a small sheep population because demographic recovery depends directly on the ability of breeding females to produce and successfully rear replacement animals. Historical observations indicate a multifaceted reproductive profile characterized by relatively late maturation, good maternal performance, prolificacy and substantial milk yield (SCHANDL and BEREK G-né, 1961).

Nineteenth-century reports from Hétfalu described the regular occurrence of twins, while a 1896 report from Somogyszentimre stated that the number of offspring, including twins, normally exceeded the number of ewes (SZENTKIRÁLYI, 1923).

Later quantitative observations provide more precise evidence. PÓCZOS (1934) reported that approximately 15% of ewes produced twins, while a more recent survey by NAGY et al. (2008) estimated lambing performance in Milking Tsigai at approximately 150%. These data indicate useful reproductive capacity, although prolificacy estimates should be interpreted in relation to management conditions, season, parity and flock structure.

Maternal ability is another relevant characteristic. GYIMÓTHY (2011) reported a markedly higher proportion of lambs weaned relative to the number of ewes in Tsigai than in prolific Merino ewes, 81.2% compared with 52.5%. This observation illustrates an important distinction between prolificacy and reproductive efficiency. Producing multiple lambs has limited value if maternal ability is inadequate. A breed capable of combining acceptable prolificacy with effective lamb rearing may therefore have greater practical value under extensive conditions (SCHANDL and BEREK G-né, 1961). The combination of milk production and maternal ability is particularly valuable in low-input systems because reproductive output cannot be evaluated independently from the ability of the ewe to rear multiple offspring.

An interesting relationship has also been reported between temperament, physiological stress and milk production in Native Tsigai sheep. PAJOR et al. (2010) found that calmer Tsigai ewes produced more milk during a 106-day milking period than animals classified as nervous, with mean yields of 52.4 and 46.1 litres, respectively. The calmer animals also showed lower blood cortisol and lactate concentrations, and their lambs subsequently achieved better weaning and fattening performance. These findings suggest that behavioural and physiological

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characteristics may interact with productive performance and could therefore be relevant to future breeding and management strategies, warranting further investigation of these relationships in the breed.

Male reproductive performance is another relevant component. OLÁH (2010) compared quantitative and qualitative semen characteristics of rams from seven sheep breeds used in Hungary, including semen concentration, motility, freezing capacity and post-thaw motility. Tsigai rams ranked among the three best-performing breeds for several semen-quality traits. Nevertheless, seasonal variation was evident, consistent with the reproductive seasonality characteristic of sheep.

Current population status

The major conservation problem facing Milking Tsigai in Hungary is its small and fluctuating population size. The latest available evidence indicates that the Hungarian population remains small, making conservation a central issue. Data presented from the Hungarian breeding records (Figure 1) show substantial variation in the number of registered ewes over the past three decades. In 1993, 121 ewes were registered in one farm. The number increased to 840 ewes in seven farms in 1998 reaching the highest number recorded in the presented series in 1999 at 1020 animals, before declining to 437 in 2005 and 325 in 2009. A temporary increase to 731 ewes occurred in 2015, followed by further declines to 255 in 2016 and 245 in 2019. The population increased to 369 ewes in 2020 but declined again to 229 in 2023. In 2024, 243 registered ewes were distributed among four farms.

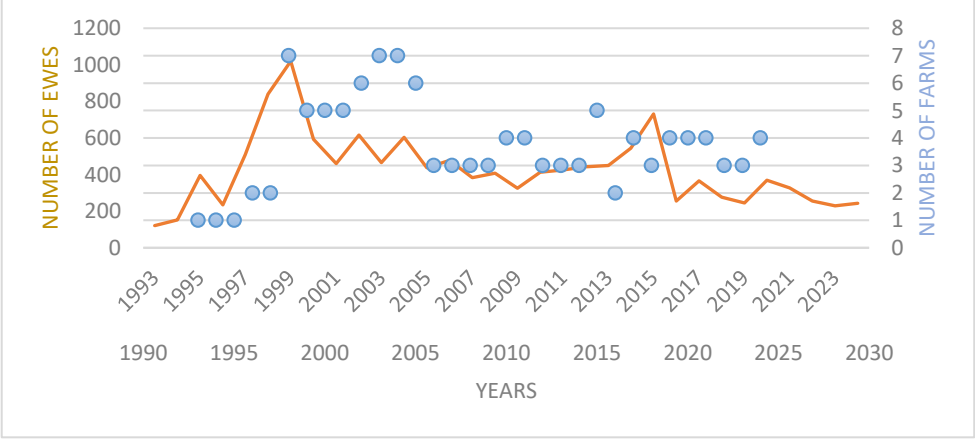


Figure 1: Number of registered Milking Tsigai breeding ewes and breeding farms in Hungary from 1993 to 2024. Data source: Hungarian Sheep and Goat Breeders' Association (MJKSZ, 2024) and GÁSPÁRDY and SÁFÁR (2014).

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These numbers demonstrate that the breed remains vulnerable to demographic fluctuations and that population size alone is not a sufficient measure of conservation success: a temporary increase can be followed by rapid contraction, particularly when the number of breeding farms is small. A population of only a few hundred registered ewes is inherently vulnerable to demographic stochasticity, loss of breeding families and excessive reproductive contribution of individual rams.

Although the Tsigai as a transboundary breed is represented by much larger populations internationally (ĆINKULOV et al., 2007), the Hungarian population is sufficiently small to require active conservation measures. The small population size is particularly important because conservation involves more than simply maintaining a numerical population threshold. Effective conservation requires maintaining genetic variation, controlling relatedness and ensuring that breeding decisions do not excessively favour a limited number of breeding animals (SZALAY and KOPPÁNY, 2017). For this reason, herd-book recording, planned mating and the exchange of breeding rams between flocks are important components of the conservation programme.

