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DAGENE
International Association for the Conservation
of Animal Breeds in the Danube Region
1078 Budapest, István street 2.
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Table of content – Volume 9, Issue 1 (2024)

Research articles

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Bulgaria as a part of the world's poultry genetic resources – Bulgarian chicken breeds

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Abstract

Today, only a few major chicken breeds are used in industrial poultry farming, which leads to a considerable reduction in the genetic diversity of domestic chickens in the world. This trend is also affecting rural poultry farming. On the other hand, there is exhibition and ornamental poultry breeding, where productivity is not so important. Amateur poultry breeders play an important role in maintaining genetic diversity in domestic birds, especially in domestic chickens. In Bulgaria there are 10 known chicken breeds, two of which are recognized by the state institutions (Black Shumen and Stara Zagora Red Chicken) and the others are recognized or in the process of recognition by the Union of Fanciers and Small Domestic Animal Breeders in Bulgaria, which is a part of the Entente Européenne d'Aviculture et de Cuniculture (EE). Depending on the presence or absence of dwarfism, the breeds can be divided into standard breeds (Black Shumen Chicken, Stara Zagora Red Chicken, Katunitsa Chicken, Struma Chicken, Rhodope Painted Chicken, Southwest Bulgarian Chicken, and Bulgarian Longcrower) and miniature breeds (Bregovska Dzhinka, Struma Bantam, and Southwest Bulgarian Dzhinka). The breeds with attractive appearance have higher populations compared to the productive breeds, which are at risk of extinction.

Keywords: genetic diversity, Bulgarian chicken breeds, poultry genetic resources

Introduction

The large-scale globalization in the meat and egg production industry has greatly reduced the number of companies engaged in poultry breeding (GURA, 2007). This process has concentrated a huge genetic resource in a small number of companies,

DOI: https://doi.org/10.59913/dagr.2024.17293

which has led to a strong reduction in genetic diversity in poultry farming (BESBES et al., 2007). Highly productive hybrid chickens are gradually replacing local breeds, thereby further narrowing the genetic diversity of domestic chickens. Nevertheless, in some parts of the world, such as Africa, South America and some countries in Asia and Europe, significant heterozygosity is still observed among local chicken populations (MALOMANE et al., 2019). More than 1600 chicken breeds are known worldwide (DAD-IS, 2021), but only a few of them have been introduced and used in the global industrial poultry farming (TENEVA et al., 2015). Local breeds account for up to 95% of the total poultry population in developing countries contain a wealth of genetic information for phenotypic traits, some of which may eventually prove valuable for poultry breeding and humans in the future. Such examples include the use of mutations in plumage type and colour, eggshell colour, dwarfism in broiler parents, etc., to provide more attractive products for the consumer or birds better adapted to specific rearing conditions. A large number of chicken breeds have been created in Europe, but many other breeds that originated outside the continent are also bred and maintained. More than 60% of all chicken breeds in the world are bred by hobby breeders in Europe, making them an important factor in the preservation and conservation of genetic diversity in domestic chickens (LUKANOV, 2017a). Bulgaria, as a part of European hobby breeders' society, contributes with its ten local breeds of chickens to the maintenance of the genetic diversity in poultry breeding.

Table 1: Bulgarian chicken breeds by type

Breed	**Breed type**		
	Productive	**Ornamental**	**Miniature**
Black Shumen chicken	✓		
Stara Zagora Red chicken	✓		
Katunitsa chicken	✓	✓	
Struma chicken		✓	
Southwest Bulgarian chicken		✓	
Bulgarian longcrower	✓	✓	
Rhodope painted chicken	✓	✓	
Bregovska dzhinka		✓	✓
Struma bantam		✓	✓
Southwest Bulgarian bantam		✓	✓

Scientifically based study of local breeds in Bulgaria began in 1927, when the Central Poultry Experimental Station was established near Sofia (TABAKOV and HLEBAROV, 1930). Research at the station was carried out with local chicken breeds: Buff Elena chicken, White Sevlievo chicken, and Black Shumen chicken. At

DOI: https://doi.org/10.59913/dagr.2024.17293

a later stage, the development of some Bulgarian dual-purpose breeds was started: Stara Zagora Red and “Chernaedra” chickens (HLEBAROV and TOTEV, 1963; NOZHCHEV and TSONKOV, 1969). Of all the chicken breeds bred back then, only two remain today: Black Shumen and Stara Zagora Red chickens (LUKANOV, 2011a). At that time, the local Bulgarian breeds that were kept for ornamental purposes had not yet been researched. The first more detailed descriptions of these breeds have only been produced in the last 15 years (LUKANOV, 2011ab; LUKANOV, 2012; TENEVA et al., 2015; LUKANOV et al., 2021ab). Today there are the following Bulgarian chicken breeds: Black Shumen chicken, Stara Zagora Red chicken, Katunitsa chicken, Struma chicken, Southwest Bulgarian chicken, Bulgarian longcrower, Rhodope painted chicken, Bregovska dzhinka, Struma bantam, and Southwest Bulgarian bantam (Table 1).

In Bulgaria, poultry genetic resources in the field of scientifically-based breeding are managed by two state centres that are part of the National Gene Pool which is under the control of the Executive Agency "Selection and Reproduction in Animal Breeding". The first centre is located near the town of Stara Zagora and is part of the Agricultural Institute. It maintains flocks of the two nationally recognized chicken breeds and 12 strains of egg-producing, meat-producing and dual-purpose chickens (NIKOLOV et al., 2011; GERZILOV, 2011; LALEV et al., 2011; LALEV et al., 2012; MIGINEISHVILI et al., 2021; LUKANOV et al., 2021a). Some of them are industrial strains that have been preserved in their original form for decades, while others are newly created. Ten strains are kept at the second state centre - Institute of Animal Science near the town of Kostinbrod, some of which are also original productive lines or newly created, mainly intended for dual purpose and suitable for meat production under free-range and organic farming conditions (PETKOV et al., 2020; MIGINEISHVILI et al., 2021; LUKANOV et al., 2021a; PETKOV et al., 2022). The country's amateur poultry breeders are also part of the National gene pool and support the 10 local chicken breeds and 45 Bulgarian pigeon breeds. Many breeds created and bred by amateur poultry breeders remain outside the field of vision of scientists and government agencies (PAVLOVA and LUKANOV, 2024). The scarce and incomplete information on the distribution, exterior and productivity of the Bulgarian chicken breeds determined the aim of this study: to describe the main characteristics of the Bulgarian chicken breeds that are part of the global genetic diversity of domestic chickens.

Egg-laying type

Black Shumen Chicken

This is the oldest Bulgarian chicken breed (Figure 1.). It was bred at the end of the 19th and beginning of the 20th century in North-Eastern Bulgaria (PETROV et al., 2011). The birds of this breed have a compact, elegant-looking body of medium size. The comb is single and of medium size. The earlobes are red. A little white in the

DOI: https://doi.org/10.59913/dagr.2024.17293

central area of the earlobe is acceptable in roosters. The eyes are orange-red. The shanks and beak are dark grey. The plumage is black. Birds of this breed are extremely adaptable and undemanding (LUKANOV, 2017a; LUKANOV et al., 2021a). The live weight of roosters is 1.7-2.5 kg, that of hens - 1.3-1.9 kg (LUKANOV et al., 2018). They start laying eggs at the age of 5-6 months. Egg production is relatively low for an egg-laying type - 150-160 eggs per year weighing about 50-55 g with an almost white shell (LUKANOV, 2017a). The hens show the brooding instinct very rarely. A blue plumage colour in the breed was created (LUKANOV, 2013).

Figure 1. Black Shumen Chicken - a rooster and a hen and area of creating of the breed.

Population status: The population of the breed accounts for about 8% of the total number of purebred chickens in the country. Most of the animals are bred by hobby breeders (57.8%), the rest in the Agricultural Institute of Stara Zagora. The Black Shumen chicken is represented by about 70 sexually mature males and 400 females, which puts them on the list of endangered chicken breeds according to the risk categorization methodology proposed by NIKOLOV and DUCHEV (2022) for Bulgaria (PAVLOVA and LUKANOV, 2024).