The economic context presents an additional challenge. The Hungarian sheep sector has experienced a general decline, while the number of specialized dairy sheep remains limited, resulting in domestic sheep milk and dairy products occupying a relatively small market niche (KUKOVICS and NAGY, 2000; NAGY et al., 2008; NASTIC et al., 2020). Even when sheep dairy products are available, their market presentation and promotion have often been insufficient (GÁSPÁRDY, 2003).

For Milking Tsigai, this creates a paradox. Its strongest distinguishing feature - milk production - could support conservation if linked to economically viable products, but low market demand reduces the financial incentive to maintain specialized dairy flocks. Conservation subsidies are therefore important, but long-term survival is more likely when conservation is combined with productive utilization.

Genetic diversity

Genetic characterization has been used to distinguish Hungarian Tsigai populations and to monitor their genetic status. Earlier studies relied on blood-group and biochemical protein polymorphisms (Fésüs, 1974). These investigations were initiated in Hungarian Native sheep during the second half of the twentieth century and were subsequently used to characterize the Native and Milking Tsigai populations. The Milking Tsigai was first specifically characterized using these approaches in 1990 (Fésüs and Al Dabbagh, 1991). These studies identified differences in systems such as haemoglobin and transferrin and demonstrated that the Milking Tsigai population differed from several Native populations.

The subsequent application of DNA-based markers considerably increased the resolution of these investigations. During the 1990s, protein polymorphisms in Milking Tsigai were characterized and genotyped (ANTON et al., 1999). GÁSPÁRDY et al. (2013) used microsatellite polymorphisms to compare Hungarian

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Tsigai flocks, while KUSZA et al. (2010) examined ten Hungarian populations using 16 microsatellite loci. The latter study detected 262 alleles and 15 population-specific alleles, demonstrating substantial allelic variation. Genetic distances and clustering revealed relationships among populations associated with geographical and breeding history, and did not indicate an immediate threat from inbreeding under the conditions examined (KUSZA et al., 2024).

More recently, GÁSPÁRDY et al. (2022) investigated the maternal genetic background of Native Tsigai using mitochondrial cytochrome *b* and control-region sequences. Further studies examining DNA-based markers amongst the Milking Tsigai population could provide useful insights into the genetic background and evaluation of the maternal families and paternal lines could provide valuable information for future breeding-program decisions.

Future perspectives

The future of Milking Tsigai depends on resolving the apparent conflict between conservation and economic viability. A population cannot be sustainably maintained indefinitely if keeping the animals has no economic rationale. Conversely, intensive selection for maximum production could undermine the very characteristics that justify conservation.

The first priority should therefore be demographic stabilization. The number of breeding ewes should increase, but population growth must be accompanied by an effective mating strategy. Pedigree analysis should be combined with molecular information to identify underrepresented lines and minimize unnecessary loss of genetic variation.

Second, production should emphasize the strengths of the breed (BODÓ, 1991). The Milking Tsigai has historically been selected under relatively extensive conditions and retains characteristics compatible with low-input farming. These characteristics suggest potential suitability for organic and other environmentally oriented production systems in which resilience, maternal ability and efficient use of pasture are important alongside milk yield.

Third, milk should be regarded as a means of conservation rather than simply as a production trait (KUKOVICS and NAGY, 2000). The breed's relatively high milk fat and protein concentrations make it suitable for value-added dairy products (ANTON et al., 1998; CSANÁDI, 2005; NAGY et al., 2008; BAJÚSZ, 2009). Small-scale cheese and fermented dairy products may offer opportunities for higher-value utilization than commodity milk (CSANÁDI et al., 2007). The objective should not be to compete directly with highly specialized dairy sheep breeds in maximum milk yield, but to conserve the breed’s character alongside sensible production use.

Fourth, the genetic identity of the Milking Tsigai requires continued monitoring. In particular, genome-wide analyses could further clarify relationships among Hungarian Milking Tsigai, Native Tsigai, Serbian and other Balkan Tsigai

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populations, as well as the possible contribution of historically proposed source populations (ĆINKULOV et al., 2008), and potential historical contributor breeds. Such work would help distinguish genuine historical introgression from convergent phenotypic similarity.

Finally, the potential role of Milking Tsigai in pasture and landscape management deserves further investigation. Extensive sheep production can contribute to the maintenance of grassland habitats (PENKSZA et al., 2024), while the preservation of locally adapted breeds can support production systems requiring relatively low external inputs, although the specific ecological contribution of Milking Tsigai remains to be evaluated. The breed therefore has potential value at the intersection of genetic conservation, sustainable agriculture and rural heritage.

The central principle should be that conservation and utilization are complementary. The historical development of Milking Tsigai demonstrates that productive selection can create a valuable population without eliminating its local character. Future breeding should retain this balance rather than pursuing either genetic isolation or unrestricted improvement.

Conclusion and recommendation

The Milking Tsigai represents a distinctive component of Hungary's sheep genetic resources. Its history extends from the introduction of Tsigai sheep into Hungary at the end of the eighteenth century through the development of regionally differentiated populations and, ultimately, the emergence of a strongly milk-oriented type. The modern Milking Tsigai is phenotypically distinct from the Native Tsigai, particularly in body size, head and ear characteristics, fleece properties and production orientation.