Dual-purpose type

Stara Zagora Red Chicken

The breed was developed in 1960s in the area of Stara Zagora, using local red hens, which were crossed with Rhode Island Red roosters (NOZHCHEV and TSONKOV, 1969). It was recognized as a breed in 1970 (NIKOLOV et al., 2011). Birds of this breed have a medium-sized body, which is set medium high (Figure 2.). The pectoral and leg muscles are well developed. The comb is single and of medium size. The earlobes are red. The eyes are red-brown. The legs are pale yellow with a brownish anterior part. The plumage is red-brown with a black tail (NOZHCHEV and TSONKOV, 1969; LUKANOV, 2011b). The breed is very well adapted to free-

DOI: https://doi.org/10.59913/dagr.2024.17293

range conditions. The body weight of roosters is 2.5-3.0 kg, that of hens is 2-2.5 kg (LUKANOV et al., 2021a). Hens are early matured and begin to lay eggs at 5-6 months of age. Egg production is about 240-260 eggs per year weighing about 60 g with a brown shell. The meat has an excellent taste (LUKANOV, 2017a).

Figure 2. Stara Zagora Red Chicken – a rooster and a hen and distribution area of the main population.

Population status: According to PAVLOVA and LUKANOV (2024), the breed has a local distribution in the region of the city of Stara Zagora, with almost 60% of the entire population bred at the Agricultural Institute - Stara Zagora, and the rest by amateur poultry breeders from the region. According to the authors, the breed is represented by about 70 roosters and 450 hens, which, together with the concentration of the population in one region, poses a serious risk to its existence, classifying it as an endangered breed.

Rhodope Painted Chicken

This interesting breed was created in the Eastern Rhodope region (the settlements around the towns Momchilgrad and Kardjali). According to unofficial information, it can be assumed that they were bred in this region more than 40 years ago. The birds have an unmistakable black and white spotted plumage colour (Figure 3). A red patterned plumage colour is also known, but it is not widespread (PAVLOVA and LUKANOV, 2024). The body is medium-sized with a horizontal position. Breasts are rounded and moderately well developed. The comb is medium large, single, but birds with a rose-shaped comb are also found. The earlobes are red. The eyes are orange-red. The beak is yellow with dark areas. The legs are yellow, massive, without feathering, with the presence of individual darker scales (LUKANOV et al., 2021a). The Spanish Asturian painted chicken breed has a similar

DOI: https://doi.org/10.59913/dagr.2024.17293

appearance but it is larger and more massive (ANONYMOUS, 2019). Rhodope painted chickens are unpretentious and perfectly adapted to the harsh climate of the mountains. The weight of roosters is 2.7-3.5 kg, and that of hens 2.5 kg. Hens start laying eggs at 5-6 months of age. Egg production is 180-200 eggs per year. Eggs weigh about 58-60 g, with a light cream shell colour (LUKANOV et al., 2021a). The hens exhibit a brooding instinct and take good care of their offspring. The breed is characterized by its very good reproductive characteristics (LUKANOV, 2023).

Figure 3. Rhodope Painted Chicken – a rooster and a hen and distribution area of the main population.

Population status: The population of the breed accounts for about 4% of the total number of sexually mature representatives of the Bulgarian chicken breeds, numbering about 40 roosters and 200 hens (PAVLOVA and LUKANOV, 2024). The concentration of most of the population in an area with a radius of less than 25 km - the settlements around the city of Momchilgrad makes it extremely vulnerable according to data from 2009 (ALDERSON, 2009), ranking it among the endangered species (PAVLOVA and LUKANOV, 2024).

Meat type

Katunitsa Chicken

This is one of the most recognizable Bulgarian chicken breeds. It was officially recognized by the Union of Fanciers and Small Domestic Animal Breeders in Bulgaria (UFSDABB) in 2014 and it was presented at the European Exhibition in Metz, France, in 2015 (LUKANOV, 2017a). Katunitsa chicken (AN line) is a new chicken breed created by Alexander Nikolov from Katunitsa (Plovdiv region) for over a decade by selecting local heavy chickens from the Plovdiv region (NIKOLOV and GERZILOV, 2011; TENEVA et al., 2015). It is a typical meat breed with high live body weight, relatively good egg laying capacity and characteristic plumage colour except black and blue (Figure 4.). These birds have a large, deep and wide body. The pectoral and leg muscles are very well developed. The back is straight,

DOI: https://doi.org/10.59913/dagr.2024.17293

wide, smoothly turning into a tail. The comb is medium large, single. The earlobes are red. The eyes are orange red. The shanks are thick, without feathering, yellow in colour with variations of yellow with dark areas. Recognized plumage colours are dark red, yellow-black, blue and black. A characteristic feature of the first two colours is the manifestation of multiple lacing (LUKANOV, 2017a). This breed is one of the heaviest purebred chickens. Birds have excellent meat characteristics. Roosters weigh 5.5-6.5 kg, and hens 4.5-5.3 kg (UFSDABB, 2014a). The meat has an excellent taste. Chicks grow intensively and reach live weight of around 2.5 kg for males and around 1.8 kg for females at the age of 12 weeks (NIKOLOV and GERZILOV, 2011; GERZILOV et al., 2015). The egg production is relatively low - 140-150 eggs per year, with an egg weight of 60-65 g and cream-colored shells (LUKANOV et al., 2021a). Chickens exhibit moderate brooding instinct (LUKANOV, 2017a).

Figure 4. Katunitsa Chicken – a rooster and a hen and distribution area of the main population.

Population status: The Katunitsa chicken has the third largest population among the Bulgarian chicken breeds, with a share of about 11%, which corresponds to about 90 roosters and just over 620 hens (PAVLOVA and LUKANOV, 2024). According to the methodology proposed by NIKOLOV and DUCHEV (2022) for determining the risk categorization for Bulgaria, it can be said that the breed is not threatened with extinction, but the concentration of most of the population in the central parts of southern Bulgaria is a cause for serious concern (PAVLOVA and LUKANOV, 2024).

Ornamental type
Struma Chicken
This is the most popular of the Bulgarian standard chicken breeds (PAVLOVA and LUKANOV, 2024). The breed was probably created about 40-50 years ago in the settlements along the Struma River (TENEVA et al., 2015; LUKANOV, 2017a). It has distinctive features such as feathered shanks, the presence of a beard and crest (LUKANOV, 2011a). The breed was recognized by the UFSDABB in 2013

DOI: https://doi.org/10.59913/dagr.2024.17293
(LUKANOV et al., 2021a). Birds of this breed have a large, deep and wide body (Figure 5.). The crest is very well defined, voluminous, wide, and does not completely cover the eyes. The beard is very well developed, with well-defined sideburns that cover the earlobes. The comb is simple-shaped, medium size. The eyes are orange red. Birds of this breed have very well-defined feathered shanks. Recognized plumage colours are red-and-white spotted, yellow-and-white spotted, black-and-white spotted, and striped. Depending on the coloration, the colour of the metatarsus varies from yellow to flesh coloured (UFSDABB, 2013a). The body weight of roosters is 3.2-3.8 kg, that of hens 2.8-3.2 kg (UFSDABB, 2013a). Egg production is about 120-140 eggs weighing about 55 g, with a cream to brownish shell. Struma chickens are medium-mature birds. They begin to lay eggs at about 7 months of age. Hens exhibit a brooding instinct and take good care of their offspring (LUKANOV, 2017a). The breed is characterized by relatively unfavourable reproductive indicators, which is relatively common in ornamental chicken breeds (LUKANOV, 2023).

Figure 5. Struma Chicken – a rooster and a hen and distribution area of the main population.

Population status: The attractive appearance makes the breed widespread in Bulgaria and recognizable abroad. The population of the breed comprises about 130 roosters and 660 hens, which is about 12% of all representatives of Bulgarian chicken breeds. Depending on the established parameters, the breed can be classified as endangered or not at risk of extinction (PAVLOVA and LUKANOV, 2024).

Southwest Bulgarian Chicken

This breed was recognized in 2021, but the data on the breeding of these chickens indicate that they have been bred in the Western Rhodopes for decades (LUKANOV et al., 2021a). They show an attractive variety of plumage colours, combined with the presence of some mutations such as feathered shanks and beard (Figure 6.). The comb is strawberry type. The birds are medium-sized, with a relatively high-set body and tail. The main plumage colours are red-mottled and black-and-white mottled.

DOI: https://doi.org/10.59913/dagr.2024.17293

The red-mottled colour dominates in the breed, which has two variants - lighter and darker. The shanks are yellow or light flesh-coloured, which matches the colour of the beak. The eyes are red to orange-yellow (UFSDABB, 2021a). Live weight of roosters is 2.5 kg, hens 2.0 kg (UFSDABB, 2021a). Birds are medium-early matured, they begin to lay eggs at the age of about 6 months. Egg production is quite high 160-180 eggs per year. Eggs have a cream shell and weigh about 50-55 g. Hens are good brooders (LUKANOV et al., 2021a). Despite the decorative nature of the breed, no serious negative impact on reproductive characteristics is reported (LUKANOV, 2023).

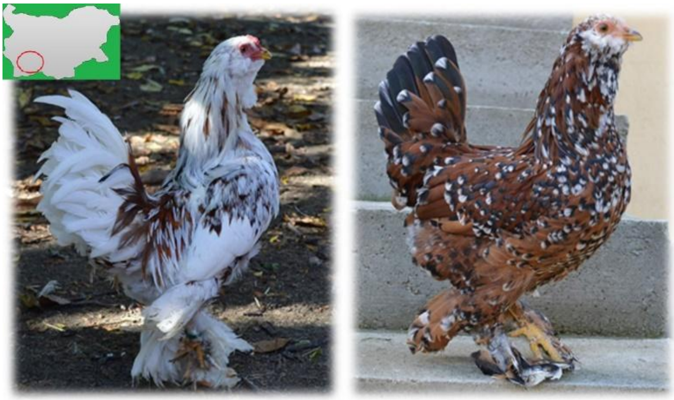

Figure 6. Southwest Bulgarian Chicken – a rooster and a hen and distribution area of the main population.

Population status: The breed is one of the most popular in the country and accounts for about 10% of the total number of representatives of Bulgarian chicken breeds with about 70 roosters and 560 hens it makes up (PAVLOVA and LUKANOV, 2024). According to the methodology of NIKOLOV and DUCHEV (2022) for determining the risk category of breeds developed for Bulgaria, the Southwest Bulgarian chicken is not threatened with extinction. Taking into account the methodology of FAO (2013), it can be defined as "Endangered".

Bulgarian Longcrower

This breed is in the recognition process by the UFSDABB and is one of the few longcrowing breeds in the world. It originates from southern Bulgaria (LUKANOV et al., 2021a). Now it is mainly bred in South (Plovdiv and Kardzhali region) and West (Blagoevgrad region) Bulgaria. These birds are very vigorous and hardy and have an appearance that differs from the other longcrowing breeds of chickens (LUKANOV, 2017b). The body is medium-sized, elegant, not very tall, with a horizontal to slightly sloping back line (Figure 7.). The plumage is black with a silvery or yellowish-golden neck, back, saddle, and shoulder area in the roosters and

DOI: https://doi.org/10.59913/dagr.2024.17293

black in the hens, resembling that of the Turkish Denizli breed. The comb is pea-shaped. The birds have a medium-sized beard and moderately to slightly pronounced feathers on the shanks. The shanks are yellow or black. The eyes are red to orange-red (LUKANOV et al., 2021a). The beak is dark in birds with black coloration of the plumage and black and yellow in black-silver birds. The crowing of roosters lasts about 12-15 seconds, but there are some individuals in which it reaches 30 seconds or even more. Live weight of roosters is 2.5 kg, that of hens 2.0 kg. Hens start laying eggs at the age of 5.5-6 months. Egg production is good, about 160-180 eggs with almost white (very pale cream) shells, weighing 50-55 g (LUKANOV et al., 2021a). The breed shows relatively good results in the study of incubation characteristics (LUKANOV, 2023).

Figure 7. Bulgarian Longcrower – a rooster and a hen and distribution area of the main population.

Population status: The Bulgarian longcrower is one of the least represented Bulgarian chicken breeds with only about 270 individuals (about 40 roosters and 230 hens), which is about 4% of the total number of representatives of Bulgarian chicken breeds, which makes it vulnerable with the status - Endangered (PAVLOVA and LUKANOV, 2024).

Bantam chicken breeds
Bregovska Dzhinka
The term “dzhinka” (in BG) refers to local true bantam chickens (LUKANOV and PAVLOVA, 2021). Of all the small chicken breeds bred in Bulgaria, the Bregovska dzhinka is the most widespread and most popular outside the country (PAVLOVA and LUKANOV, 2024). It was most probably created in the 70s of the last century on the basis of local dzhinki in the area of the town of Bregovo and Vidin (LUKANOV, 2012; TENEVA et al., 2015; LUKANOV et al., 2021b). The breed belongs to the group of true bantams (they do not have a large version). It was

DOI: https://doi.org/10.59913/dagr.2024.17293

recognized as a breed by the UFSDABB in 2013. The birds are visually attractive, extremely unpretentious, and very vital. The body is small, compact, wide and low-set (Figure 8.). The breasts are well formed, rounded, wide and convex in both sexes. The back is short, especially noticeable in roosters. The tail of roosters is relatively short and set high. The comb is single-shaped, medium-sized. The earlobes are white. The eyes are orange-red (UFSDABB, 2013b). The known plumage colours are red-mottled, black-and-white mottled, and white. The shanks are light flesh-coloured, and in birds with black-and-white mottled plumage colouring they are dark (LUKANOV, 2017a). The live weight of roosters is 500-750 g, that of hens around 400-600 g (UFSDABB, 2013b). Egg production is low - about 80-100 eggs per year weighing about 30 g with a creamy shell. The hens are good brooders (LUKANOV et al., 2021b).

Figure 8. Bregovska Dzhinka – a rooster and a hen and area of creating of the breed.

Population status: The breed has the largest population compared to other Bulgarian chicken breeds - about 34% and about 2,200 sexually mature individuals, which makes it the only breed that is not threatened with extinction according to all three methods presented by PAVLOVA and LUKANOV (2024).

Struma Bantam

This breed of chicken was created in Western Bulgaria, by the inhabitants of the settlements along the Struma River (LUKANOV, 2012). It was recognized as a breed by the UFSDABB in 2014. The birds of this breed have a small, wide and relatively deep body (Figure 9.). The crest is well formed, medium in size, does not cover the eyes. The beard is also very well formed. The comb is medium in size, single by type (UFSDABB, 2014). There are also individuals with a rose-shaped comb (LUKANOV et al., 2021b). The eyes are orange-red. They have very well-defined feathered shanks. Struma bantams are recognized with black and white spotted, with red-mottled and white plumage (LUKANOV, 2017a). Birds of this breed are unpretentious and have an attractive appearance. The live weight of roosters is 750-950 g, and hens about 650-850 g (UFSDABB, 2014). Egg production is low - about

DOI: https://doi.org/10.59913/dagr.2024.17293

80 eggs per year, which have a cream shell and weigh about 30 g. Hens are good brooders (LUKANOV, 2017a).

Figure 9. Struma Bantam – a rooster and a hen and distribution area of the main population.

Population status: according to PAVLOVA and LUKANOV (2024), it is the second most numerous Bulgarian chicken breed, with a share of about 12%, composed of about 90 roosters and 350 hens. The Struma bantam is a breed threatened with extinction according to all three methods presented by the cited authors.

Southwest Bulgarian Dzhinka

This is a relatively new breed in the poultry world, but it is descended from the local Dzhinka chickens that have been bred in the Western Rhodope region for decades (LUKANOV and PAVLOVA, 2021). It was officially recognized as a breed in 2021 by the UFSDABB (UFSDABB, 2021b). The birds have a small, low-set body (Figure 10.). The breasts are rounded, strongly convex. They have a rose comb, beard and well pronounced feathered legs (LUKANOV et al., 2021b). The breed is characterized by an attractive plumage colour - the most common plumage colour is red-mottled, but black-and-white spotted is also common (LUKANOV and PAVLOVA, 2021). There is a similar breed bred in Turkey - Ispench, which is characterized by the presence of a fifth toe, which the Southwest Bulgarian Dzhinka does not have (PAVLOVA et al., 2021). The live weight is 600-800 g for the roosters and 500-600 g for the hens (UFSDABB, 2021b). Egg production is low 60-80 eggs per year with a cream-coloured shell and a weight of about 30 g. The hens show a very strong brooding instinct (LUKANOV et al., 2021b).