One of its principal distinguishing values is its milk yield, supported by reproductive performance and good maternal ability. At the same time, the Hungarian population remains very small and fluctuates considerably between years, placing the breed in a vulnerable position. Genetic studies imply that flocks retain considerable genetic variation, but further studies are needed to evaluate the current state of the genetic resource, especially considering the maternal families and paternal lines.

The long-term conservation of Milking Tsigai should therefore combine demographic expansion with controlled breeding, pedigree management and genetic monitoring. Economically viable low-input and organic production, especially through high-value dairy products, could provide an important foundation for maintaining the breed in active agricultural use. Conservation should ultimately aim not merely to preserve the name “Milking Tsigai”, but to retain the population's distinctive genetic, phenotypic and productive characteristics as a functional Hungarian genetic resource.

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Exterior and production characteristics of Brač sheep

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Abstract

The Brač sheep is a locally adapted population within the Dalmatian Pramenka breed, shaped by the specific environmental conditions of the Croatian island of Brač. Despite its cultural and agricultural significance, this sheep population has not yet been fully described in scientific literature. This study aimed to record the exterior and productive traits of the Brač sheep to support its recognition and conservation as an autochthonous genetic resource. A total of 65 adult ewes, 13 adult rams, 12 male lambs and nine female lambs from nine flocks were included in the study. The animals were kept primarily on pasture and used either for meat production or as dual-purpose, for milk and meat. Body weight and seven morphometric traits were recorded. Measurements were conducted using a scale, Lydtin stick, measuring tape, and callipers. Lactation yield was assessed in five ewes after weaning of lambs. Milk was measured every 30 days until drying-off, and post-weaning milk yield was calculated using the Fleischmann formula. The average body weight of ewes was 41.08 kg, and that of rams was 54.55 kg. Rams were larger than ewes in all measured exterior traits. Withers height averaged 61.94 cm in ewes and 68.04 cm in rams, while sacral height was 62.85 cm and 68.00 cm, respectively. Hip width showed the greatest variation, with a coefficient of variation of 15.71% in rams and 8.11% in ewes. Average milk production from weaning of lambs until drying was 68.7 litres per ewe. All examined lambs were singletons. The Brač sheep is characterized by a small body frame and modest productive performance, which are comparable to those of other Croatian Pramenka-type breeds.

Keywords: Brač sheep, Dalmatian Pramenka, morphometry, production

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Introduction

Local livestock populations represent an important component of animal genetic resources because they have evolved under specific environmental conditions and traditional management systems, resulting in valuable adaptations to local production environments. Their phenotypic characterisation is an essential prerequisite for the conservation, sustainable utilisation, and future improvement of indigenous sheep populations. Exterior characteristics and productive performance remain the primary criteria for describing local sheep populations and distinguishing them from closely related breeds.

The Brač sheep is a locally adapted population of the Dalmatian Pramenka traditionally reared on the Croatian island of Brač. The island is characterized by a typical Mediterranean climate, extensive karst pastures and sparse natural vegetation, conditions that have shaped sheep production and influenced the adaptation of this population over centuries (DERADO, 1984; KEČKEMET, 1998; VOJNOVIĆ, 1991). Traditionally, the Brač sheep has been managed under extensive grazing systems as a dual-purpose animal, producing both lamb and milk, while wool has gradually lost its economic significance. Today, lamb and traditional Brač sheep cheese remain the most valuable products of this production system.

Historical records indicate that sheep breeding has been among the most important agricultural activities on the island for centuries and has played a significant role in the local population's economy and cultural heritage (KURSCHNER, 1937; ZDANOVSKI, 1947; GAMULIN and VIDOVIĆ, 1975). Nevertheless, despite its long tradition and local importance, the Brač sheep has never been comprehensively described in the scientific literature. Available information is limited to historical reports and local publications, whereas recent data on its exterior characteristics and productive performance are scarce.

Therefore, this study aimed to provide an updated phenotypic characterisation of the Brač sheep by evaluating its exterior characteristics and productive performance under current breeding conditions.

Material and methods

Animals

The study included 99 Brač sheep: 65 adult (2-6 years) ewes, 13 adult (2-6 years) rams, and 21 lambs. The animals were randomly sampled from nine privately owned flocks located on the island of Brač, Croatia. All flocks were managed under traditional extensive production systems, with natural pasture serving as the primary feed resource supplemented by meadow hay when necessary. Depending on the production system, animals were reared either for lamb production or under a dual-

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purpose system for both milk and meat production. All lambs included in the study originated from the same nine flocks and were born during the same lambing season. Data was collected from January to November 2024.

Morphological measurements

Body weight was recorded for all rams and lambs. Out of 65 ewes, 45 were in early pregnancy and were weighed, while 20 were in late pregnancy and were not suitable for weighing. Body weight was measured using an electronic scale and recorded to the nearest 0.01 kg.

Morphometric measurements were recorded in centimetres following standard livestock evaluation procedures. Withers height, sacral height and body length (measured from the cranial edge of the shoulder joint to the caudal edge of the pin bone) were determined using a Lydtin measuring stick. Chest circumference and cannon bone circumference were measured using a measuring tape, whereas chest depth and hip width were determined with a livestock calliper.

Average daily gain was calculated as follows: birth weight was subtracted from weaning weight, and divided by the number of days from birth to weaning.

Milk production

Milk production was evaluated in five 3-year-old ewes after weaning (approximately two months after lambing), as lambs remained with their dams throughout the suckling period and milking commenced only after weaning. Individual daily milk yield was recorded at approximately 30-day intervals until drying off (three months after weaning), in accordance with the official Croatian milk recording guidelines (JURKOVIĆ *et al.,* 2021). Post-weaning milk yield (total amount of milk from weaning of lambs until drying) was calculated using the Fleischmann method.