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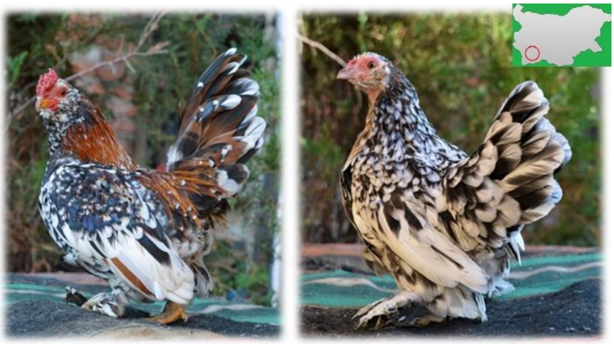

Figure 10. Southwest Bulgarian Dzhinka – a rooster and a hen and distribution area of the main population.

Population status: This is the least represented Bulgarian chicken breed with a share of only about 2%, represented by about 30 males and 115 females concentrated in the Razlog area (PAVLOVA and LUKANOV, 2024). The breed can be defined as "endangered", but considering its concentration in an area with a radius of <25 km, it can be classified as critical (FAO, 2013).

Conclusion and recommendation

The genetic resources of the Bulgarian chicken consist of 7 standard and 3 miniature breeds and 22 productive strains. The main part of the Bulgarian chicken breeds is bred by hobby breeders, while the industrial lines are subject to the institutes, part of the National Gene Pool – Agricultural Institute – Stara Zagora and Institute of Animal Science - Kostinbrod. All Bulgarian chicken breeds, with the exception of the Bregovska Dzhinka, are now threatened with extinction. Due to the outbreaks of avian influenza in our country and the ban on national chicken exhibitions in the last 3 to 4 years, the future trend of the population status of our breeds is unfortunately not positive.

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Results of an expedition survey on population of rare, aboriginal, local and endangered Brown Carpathian cows

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Abstract

From the expedition survey of the cow’s population, it appears that 7 breeds and genotypes of cattle are bred in Transcarpathia, part of the Ukrainian Carpathians. The materials presented in the work and their analysis show that during the years 1911-2023 there were changes in the breed composition and numbers. The state of distribution, the number of breeding stock of one of them - a rare, aboriginal, local and disappearing brown Carpathian breed is currently insufficiently studied. From a scientific point of view it represents a valuable object of preserving the biological diversity of cattle and establishing the history of the development of cattle breeding. From a practical point of view - with low-cost production technologies, it provides high-quality milk and meat to the local population, the stability of ecosystems and the biosphere as a whole. It provides also the sustainable development of the regional communities and international cooperation. In the current and future global warming and energy crisis, this breed is suitable for operation under the conditions of application of new "Green Architecture" technologies recommended by the European Commission, which fundamentally change traditional approaches in dairy farming. The results of our research will help clarify the status of the brown Carpathian breed, the number of cows and the breeding area as well as draw attention to the problems of implementation of the provided protection and conservation measures, their financing mechanisms.

Keywords: aboriginal breed, closed population, preservation of biodiversity, gene pool

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Introduction

Cattle is bred in 144 countries of the world. Unfortunately, progressive erosion of genetic diversity is observed in the vast majority of them. Therefore, the strategy of balanced economic development adopted by the conference of the international agricultural and food organization of the United Nations in 1992 gives decisive importance to the inventory, protection and reproduction of breeds of agricultural animals. It is cattle that provide the biological basis on which milk and beef production systems are built, the development of the livestock industry and rural areas of the world (FAO, 2011). To the greatest extent, in the microregion of the Upper Potyssia of the Carpathians, this applies to the brown Carpathian breed, a component of the economy and ecosystem of the Transcarpathian region of Ukraine with a population of 1250.1 thousand people, an area of 12.8 thousand km² with a specific relief structure. Natural factors in terms of hydrological, climatological parameters, soil and grass complexes are also very different here. In these conditions, animals of the brown Carpathian breed, occupying a wide amplitude of significant differences in altitude, from floodplain meadows to high-altitude meadows, feel comfortable and produce high-quality milk and beef. Thanks to their strong legs and hoofs, they can move through wetlands and climb stony paths in the mountains, the lowest grazing mark is 101 m above sea level, the highest is 1880 m above sea level. However, the breed is limited in its distribution area, localized within the borders of the region, bred in a closed population. According to the United Nations (FAO) assessment methodology, it is endangered (FAO, 2010). It is known that the main indicator of the protection and preservation of the breed of the first level in situ is the mother stock. But literary and other sources, inventory materials indicate that during the period of reforming the agrarian sector in the state, breeding farms were liquidated, the population of cows of the brown Carpathian breed is undergoing negative changes, especially in recent years it has been sharply reduced. For various reasons, negative trends do not stop, its protection and preservation are insufficient. Taking into account the multifaceted scientific and practical importance of the breed, the problem of increasing research attention, establishing territorial and numerical representation of cows arises. The task of our work was to clarify the status of the brown Carpathian breed, to obtain new data by clarifying the number of breeding stock and to assess changes and geography of distribution. In the following, these materials will provide an opportunity to correctly understand its current state, determine the optimal quantity of cows for the creation of a database and the development of a management strategy for the genetic resources of the brown Carpathian breed. The purpose of this - sustainable use, discovery of potential opportunities for protection, preservation and development in the purity of the genotype of animals in situ and ex situ, as well as establishing a tireless system of natural food production at the local and regional level in the situation of climate change and energy crisis using the technologies of "Green Architecture"

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recommended by the European Commission. The priority is climate neutrality, preservation of valuable natural complexes, landscapes and biodiversity, production of organic products (KRAVCHIK, 2021). Especially now and in the future, the industry must also be competitive, so the relative value of the brown Carpathian breed changes rapidly with the progress of knowledge and technology. In our opinion, it is necessary in the near future to find and apply tools that will avert regression and support the existing mother stock of the brown Carpathian breed and even give an incentive for its growth.

Material and methods

The object of research was the brown Carpathian breed of cattle. In the first part of the work, its status was clarified according to the new internationally recognized system of approaches in the classification of gene pool objects, "State of the world genetic resources of animals in the field of Food and Agriculture Organization" (FAO) of the United Nations (FAO, 2011) and "Program for the conservation of the gene pool of the main species of agricultural animals in Ukraine for the period until 2015" (GUZEV, 2009). Organized and conducted an expedition on the specified topic according to the developed model in three stages: preparatory, field stage and processing of collected materials, drawing up a report. At the first stage, literature, archival documents, materials of the Agency for Identification and Registration of Animals, veterinary institutions were worked out, routes were planned. During the second stage, animal owners, veterinary workers, artificial insemination technicians, shepherds were interviewed. The expedition survey covered all 3 ecological zones and 13 administrative districts of the Transcarpathian region according to the division of territories before consolidation. The inventory and dynamics of the herd of cows, quantitative ratio of breeds, geographical location in the studied territory were carried out during 2023 using the route method with the determination of the belonging of animals to the corresponding breed by phenotype (according to the standardized description of the phenotype and the protocol recommended by the FAO outlined in the publication "Phenotypic characteristics of genetic of animal resources", 2012) and recorded the number with an entry in a field diary. In conclusion stage, a comparative-analytical generalization of the obtained data was made on the background of a number of materials, including retrospective ones, statistical processing was carried out, and a report was drawn up.

Results and discussion

A comprehensive assessment of the status of gene pool objects of cattle in Transcarpathian region established that the brown Carpathian breed counts two gene

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pool objects – lowland and mountain. In terms of threats to existence, it belongs to category 1 [1], which is already on the verge of extinction, local [M] – which is bred only in one country, regional [TR] – Is present only in one region. Thus, as of the end of 2023, the brown Carpathian breed belongs to rare, local and endangered species. Counts two gene pool objects. Since ancient times, pastoralists lived in the current territory of Transcarpathia and had a lot of oxen, cows and other livestock, as evidenced by the registers of the 16th century, and they were good experts in their field. Animals gave them clothes and food, served as traction force. A cow was considered a measure of wealth. The family craft was passed down from generation to generation and this affected the mentality of the local people, their culture and socio-economic organization. Undoubtedly, one-person farming has become the most widespread. The facts confirm that, according to official documents, back in 1895 there were 250.8 thousand heads of cattle. In the calculation, there were 51 heads per 100 people. Improvement of imported animals begins, breeding work is carried out. In 1903, on the basis of the estate of Count SCHÖNBORN, a state breeding farm for Carpathian grey (in the future Carpathian brown) cattle was organized and mating stations were established in the villages. These initiatives carried out had a positive effect on the growth of the productivity of cows. It became profitable to engage in dairy farming, and already since 1911 there has been a positive trend in the growth of farms that kept cows, including animals of a new genotype, which is indicated by data per 100 farms (IRENEY et al., 1925), Table 1.

Table 1. Growth dynamics of farms that kept cattle in the years 1911-1933 per 100 farms (%)

Sex-age groups	**Years**		
	1911	**1925**	**1933**
Cows	43.7	48.4	53.6
Oxen and bulls	30.5	24.9	19.5
Growers	25.8	26.7	26.9

Over the course of 22 years, the number of farms that kept cows (per 100 farms) increased by 22.7%. But there were also unfavourable periods. For example, the World War I led to the decline of the industry. By the end of 1916, only in the Uzhan Committee, the number of cattle decreased by 32% compared to 1914. In the post-war years, the polonynska economy developed widely, which gave a new impetus to development. Thus, in 1933, 23,300 heads of mainly grey Carpathian cattle, including 4,130 cows, were grazed on the subalpine and alpine meadows from May 15 to June 1 and until September 21, depending on the polonyna and weather. On pastures located along the Teresva River, 14 cows were grazed for every 100 head of cattle, 61 on the Tysa. One shepherd served 14 cows or 28 oxen. A total of 137 large oxen farms operated. The herding of animals for grazing was accompanied by celebrations and ceremonies. V. GOSNEDL (1936) reported on the breeding of 68.7% Carpathian grey cattle, 15% Grey steppe cattle, 12.2% Pinzgau and 4.1%

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Simmental cattle. During the World War II, population degradation took place again. 78,300 heads of grey Carpathian, Simmental, Grey steppe and Pinzgau cattle were exported to Germany alone. After the end of the war, the situation gradually stabilized and acquired positive dynamics. The herd increased sharply, taking into account purebred brown Carpathian animals. Statistical data on the quantitative and breed composition are given in Table 2. It should be added that in 1948 the State Breeding Book of Brown Carpathian Cattle was published.

Table 2. Changes in the number of brown Carpathian cattle and their specific weight to all breeding cattle in the Transcarpathian region for the years 1950-1968

Genotypes	**Years**		
	1950	**1959**	**1968**
Total brown Carpathian cattle, heads x1000	17.6	89.5	136.3
Specific weight of all breeding cattle, %	81.0	86.0	99.0

In the specified period (Table 2) the number of animals increased rapidly (7.7 times) and the specific weight of purebred brown Carpathian cattle increased by 18% to all available purebred cattle. This phenomenal breed is a genotype on which scientists and practitioners worked in the last century and many years of breeding "in itself" formed the original gene pool. Let's dwell on the most characteristic features. A mature cow is entered in the 8th volume of the State studbook of brown Carpathian cattle, dark and light brown colour, nose mirror dark with a mandatory light ring, live weight 485 kg, and height at the withers 133 cm. It presents milk-meat type with strongly emphasized indicators: weight 5680 kg, fat 3.72%, average daily fattening gain 800 g. It has a harmonious body structure, a well-developed udder, strong limbs and hoof horn, the possibility of increasing the milk yield of high-quality, improves the feedlots (Gosplemkniga, Vol. 8, 1992). Resistant to diseases and parasites. Maintains live weight for a certain time when faced with seasonal stresses caused by interruptions in feeding, has a high reproductive capacity, a long period of productive use. Milk and beef have high nutritional and technological properties. The breed is an important element of local farming culture, a historical heritage, adapted to local soil and climatic conditions, etc. The best specimens of the breed were presented at state and regional exhibitions. It should be noted that in the 90s of the last century, the largest share of income in the agriculture of the Transcarpathia region came from animal husbandry, including the sale of live breeding and improved young animals within the local and national markets. In the studied territory, the specific weight of animal husbandry in the produced gross agricultural products was 51.3% in monetary terms, while cattle breeding accounted for 89.1% in the structure of the industry's gross products. A favourable factor for keeping cattle is the abundance of types of grass cover on an area of more than 200,000 hectares of natural meadows and pastures. However, during the time that has passed since the radical reformation

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of property and land ownership relations, in accordance with the laws of Ukraine "On Business Societies", "On Rural (Farm) Economy", "On Entrepreneurship", a price disparity has arisen for industrial, crop and livestock products and shift occurred to the development of crop production. The collapse of the livestock industry caused a decline in milk production with a shortage of milk and milk products, covering the region's need by 63.8% (TERPAY, 2021). In addition, the low interest of investors in the agrarian sector for development of cattle breeding was found out. As a result, the number of farms of various forms of ownership and the total number of cows decreased significantly from 141,500 to 42,034 in 2023, or by 3.4 times, Table 3.

Table 3. Dynamics of the cow population in Transcarpathia oblast for 1990 and 2023, farms of all forms of ownership

Eco zones	**Districts**	**Quantity, headsx1000, years**			**Per 100 ha of agric. land**		
		1990	**2023**	**fold**	**1990**	**2023**	**fold**
Mountains	Volovetskyi	6.8	2.0	3.4	40.0	11.8	3,4
	V.Bereznyansk.	7.0	1.8	3.9	38.9	10.0	3,9
	Mizhhirskyi	10.2	6.6	1.5	32.5	21.0	1,5
	Perechynskyi	5.3	1.3	4.1	31.2	7.6	4,1
	Rakhivskyi	9.8	5.9	1.7	29.5	17.8	1,7
	Svaliavskyi	5.8	1.4	4.1	37.4	9.0	4,2
In the mountainous area		44.9	19.0	2.4	34.0	14.4	2.4
Pre-mountains	Irshavskyi	12.0	2.6	4.6	33.5	7.3	4,6
	Tiachivskyi	18.6	5.4	3.4	35.1	10.2	3,4
	Khustskyi	13.8	6.0	2.3	36.4	15.8	2,3
In the foothill zone		44.4	14.0	3.2	35.0	11.0	3.2
Lowlands	Berehivskyi	10.7	1.5	7.1	23.0	3.2	7,2
	Vynogradivsk.	13.0	2.6	5.0	28.3	5.7	5,0
	Mukachivskyi	16.4	3.3	5.0	28.8	5.8	5,0
	Uzhhorodskyi	12.1	1.6	7.6	24.5	3.2	7,7
In the lowland zone		52.2	9.0	5.8	26.3	4.5	5.8
In the region		141.5	42.0	3.4	30.9	9.2	3.4

The reduction applies to all three ecological zones, 13 administrative districts and 561 rural settlements. The changes did not take place evenly. As for the ecological zones, the greatest decline occurred in the lowland – by 43.2 thousand cows or 5.8 times, the lowest in the mountain – 2.4 times. Both in absolute values and in percentage calculation, in particular, Tiachivskyi, Mukachivskyi, Uzhhorodskyi, Irshavskyi and Berehivskyi districts decreased by 13.2 – 9.2 thousand heads, in percentage Uzhhorodskyi by 7.6, Berehivskyi by 7.1 times. It should be noted that the lowest number of cows is kept in the lowland ecological zone – 9,000 heads, Perechynskyi – 1130, Berehivskyi – 1136, Svalyavskyi – 1167 heads, separate settlements. Thus, the author found that in the village of Runia and Dibrova of

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Tiachivskyi district, with a population of 678 and 440 men, there are 5 and 3 cows, respectively, in the village of Glyniany, where 301 people live, none. It is also necessary to assess the low volume of livestock per 100 hectares of agricultural land. With average indicators in the region of 9.2 cows, again, there is only 4.5 heads in the lowland ecological zone, 3.2 each in the Berehivskyi and Uzhhorodskyi districts. The situation is a little better in Vynogradivskyi – 5.7, Mukachivskyi district – 5.8 cows. Dispersion is observed, on average there are 23.4 heads for one farm, and 1.01 heads for individual farms, in which dairy farming is not the main source of income. Although the specific weight of cows kept in the latter, as of January 1, 2023, is 96.8% and in enterprises of various forms of ownership 3.2%. As a result of a sharp reduction in the number of cows and young cattle of different sex and age groups, many owners of hayfields have nowhere to put the hay harvested from them and burn it en masse, harming the microbiota and the environment, Figure 1.