Results and discussion

The body weight and morphometric characteristics of adult Brač sheep are presented in Table 1. Adult ewes had an average body weight of 41.08 kg, whereas rams averaged 54.55 kg. As expected, rams exhibited greater values for all recorded body measurements, including withers height, sacral height, body length, chest circumference, chest depth, hip width and cannon bone circumference. The greatest variability was observed in hip width, particularly in rams, with a coefficient of variation of 15.71%. Cannon bone circumference showed no recorded variation within either sex, with all measured ewes having a value of 7 cm and all rams a value of 8 cm.

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Table 1: Body weight and morphometric characteristics of adult Brač sheep

Variable	Sex	n	Mean	St.dev.	Min.	Max.	Coefficient of variation, %
Body weight (kg)	F*	45	41.08	7.40	28.30	58.00	18.00
	M**	13	54.55	8.30	38.00	70.00	15.21
Withers height (cm)	F	65	61.94	3.04	53.00	68.00	4.91
	M	13	68.04	2.76	63.00	72.00	4.06
Sacral height (cm)	F	65	62.85	3.06	56.00	69.00	4.87
	M	13	68.00	2.78	64.00	73.00	4.08
Body length (cm)	F	65	68.14	3.65	56.50	78.00	5.36
	M	13	74.08	4.61	66.00	82.00	6.22
Chest circumference (cm)	F	65	83.54	5.87	72.00	100.00	7.03
	M	13	89.46	6.74	77.00	104.00	7.53
Chest depth (cm)	F	65	29.97	2.10	25.00	35.00	7.02
	M	13	32.92	2.18	28.00	36.00	6.62
Hip width (cm)	F	65	17.25	1.40	14.00	21.00	8.11
	M	13	18.31	2.88	15.50	27.00	15.71
Cannon bone circumference (cm)	F	65	7.00	0	7.00	7.00	0
	M	13	8	0	8.00	8.00	0

*F – Female; **M – Male

The average body weight of adult ewes falls within the range reported for the Dalmatian Pramenka breed (35–45 kg), while the average body weight of rams corresponds to the upper limit of the published breed standard (MIOČ *et al.,* 2011). Compared with other Croatian indigenous sheep breeds, the body weight of Brač ewes is lower than that reported for Lika Pramenka and Pag sheep but comparable with Cres sheep and slightly higher than Krk and Rab sheep (MIOČ *et al.,* 1998; MIOČ *et al.,* 2004; PAVIĆ *et al.,* 2005; PAVIĆ *et al.,* 2006). The average withers height of ewes (61.94 cm) was similar to that reported for Lika Pramenka, Pag and Cres sheep, although slightly higher than the values described for the Dalmatian Pramenka breed standard and Krk sheep (MIOČ *et al.*, 1998; MIOČ *et al.*, 2004; PAVIĆ *et al.,* 2005; PAVIĆ *et al.,* 2006; MIOČ *et al.,* 2011). As in other Pramenka-type sheep, body length exceeded withers height, reflecting the typical body conformation of this group of breeds. The slightly greater sacral height than withers height observed in ewes indicates a mildly elevated hindquarters, whereas these measurements were almost identical in rams.

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Table 2: Body weight of Brač lambs at birth and at weaning (kg)

Traits	Sex	n	Average age (days)	Mean	St.dev.	Min.	Max.	Coefficient of variation %
Birth weight (kg)	F*	9	0	2.66	0.12	2.5	2.9	4.65
	M**	12	0	2.81	0.14	2.6	3.1	5.13
Weaning weight (kg)	F	9	52	16.67	2.38	13	20	14.30
	M	12	52	16.92	2.31	14	20	13.68

*F – Female; **M – Male

The body weights of lambs at birth and at weaning are summarised in Table 2. Male lambs were slightly heavier than females both at birth (2.81 vs 2.66 kg) and at weaning (16.92 vs 16.67 kg), although the differences between sexes were small. Average daily weight gain was nearly identical, at 271 g in males and 270 g in females.

Birth weights recorded in the present study were lower than those previously reported for Pag sheep (SUŠIĆ *et al.,* 2008) and lower than the official performance recording data available for Dalmatian Pramenka (PRPIĆ *et al.,* 2019). Nevertheless, these comparisons should be interpreted cautiously because the national database mainly includes selected male lambs used for performance testing, and those tend to be the best animals reared for reproduction. Likewise, weaning weights of Brač lambs were slightly lower than the reference values reported for Dalmatian Pramenka (MIOČ *et al.,* 2011), reflecting the extensive production conditions under which the investigated flocks were managed.

Milk production results are presented in Table 3. Average post-weaning milk yield during the milking period was 68.7 L per ewe, with individual production ranging from 56.6 to 95.3 L. Since lambs remained with their dams until weaning, only the milk produced after weaning was included in the calculation of post-weaning milk yield. Under these management conditions, the milking period lasted approximately two to three months, corresponding to a total lactation length of about five months. Milk production results should be interpreted with caution because milk recording was performed in only five ewes, and therefore these results should be considered preliminary.

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Table 3: Milk production of Brač sheep

Sheep	Post-weaning milk yield (L)
Ewe no. 1	61.9
Ewe no. 2	60.2
Ewe no. 3	56.6
Ewe no. 4	95.3
Ewe no. 5	69.5
Average	68.7

Conclusion and recommendation

The present study provides an updated phenotypic characterization of the Brač sheep, a locally adapted population of the Dalmatian Pramenka breed reared under extensive Mediterranean production systems on the island of Brač. Adult animals exhibited small body frame, while productive performance was comparable to that reported for other Croatian Pramenka-type sheep.