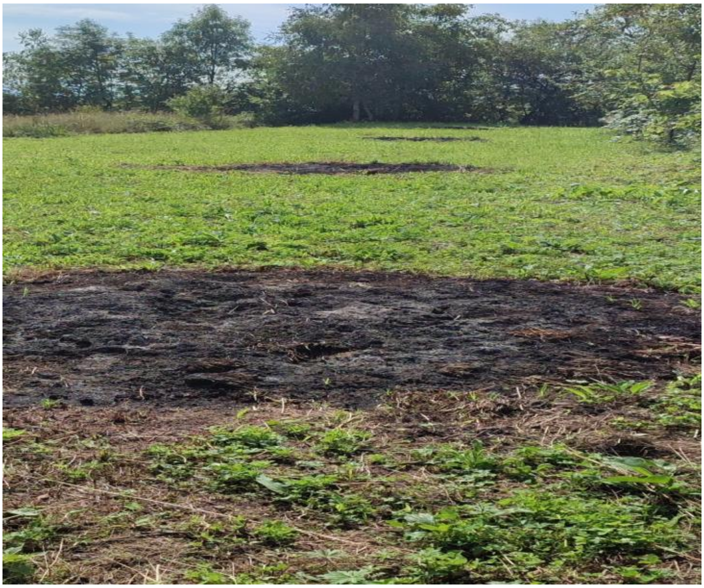

Figure 1. Burns after burning mowed, dried and collected grass in natural hayfields and even areas of sown perennial grasses.

At the same time, first of all, it should be noted that the region is rich in natural hay meadows, and this industry affects the level of employment of the population, food supply, ecological and social criteria, and is a source of organic matter. Farms of all organizational forms use a mixed system of maintenance: on self-grown forage and pastures, the purchase of concentrates outside the region; manual milking, in a small part of individual farms – machine milking, controlled manual pairing and artificial insemination. Repairs instead of lost cows are carried out at the expense of raised own heifers. The production of milk and meat is only partially oriented to the local

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market. Summarizing the above indicators, we note that cattle have played an important role in the life of the local population since ancient times. Since the reform of the agrarian sector of the economy in the 1990s, the total number of cows in Transcarpathia region has decreased by 3.4 times. The vast majority of cows are kept in individual farms to provide livestock products for their own needs and for small sales to neighbours and local markets. Geographical distribution of brown Carpathian and other breeds, quantitative ratio, were studied on the territory of Transcarpathia. We recorded quite high breed heterogeneity and diversity, breeding of 7 different specialized and combined breeds and genotypes of cattle. It should be noted that they underwent constant changes in the place of distribution, ratio and number, which can be traced in the statistical indicators of occurrence. The presence and predominance of individuals of the brown Carpathian breed with different levels of variation in all ecological zones and over the region is shown in Table 4.

Table 4. Representation of breeds and genotypes by ecological zones and by region

Breeds and genotypes	**Ecological zones**			
	Mountains	**Foothills**	**Lowland**	**Presence in region, %**
Brown Carpathian	+	+	+	86
Simmental	+	+	+	2.8
Ukrainian brown/dairy	few animals	crossed	+	8.3
Ukrainian red/dairy	few animals	crossed	+	1.3
Holstein	-	-	+	0.4
Aberdeen Angus	-	-	+	0.3
Hungarian Grey	-	-	+	0.9

The heterogeneity of the breeds varies depending on the location of the zone relative to the height above sea level. Indicatively, the high saturation of local cattle cows with a small proportion of Simmental cattle is characteristic of the mountainous area. This is explained by the harsher environmental conditions to which it has adapted well due to its endurance, optimal size and weight of adult animals, short legs, the ability to overcome long distances on a rocky surface, resistance and strength constitution The criterion of functional characteristics is that it can withstand heat, which is very important in times of global warming. To understand the problem, in Holstein animals heat stress begins at an air temperature of +22 C°, at +28 C° it becomes critical, while the brown Carpathian feels only minor discomfort. In addition, according to the Federation of Brown Breeds of Europe, brown cattle cause less emissions of greenhouse gases per unit of produced protein.
The allelic frequency of the kappa casein B variant obtained by genotyping (63.8% BB and 32.6 AB). Beta casein is 30% of milk protein. The difference in the yield of

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hard cheese brown French – Holstein + 13% in favour of the first. The fat/protein ratio is between 1.15 and 1.23 (Brown Swiss News, 2020, 2021, 2022, and 2023). Although brown Carpathian cattle predominate on the plain, the percentage is no more than 67.7% with the presence of all available breeds and genotypes in the region. The foothills occupy intermediate positions. Despite the fact that the regions have a similar breed composition in our day, they are distinguished by their specificity and difference in the percentage ratio between them. Specialized factory breeds and types imported in different ways turned out to be biologically unstable. The ecological conditions of breeding differ sharply from their needs, accordingly, health, productivity, quality of products are deteriorated and the population is insignificant, accordingly, the specific weight in the total share is 11.2 percent. The negative influence of this factor is observed. Thus, the brown Carpathian breed of cattle is one full-fledged representative in the studied territory and corresponds to the main regularity, which consists in the differentiation of relationships in the system of cattle breeding – ecology – climate – plant – animal breed. The assessment of the presence of breeds and the population of cows of the brown Carpathian breed, which has been well adapted to the conditions of Transcarpathia for over 100 years, reflects the degree of threats to it. In particular, the introduction of new diseases, uncontrolled crossbreeding of imported breeds with local livestock violates its genetic purity and stability. Anticipating such a situation, far-sighted Carpathian scientists and breeders in the 70s and 80s of the last century performed exclusively breeding the Carpathian brown, which, starting from the 90s of the specified century, has been destroyed under the influence of various factors, Table 5.

Table 5. Dynamics of the breed composition of cows in the Transcarpathian region since the official existence of the grey bay Carpathian (GC) in the subsequent brown Carpathian (BC) breed, thousands of heads, %

Breeds	**1911**	**1937**	**1980**	**1990**	**2023**
	Heads (%)	**Heads (%)**	**Heads (%)**	**Heads (%)**	**Heads (%)**
GC and BC	45.0 (22.5)	76.8 (70.5)	138.5 (97.6)	110.8 (78.3)	36.1 (86.0)
Others	155.0 (77.5)	32.1 (29.5)	3.4 (2.4)	30.7 (21.7)	5.9 (14.0)
Total	200.0 (100)	108.9 (100)	141.9 (100)	141.5 (100)	42.0 (100)

In particular, in 1992 alone, 16,200 cows and 12,000 beef cows and heifers were inseminated with the semen of Holstein bulls. Through barter operations, more than 4,000 different Holstein genotypes, mainly commercial heifers, were purchased directly by farms from Hungary. Inbred depression acquires special relevance in a territorially and numerically limited breed. The real pressure of which can be avoided by rethinking traditional approaches to breeding, maintaining the optimal number of breeding stock of the brown Carpathian breed, today classified as a breed that is preserved and protected by various international and state documents of Ukraine. At the same time, it should be noted that the research of the last decade has changed the above representations. Using various sources of information and the results of own

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research, in 2023 it was established that the structural share in the spectrum of breeds by specific gravity increased significantly in favour of the local one, which is not found anywhere else in the state. For example, if in 2013 the specific weight of cows of the brown Carpathian breed was 78.6 percent, then in 2023 it was 86.0 percent. Summarizing the above, we note that the indicators for 2023 characterize Transcarpathia as the main centre of breeding and distribution of genetic resources of the brown Carpathian breed, which indicates the significant importance of the region in the development and preservation of its gene pool, and the determining importance of the breed in its sustainable development. Due to its biological characteristics, the brown Carpathian breed has occupied and still occupies dominant positions in terms of number and specific weight, almost since the time of breeding. However, the processing of research results, the total assessment by all methods revealed the process of a rather significant narrowing of brown Carpathian cows in 2023 to 36,100 heads. At the same time, accelerating the pace of livestock limitation may lead to a change in the evolutionarily formed ecological balance, especially in the highlands, and the loss of alpine and subalpine meadows, unique for Ukraine, where these animals were grazed. STOYKO et al. (1982) emphasize that there is already a gradual change of grassy vegetation to tree-shrub vegetation, which leads to a reduction in their area. This can be prevented by stabilization of the population of the Carpathian brown breed, which is tolerant to the extreme conditions of the Carpathians, accurate information about the current condition, constant monitoring, deepening of research and restoration of traditional management of polonyna farming. In addition, brown Carpathian cattle adapt to various systems of production and maintenance, are an important resource that determines the possibilities of further socio-economic and ecological development of the region using modern technologies of "Green Architecture" recommended by the European Commission. At the same time, valuable organic fertilizer is obtained from the animals, which is so necessary for the poor soils of Transcarpathia. Below we present the absolute values, the specific area of territorial distribution and the specific weight of the herd of cows of the brown Carpathian breed in the section of ecological zones, administrative districts and individual settlements according to the results of the expedition survey in 2023, Table 6.
The results presented in the paper and the comparative quantitative analysis, as can be seen from Table 6, show that the distribution of ecological zones, administrative districts by the number and specific weight of the breed is significantly different, and distinguished by a significant uneven distribution of livestock territorially. At the time of the study, the mountainous zone was noted to have the largest number of brown Carpathian cows - 18,200 cows. Among the districts Mizhhirskyi – 6.4, Khustskyi – 5.9 and Rakhivskyi – 5.8 thousand heads, where the maximum number is. Instead, the opposite trend, the presence of a noticeable negative impact, which is expressed in the intensive reduction of the number of cows, breeding of other breeds,

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Table 6. The total number of cows and the brown Carpathian (BC) breed and its specific weight in the section of ecological zones and administrative districts of the Transcarpathian region as of the end of 2023

Eco zones	**Districts**	**Quantity, thousands of heads**		
		Total	**BC**	**% BC**
Moun-tains	Volovetskyi	2.0	1.8	90.0
	V.Berezniansk.	1.8	1.7	94.4
	Mizhhirskyi	6.6	6.4	96.9
	Perechynskyi	1.3	1.2	92.3
	Rakhivskyi	5.9	5.8	98.3
	Svaliavskyi	1.4	1.3	92.9
In the mountainous zone		19,0	18.2	95.8
Pre-moun-tains	Irshavskyi	2.6	2.5	96.2
	Tiachivskyi	5.4	3.4	63.0
	Khustskyi	6.0	5.9	98.3
In the foothill zone		14,0	11.8	84.3
Low-lands	Berehivskyi	1.5	1.1	73.3
	Vynogradivsk.	2.6	1.7	65.4
	Mukachivskyi	3.3	2.3	69.7
	Uzhhorodskyi	1.6	1.0	62.5
In the lowland zone		9.0	6.1	67.7
In the region		42.0	36.1	86.0

absorption crossbreeding with Holstein, unsystematic use in the reproduction of home-bred bulls (TERPAY, 2013) caused a critical situation in the lowland areas, since here there are 6.1 thousand of cows. The lowest number of cows here is also connected with the high ploughing of the land. Going back to the data and comparing the results in other districts we see close values. A small number of cows were noted in the Perechynskyi, Berehivskyi and Uzhgorodskyi districts, 1.2 – 1.0 thousand each, while in Mizhhirskyi it was almost 6 times more, as many as 6.4 thousand heads. Uzhgorodskyi district has about half the population of neighbouring Mukachivskyi district. Tyachivskyi, the largest in terms of population, keeps 3,400 cows. In terms of saturation, again, the primacy is in the mountainous ecological zone – 95.8%, Rakhivskyi – 98.3% and Mizhhirskyi districts – 96.9%. Diametrically opposite indicators are in the lowland ecological zone – 67.7%, in the Uzhgorodskyi district – 62.5%. The average level of specific weight is characteristic of the foothill zone with a slight fluctuation of the indicator. Its almost exclusive presence is observed in the examined settlements of Rakhiv district: Vyshka – 90, Kniahynia – 39, Velyko-Bereznianskyi, Luhy – 138, Trostianets – 12, Strymba – 129 cows. Unique centers of breeding are villages Bohdan – 281, Yasinia – 547 of the Rakhivskyi district. The same number of cows are in the villages of Ruske Pole – 92, Vilkhivtsi – 96, Lazy – 147, Bedevlia – 133 of the Tyachivskyi district. Similar values are found in the villages of Horinchevo – 103, Berezovo – 155, Dragovo –

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87, Nankovo – 104 cows in Khustskyi district. Strengthens its position in terms of the number of cows in the herds of the villages of Tyshiv – 59, Bilasovytsia – 65 of Volovetskyi district; Klynovets – 40, Velyka Vyznytsia – 49 of Mukachivskyi district; Lysychevo – 88, Lukovo – 91 cows in Irshavskyi district. The average level of specific weight is characteristic of Izok (Mizhhirskyi district), Poroshkovo (Perechynskyi district), Trosnyk (Vynogradivskyi district), Hat (Berehivskyi district), Serednie of Uzhgorodskyi districts with a slight fluctuation of the indicator. The mentioned parameters refer to the villages of Guklyvyi (Volovetskyi district), Haydosh (Uzhhorodskyi district), which indicates the similarity of the data. It is sporadically distributed in Andriivka and Chervone settlements of the Uzhhorod district. This breed is rare in the villages of Palad and Komarivtsi of the mentioned district and Perekhrestia of Vynogradivskyi district. The situation is even worse in the village of Zabrid (Velyko-Bereznianskyi district), where 8 cows are kept with a population of 1,250 people. In village Mala Roztoka of Irshavskyi district with a population of 510 people there are only 4 cows. In village Mala Kopany of Vynogradivskyi district with a population of 1,380 people - 5 cows. Single animals are visually found in Pidvynohradiv (Vynogradivskyi district), Zhniatyno (Mukachivskyi district), V. Dobron (Uzhhorodskyi district), which is a negative signal for the safety of the brown Carpathian breed. It follows from the above that the decrease in the population of the brown Carpathian breed occurs, firstly, due to a general decline in the number of cows, and secondly, due to the introduction of other breeds and the crossing of the brown Carpathian breed with them. A very negative impact on the population is caused by the wrong breeding strategy and methods. The recent introduction of non-native breeds, limited access to natural resources of hayfields and pastures, especially in nature conservation areas, large population migration, and lack of economic measures aimed at the development of the cattle industry as a whole and the protection of the herd of Carpathian brown animals. It is known that in order to preserve the species, it is necessary to have a certain number of breed stock, a genealogical structure, zootechnical and breeding records, that is, sources of information in this regard. It should be emphasized that at the time of the expedition survey, we cannot represent the genealogical structure in the form of lines and families, because in modern times there are no subjects of the breed affairs, bearers of legal rights and obligations of the organizational form regarding the preservation of the relevant gene pool of rare, aboriginal, local and endangered brown Carpathian breed of cattle. Therefore, the next step after the expedition survey is to select animals typical for the breed, their linear evaluation and determine the optimal (from the biological, selection and economic point of view) sizes of the closed gene pool population of limited volume for its preservation, to establish performance control and milk quality control, zootechnical and breeding records. It was found that only a small part of cows of the brown Carpathian breed comes from purebred bulls and a sharp reduction, especially in recent years, means that the population needs protection, preservation and constant control of the number

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of livestock and its use. We believe that the main method of preserving the brown Carpathian breed should be purebred breeding with the use of correct selection, rotation of breeder bulls and line breeding, which will ensure the accumulation and consolidation of the necessary hereditary qualities in the offspring in subsequent generations, a constant increase in the viability of the offspring and the strength of their constitution. Support of consolidation and genetic variability while maintaining the available number of breed stock. For the practical implementation of purebred breeding by the method of artificial insemination, 190,000 doses of sperm from 17 brown Carpathian bulls are available at the regional breeding enterprise. On the basis of the concept of preservation of the local brown Carpathian breed and the development of dairy cattle breeding in Transcarpathia region until 2025, developed by the Brown Carpathian Breed Association (Chairman of the Board Vasyl TERPAI), which is part of the European Brown Breed Federation, we, together with the specialists of the breeding enterprise, have created a scheme for fixing bulls-breeders for 2023-2024 (Table 7).

Table 7. The scheme for fixing bulls-breeders of the brown Carpathian breed for 2023-2024, districts of the Transcarpathian region

Districts	**Bull's name**	**Inv. №**	**№, brand DPK**	**Date of birth**	**LW**	**Line, related group**
Berehivskyi	Bublyk	7886	ЗКЗ-629	21/101990	775	Concentrat
Vynogradivskyi	Romb	8829	ЗКЗ-489	7/6. 1983	743	Ranet
Volovetskyi	Reps	1567	ЗКЗ-610	27/7.1989	740	Eleymuni
Irshavskyi	Tsvirkun	5076	ЗКЗ-509	18/5.1984	890	Elegant
Mizhhirskyi	Reps	1567	ЗКЗ-610	27/7.1989	740	Eleymuni
Mukachivskyi	Romb	8829	ЗКЗ-489	7/6.1983	743	Ranet
Perechynskyi	Reps	1567	ЗКЗ-610	27/7.1989	740	Eleymuni
Rakhivskyi	Buton	7586	ЗКЗ-627	17/2.1990	634	Concentrat
Svaliavskyi	Romb	8829	ЗКЗ-489	7/6.1983	743	Ranet
Tiachivskyi	Buton	7586	ЗКЗ-627	17/2.1990	634	Concentrat
Uzhhorodskyi	Buton	7586	ЗКЗ-627	17/2.1990	634	Concentrat
Khustskyi	Buton	7586	ЗКЗ-627	17/2.1990	634	Concentrat

Note: There is no artificial insemination in the Velyko-Berezhnianskyi district;
LW – live weight

A gene pool sperm bank has been formed, which provides ex situ protection. A number of genetic studies are being carried out with the financial support of FAO, the Scientific Research Institute of Zootechnics in Krakow, Poland, in particular, on identifying cows that produce A_2/A_2 milk.