The results presented in this study contribute to the scientific documentation of the Brač sheep. Further investigations, including larger sample sizes and long-term monitoring of productive and reproductive performance, are recommended to support the preservation and promotion of this valuable local sheep population.

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Genetic variability of canine *NR3C1* gene and its role in glucocorticoid signaling

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Abstract

The glucocorticoid receptor is a ligand-activated transcription factor that mediates the actions of glucocorticoids and regulates genes involved in metabolism, development, inflammation, and the immune response. Glucocorticoids are lipophilic, allowing them to freely cross cell membranes and bind to glucocorticoid receptors, which, upon translocation to the nucleus, enhance or suppress transcription of target genes. The glucocorticoid receptor is encoded by the *NR3C1* gene, which has been identified in both humans and dogs. This gene is located on canine chromosome 2 and contains nine exons. The *NR3C1* gene encodes multiple glucocorticoid receptor isoforms via alternative splicing and alternative translation initiation, leading to diverse cellular and physiological responses. Previous studies of *NR3C1* gene polymorphisms have been conducted in the context of various human diseases, such as rheumatoid arthritis, multiple sclerosis, and an increased risk of major depression. In dogs, identified polymorphisms have been investigated in relation to immune thrombocytopenia and in clinical studies evaluating dogs' responses to steroid medications. Since glucocorticoids such as cortisol are key mediators of the hypothalamic-pituitary-adrenal (HPA) axis and play a central role in the physiological adaptation to stress, variations in the *NR3C1* gene may influence receptor sensitivity, feedback regulation, and overall stress reactivity in dogs.

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Investigating the occurrence of genetic variants and the relationship between *NR3C1* polymorphisms, cortisol levels, and behavioural indicators of stress may provide valuable insights into canine stress physiology and support the development of more individualized approaches to animal welfare assessment and veterinary treatment. Particular attention should be given to indigenous dog breeds, which represent unique genetic resources shaped by long-term adaptation to specific environmental conditions and management practices, and may therefore harbour distinct genetic variants associated with stress resilience and adaptive capacity.

Keywords: dogs, NR3C1 gene, stress response, corticosteroids, polymorphisms

Introduction

Glucocorticoids are steroid hormones synthesized in the adrenal cortex and are essential for metabolism, immune regulation, development, and stress adaptation. Their biological effects are mediated primarily through the glucocorticoid receptor (GR), a ligand-activated transcription factor encoded by the *NR3C1* gene (*Nuclear Receptor Subfamily 3, Group C, Member 1*) (COSTA et al., 2016). Following hormone binding, the receptor translocates to the nucleus where it regulates transcription of numerous target genes involved in inflammation, metabolism, and homeostasis.

The canine *NR3C1* gene is located on chromosome 2 and contains nine exons. Similar to humans, alternative splicing and alternative promoter usage generate multiple receptor isoforms that may differ in biological activity (VANDEVYVER et al., 2014). Studies in human medicine have demonstrated associations between *NR3C1* polymorphisms and diseases such as rheumatoid arthritis, multiple sclerosis, depression, and altered responses to glucocorticoid therapy (TAYLER et al., 2022). Comparable investigations in dogs remain limited but indicate that genetic variation may affect corticosteroid sensitivity and immune-mediated diseases.

Because cortisol is the primary glucocorticoid involved in the hypothalamic-pituitary-adrenal (HPA) axis regulation, variation in receptor structure and expression may influence behavioral and physiological responses to stress. Understanding canine *NR3C1* variability may therefore contribute to veterinary medicine, animal welfare research, and breeding strategies aimed at improving stress resilience. Despite these advances, knowledge regarding the functional significance of canine NR3C1 variation remains limited, highlighting the need for further investigation.

Material and methods

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This extended abstract is based on a comprehensive review of published scientific literature addressing glucocorticoid receptor biology, *NR3C1* gene structure, polymorphisms, alternative splicing, cortisol physiology, stress responses, and clinical applications in dogs. Relevant peer-reviewed studies were evaluated and synthesized to provide an integrated overview of current knowledge and future research directions.

Results and discussion

The glucocorticoid receptor belongs to the nuclear receptor superfamily and consists of an N-terminal transactivation domain, a DNA-binding domain, and a ligand-binding domain. Two principal receptor isoforms, GRα and GRβ, have been described. GRα is considered the primary biologically active receptor, whereas GRβ may act as a dominant negative regulator of glucocorticoid signaling (VANDEVYVER et al., 2014).

Activation of the HPA axis during stress results in glucocorticoid secretion, predominantly cortisol. Cortisol concentrations in dogs can be measured in plasma, saliva, urine, feces, and hair (CHMELÍKOVÁ et al, 2020; VAN DER LAAN et al., 2022). Although cortisol is widely used as a biomarker of stress, interpretation requires consideration of individual variation, environmental context, and previous experiences (FERRANS et al., 2025).

COSTA et al. (2016) identified six polymorphisms within the canine *NR3C1* gene, including two nonsynonymous variants predicted to affect receptor function. These findings provided the first evidence that naturally occurring genetic variation may influence glucocorticoid responsiveness in dogs. The same study also demonstrated the existence of multiple transcript variants generated through alternative promoter usage.

Subsequent research has highlighted the clinical relevance of *NR3C1* variation. TAYLER et al. (2022) investigated *NR3C1* polymorphisms in English Cocker Spaniels with primary immune thrombocytopenia and demonstrated breed-specific differences in allele frequencies. Although direct associations with treatment outcomes were not identified, the results supported the hypothesis that *NR3C1* polymorphisms are unevenly distributed among breeds and may contribute to variability in disease susceptibility.

Alternative splicing represents another important mechanism contributing to receptor diversity. KÁLLAI et al. (2021) identified a previously unknown canine ortholog of the GR-P splice variant and reported altered expression patterns in dogs with systemic inflammatory response syndrome (SIRS). These findings suggest that receptor isoforms may influence disease progression and survival.

Studies by YAMANAKA et al. (2019) further demonstrated that structural variation within canine glucocorticoid receptors can alter glucocorticoid sensitivity. Such

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observations are consistent with human studies showing that polymorphisms and receptor abnormalities influence individual responses to endogenous cortisol and therapeutic glucocorticoids.

From an animal welfare perspective, *NR3C1* variation may affect behavioral responses to stress. Cortisol serves as a major mediator of stress adaptation, and prolonged dysregulation of glucocorticoid signaling has been linked to anxiety, altered behavior, immune dysfunction, and reduced welfare (MÂRZA et al., 2024). Recent reviews emphasize that integrating genetic markers with physiological and behavioral indicators may improve assessment of stress resilience in domestic dogs (COBB et al., 2016; KASZYCKA et al., 2025).

Indigenous and locally adapted dog breeds may be particularly valuable in this context. Long-term adaptation to specific environmental conditions may have promoted the accumulation of genetic variants affecting HPA-axis function and glucocorticoid signaling. Characterizing *NR3C1* diversity within these populations could reveal novel adaptive mechanisms and support conservation of unique genetic resources.

Conclusion and recommendation

Current evidence indicates that the canine *NR3C1* gene exhibits considerable genetic and transcriptional variability. Identified polymorphisms and splice variants may influence receptor function, glucocorticoid sensitivity, stress responsiveness, and clinical outcomes. Although canine research remains less extensive than human research, available findings support the importance of *NR3C1* as a candidate gene for studies of animal welfare, stress physiology, and personalized veterinary medicine.

Future investigations should focus on large-scale population studies, genotype-phenotype associations, and the integration of molecular, endocrine, and behavioral data. Particular attention should be directed toward indigenous dog breeds, which may harbor unique genetic variants associated with adaptation and resilience. Such knowledge may contribute to improved welfare assessment, optimized therapeutic protocols, and conservation of valuable canine genetic resources.

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Study of sericin added to semen extender in sperm cryopreservation of Hungarian Giant Rabbit

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Abstract

Because of the dramatic decline of Hungarian Giant rabbit, we aim to create an *in vitro* sperm bank for safe gene preservation purposes. During testing different freezing protocols, we developed an effective extender for the cryopreservation of Hungarian Giant sperm. The present study investigated the effect of sericin as antioxidants on sperm freezability using the modified Lake's extender. Collected sperm from 10 bucks was diluted 1:4 with modified Lake's extender with 1% glycerol and 4% DMF (control group), containing sericin 0.1 and 0.3 w/v% (treated groups). Samples filled into 0.25 ml straws, and after equilibration at 5°C for 60 min static freezing (5 cm above liquid nitrogen for 15 minutes) was applied. The thawing was carried out at 40ºC for 30 sec. In the fresh and frozen/thawed sperm samples motility (CASA) and ratio of live/dead cells, sperm abnormalities (aniline blue-eosin staining) were checked. In testing two different sericin concentrations no significant difference was observed in the proportion of live, intact cells or in total and progressive motility, none of the tested concentrations of sericin supplementation improved sperm viability. Further studies are required to determine whether the concentrations were suboptimal or this rabbit breed responds poorly to these treatments.

Keywords: rabbit sperm; cryopreservation; sericin; Hungarian Giant

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Introduction

Despite the fact that research into rabbit semen cryopreservation has been ongoing for several decades, sperm cryopreservation is still applied mainly for experimental purposes and gene conservation. The dramatic decline of our native rabbit breed, the Hungarian Giant, highlights the need to establish an *in vitro* sperm bank to supplement the *in vivo* nucleus population already maintained at our Institute. The establishment and maintenance of an *in vitro* sperm bank enable the safe conservation of the breed's genetic material while also supporting the *in vivo* population.

To this end, in recent years, we have been working to develop an efficient freezing protocol for the Hungarian Giant. Following the comparison of different extenders and cryoprotectants finally we have developed an effective extender for Hungarian Giant sperm (KISSNÉ VÁRADI et al., 2026). However, as our cryopreservation protocol did not prove to be sufficiently effective during our fertilisation experiment, we considered it necessary to further improve the viability and motility parameters of frozen/thawed sperm.

Antioxidants used in semen extenders may improve semen freezability and protect sperm from damage occurring during the cryopreservation process; therefore, they may be suitable for improving the efficacy of sperm freezing protocols (KHAN, 2011; PEGG, 2015).

Sericin is a glycoprotein rich in amino acids that is extracted from the silkworm cocoon and is believed to have antioxidant and antimicrobial effects (ZHANG, 2002). Due to its bioactive, mechanical, and chemical properties, it is used in a wide range of fields, including the beauty, food, and pharmaceutical industries, as well as in various biological research applications (KUNDU et al., 2008). Since sericin is water-soluble, it can be easily applied as an antioxidant supplement in extenders during the sperm freezing of rabbits (RAZA et al., 2019) and other mammalian species (SASAKI et al., 2005). During the cryopreservation of stallion sperm, sericin supplementation increased DNA integrity and protected sperm from the damaging effects of reactive oxygen species (NASIRABADI et al., 2019). Similar protective effects have been observed during mouse and bull sperm freezing (GHASEMI, et.al. 2018; YANGNGAM et al., 2021).

Sericin may also offer a solution to a problem that arises during both cold storage and sperm freezing of rabbit semen, namely the 'dilution effect'. When semen is diluted to a greater extent, adverse changes occur in the structure and function of the spermatozoa's plasma membranes (AKSOY et al., 2010). Moreover, dilution may reduce the amount of plasma proteins available to sperm, which may lead to reduced fertility (DI IORIO et al., 2024). Due to the specific molecular properties of sericin, it may be suitable for repairing damage to the plasma membrane (AKSOY et al., 2024), particularly during treatment with extenders.

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In our study, we investigated the effect of sericin supplementation on sperm viability during cryopreservation and aimed to improve sperm motility by exploiting the potential protective effects of sericin. Improved sperm motility is essential for the successful use of frozen/thawed sperm in artificial insemination, which represents the ultimate purpose of sperm banking.

Material and methods

Animals and semen collection

The study was conducted at the National Centre for Biodiversity and Gene Conservation, Institute for Farm Animal Gene Conservation, Hungary. Ten sexually mature Hungarian Giant bucks, aged 10-22 months, were housed individually under 12L:12D photoperiod. The animals were fed 150-200g/day of a special rabbit breeding diet prepared according to the institutional recipe (17.38% crude protein, 10.35 AME/MJ, 3.04% crude fat, 16.79% crude fibre, 8.78% crude ashes). Hay and drinking water supplemented with probiotics and vitamins (Gastroferm M-vit, Premium Product Ltd. Hungary) were provided *ad libitum*.

Following a two-week training period, semen was collected once weekly from each of the 10 bucks using a pre-heated artificial vagina. Two consecutive ejaculates were obtained from each buck and evaluated.

Sperm qualification

Semen volume and motility were assessed in both ejaculates, and the gel plug was removed when present. Based on subjective motility scoring (scale 0-5), only samples of adequate quality (score 4-5) were pooled and subsequently analysed using a computer assisted semen analysis system (CASA, Microptic S.L. SCA®, Spain). The freezing experiments were performed in 10 replicates using pooled semen. Sperm concentration was determined using a Makler chamber (Sefi-Medical Instruments Ltd, Israel). Aniline blue-eosin (Aniline SIGMA-Aldrich, Eosin Y Merck Hungary) vital staining was used to assess the proportion of live and dead spermatozoa, with 200 cells evaluated per sample on smears (VÁRADI et al., 2013). The live/dead cell ratio was assessed in fresh pooled semen, after equilibration and after freezing/thawing. CASA parameters were determined before freezing (fresh samples) and after freezing/thawing in all experimental groups.

Sperm freezing protocol

Pooled semen samples were divided into three equal aliquots. The control sample was diluted with modified Lake's extender developed for rabbit semen (KISSNÉ VÁRADI et al., 2026) and supplemented with 1% glycerol and 4% dimethylformamide (DMF). The sericin-supplemented groups were extended with

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modified Lake's diluent supplemented with 0.1 and 0.3 w/v% sericin. The dilution ratio was 1:4 in all groups. Samples were loaded into 0.25 mL straws and equilibrated at 5°C for 60 minutes. Following equilibration, the straws were placed 5 cm above liquid nitrogen for 15 minutes. After freezing and a 30-minute stabilization period, the samples were thawed in a 40°C water bath for 30 seconds.

2.4. Statistical analysis

Statistical analyses were performed using StataNow 19.5 software (StataCorp LLC, College Station, TX, USA). For data expressed as percentages, arcsine transformation was applied prior to statistical analysis (REICZIGEL et al., 2007). Normally distributed data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. For non-normally distributed data, the Mann–Whitney U test was used to compare the groups.

Results and discussion

Following the cryopreservation procedure total and progressive motility showed a marked decline (Table 1). In contrast an earlier study on sperm cryopreservation with sericin pretreatment reported improvements in total and progressive motility in a dose dependent manner (RAZA et al., 2019). During 72 hours of cooling storage, the addition of 0.1% sericin also resulted in an increased sperm motility (AAD et al., 2024). The effectiveness of sericin supplementation appears to be concentration-dependent; in rabbits, concentration between 0.03-0.5% to be effective, whereas higher concentrations may lead to a decline in semen quality (ALI and KHATUN, 2026).

Table 1: Results of the CASA analysis of frozen/thawed semen samples supplemented with different concentrations of sericin.

	CASA analysis			
	Progressive (%)	**Non progressive (%)**	**Immotile (%)**	**Total motility (%)**
Fresh, diluted sample	76.1[a]	8.5 [a]	15.4[a]	84.6[a]
Frozen/thawed sample Control	3[b]	13 [b]	84[b]	16[b]
Frozen/thawed sample Sericin 0.1 w/v%	3.4[b]	14.1 [b]	82.5[b]	17.5[b]
Frozen/thawed sample Sericin 0.3 w/v%	3.8[b]	14.7 [b]	81.5[b]	18.5[b]

a-b-c-d indicate significant differences between the rows $P<0.05$

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In other species, sericin supplementation has been observed to increase total and progressive motility following the cryopreservation of bull, goat, boar and chicken sperm freezing (CHATURVEDI et al., 2022; KUMAR et al., 2024; RATCHAMAK et al., 2020, 2023). In contrast, in our previous studies on sperm cryopreservation in native Hungarian roosters, sericin supplementation proved ineffective (BÁNYÁSZ et al., 2025).

At the end of the equilibration period, no differences were detected in the proportion of live, intact cells, among the fresh (87.2%), control (diluted, without sericin: 86.5%), and sericin-supplemented groups (0.1 w/v%: 86.0%; 0.3 w/v%: 85.4%) (Figure 1). This finding contrasts with the results of RAZA et al. (2019), who reported that sericin pretreatment of fresh, diluted rabbit semen improved semen quality during cold storage. Increased sperm viability and a higher proportion of live, intact spermatozoa were observed during equilibration period following sericin treatment.

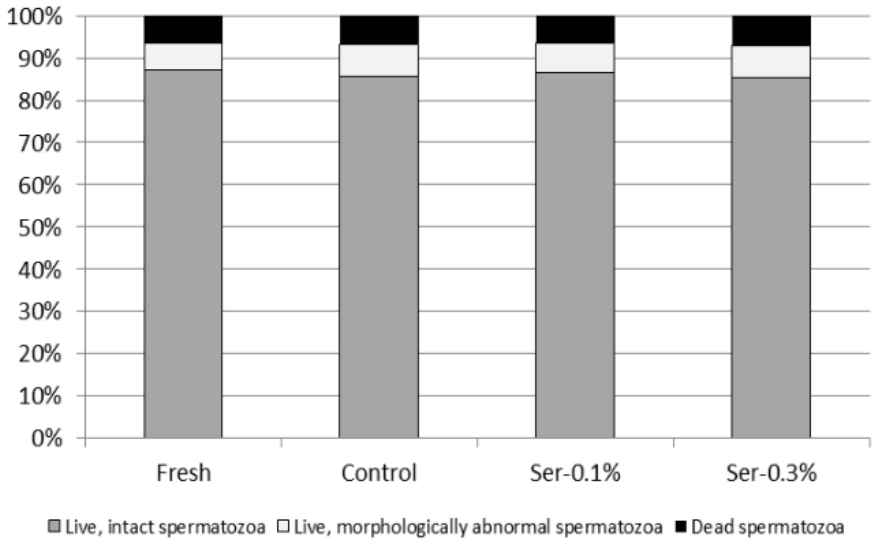


Figure 1: Changes in sperm quality following the equilibration period in samples supplemented with different concentrations of sericin.

Subsequent the freezing/thawing procedure, no differences in semen quality were found among the control and sericin-supplemented groups (Figure 2). The proportion of live, intact spermatozoa decreased significantly in all groups (Control: 33.3%; Ser 0.1 w/v%: 30.15%, Ser 0.3 w/v%: 30.17%), regardless of sericin supplementation.

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To the best of our knowledge, RAZA et al. (2019) are the only researchers to have investigated the effects of sericin supplementation during sperm cryopreservation in rabbits, reporting increased sperm viability and acrosome integrity following freezing and thawing. In other mammalian species, including mice, bull, goat, buffalo, and boar, improvements in sperm quality have also been demonstrated following the use of sericin during sperm cryopreservation (GHASEMI et al., 2018; KUMAR et al., 2015, 2024; VIJYETA et al. 2024; RATCHAMAK et al., 2020).

Unfortunately, the positive effects of sericin reported in other species were not evident during the cryopreservation of Hungarian Giant rabbit sperm. According to previous research, this may be related to the increased susceptibility of rabbit spermatozoa to oxidative damage (ROSATO el. al., 2011). However, as a positive effect has been observed in other rabbit breed (RAZA et al., 2019), it is possible that the effectiveness of antioxidant supplementation depends on breed-specific characteristics.

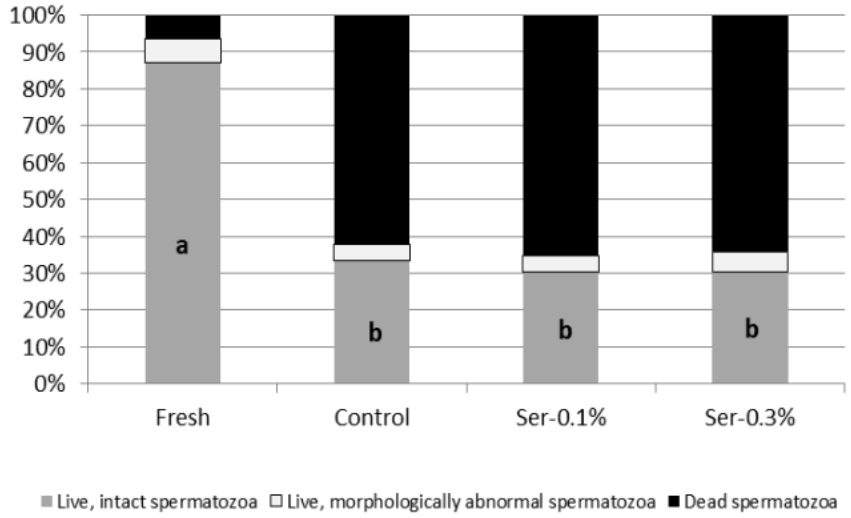


Figure 2: Proportions of live, membrane-intact; live, morphologically abnormal; and dead spermatozoa in fresh and frozen/thawed semen samples.
a-b indicate significant differences (P<0.05)

Conclusion and recommendation

At the concentrations tested (0.1 w/v% and 0.3 w/v%), sericin supplementation did not improve the outcome of sperm cryopreservation. Further studies are required to

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determine whether the tested concentrations were suboptimal or whether this rabbit breed responds poorly to sericin supplementation.

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