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Conclusion and recommendation

The results of the study made it possible to clarify the status, outline the specifics of the distribution of cows of the rare, aboriginal, local and endangered brown Carpathian breed and their number in Transcarpathia as well as changes in the breed composition. The multifaceted importance of the breed was clearly shown – it is a natural model for the restoration of a transformed ecosystem and livestock farming, plays and will play a major role in the future in providing food, improving people's nutrition, sustainable development of local communities, preserving biodiversity and mountain ecosystems of the Carpathian region. It will make it possible to adapt one of the branches of animal husbandry to climate change and apply the technologies of "Green Architecture" in the aspect of environmental and nature protection recommended by the European Commission. The urgency of measures for the protection, preservation, reproduction and more qualified use of the genetic resources of the brown Carpathian breed of cattle is to include a special provision in the Law of Ukraine "On breeding matters in livestock breeding" on rare, aboriginal, local and endangered breeds. The need to create a system and conduct constant monitoring of the breed, which include studies of changes in the genetic structure, biological features, dynamic population trends, etc., development of new approaches to financing these activities. On the basis of many years of research, develop practical recommendations and action plans for protection and preservation. Create an electronic database. Ensure popularization of this genetic object in mass media, official websites of the Ministry of Agriculture, regional state administration, scientific institutions, and publication of booklets. It is necessary to continue comprehensive research of animals and their products, to determine the specific optimal sizes of native gene pool micro populations of the brown Carpathian breed, to conduct a linear assessment of animals for the long-term preservation of genetic resources, which must continue to be protected by the legislation of Ukraine.

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Erforschung der genetischen Vielfalt von Schafen anhand mitochondrialer DNS

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Zusammenfassung

Unter den heute vorherrschenden wirtschaftlichen Bedingungen überwiegt die Haltung von Weltrassen. Diese Tiere erbringen hervorragende Leistungen und produzieren effizient und rentabel. Je mehr sich diese Rassen verbreiten, desto mehr werden die einheimischen Rassen aus der Zucht und aus dem öffentlichen Bewusstsein verdrängt. Im letzteren Fall besteht neben dem Rückgang der Anzahl dieser Rassen ein vielleicht noch größeres Problem darin, dass ohne einen geeigneten Paarungsplan auch ihre Allelvielfalt abnimmt und ihr Fortbestand gefährdet ist. Auf mitochondrialer DNS (mtDNS) basierende Untersuchungen spielen eine immer wichtigere Rolle bei der genetischen Erhaltung gefährdeter Haustierrassen. Die Autoren wollen einen Überblick über die Möglichkeiten geben, die sich hieraus ergeben, wobei ein besonderer Schwerpunkt auf Schafen liegt. Nach einer allgemeinen Beschreibung der mtDNS und ihrer Gene und von deren Funktionen wird die Spezifität der mütterlichen Linien dargestellt. Auf diese Weise informieren sie die Züchter über das vielversprechende Potenzial der mtDNS zur Kontrolle der genetischen Vielfalt gefährdeter Nutztierrassen. All dies bewahrt nicht nur einen wesentlichen Teil unserer Kultur und Geschichte, sondern trägt auch zur Nachhaltigkeit bei.

Stichworte: mtDNS, genetische Vielfalt, Gen-Erhaltung

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Einführung

Haustiere haben im Laufe der Mikroevolution im Vergleich zu ihren wilden Vorfahren erhebliche Veränderungen erfahren (Fleisch-, Milch-, Wollproduktion usw.). Unter den heutigen wirtschaftlichen Bedingungen ist die intensive Haltung der sogenannten Weltrassen die vorherrschende Nutzung. Diese Tiere sind zu Höchstleistungen fähig, und man kann hohe Gewinne von ihnen erwarten. Je weiter sie sich verbreiten, desto mehr werden unsere einheimischen Rassen aus der Zucht und dem öffentlichen Bewusstsein verdrängt. Im Gegensatz zu gezüchteten Rassen, die in der Regel hochgradig ingezüchtet und an intensive landwirtschaftliche Techniken angepasst sind, können unsere seltenen einheimischen Nutztiere wertvolle Allele in sich tragen, da sie oft resistenter gegen Krankheiten wie Prionenerkrankungen und Parasitosen und auch besser an Umweltveränderungen (Klimawandel) angepasst sind. Leider nimmt jedoch nicht nur ihre Individuenzahl ab, sondern auch ihr Alleldiversität, und sie werden noch anfälliger, da es keine geeigneten Paarungspläne gibt. Heutzutage spielt die auf mitochondrialer DNS (mtDNS) basierende Forschung eine immer wichtigere Rolle für den genetischen Schutz gefährdeter Haustierrassen. In diesem Artikel konzentrieren sich die Autoren auf das Schaf, um einen Überblick über das Potenzial dieser molekularen Forschung zu geben. Nach einer allgemeinen Beschreibung der mtDNS werden die die bei der Rassenidentifizierung und -erhaltung eine Rolle spielen vorgestellt. Ziel ist es, den Züchtern das Potenzial dieser Methode zur Kontrolle der genetischen Vielfalt unserer bedrohten Nutztierrassen bewusst zu machen. Auf diese Weise können wir nicht nur einen wesentlichen Teil unserer Kultur und Geschichte bewahren, sondern auch einen Beitrag zur Nachhaltigkeit leisten.

Molekulargenetik im Dienste der Schafzucht

Im 20. Jahrhundert hat die rasante Entwicklung von Biotechnologie und Genetik nicht nur in der Medizin, sondern auch in der Agrarwissenschaft zu großen Durchbrüchen geführt. Diese molekularen Methoden bieten eine hervorragende Grundlage für vergleichende Studien von Tiergruppen. Um die Allelhäufigkeiten in Schafpopulationen zu bestimmen und ihre Veränderungen im Laufe der Zeit zu verfolgen, wurden in Ungarn bereits in den 50er Jahren Blutgruppen- und biochemische Polymorphismusstudien durchgeführt (FÉSÜS, 1974), und in den 90er Jahren bezog man auch die einheimischen Rassen ein (FÉSÜS, 1992). Ebenfalls in den 90er Jahren wurden verschiedene Studien zum Proteinpolymorphismus ausgeführt, wobei die PCR-RFLP-Technik (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) zum Einsatz kam (ANTON et al., 1999a; ANTON

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et al., 1999b). Eine länderübergreifende vergleichende genetische Studie einer einheimischen ungarischen Schafrasse (Cigája, Zigaya) wurde unter Verwendung von Mikrosatelliten ausgeführt (KUSZA et al., 2008; KUSZA et al., 2010; GÁSPÁRDY et al. 2013; GÁSPÁRDY et al., 2014). Neben der Untersuchung der genetischen Vielfalt können auch Rückschlüsse auf die Produktion der Tiere in den Labors gezogen werden. Einige DNS-Sequenzen können auch mit Produktionsparametern wie Körpergewichtszunahme (KOMLÓSI et al., 2005), Muskelhypertrophie (FÉSÜS, 2000), bestimmten reproduktionsbiologischen Parametern (FÉSÜS, 1999), Milchproduktion (ÁRNYASI et al., 2009) und Resistenz gegen bestimmte Krankheitserreger (ZSOLNAI et al, 2003) in Zusammenhang gebracht werden. Die oben erwähnten Mikrosatelliten (JOOST et al., 2007) und Einzelnukleotid-Polymorphismen (Single Nucleotide Polymorphism – SNPs) (KIJAS et al., 2009) werden heutzutage hauptsächlich zur Diagnose der Krankheitsanfälligkeit verwendet, eignen sich aber auch hervorragend zur Bestimmung genomischer Zuchtwerte und können auch aus evolutionsbiologischer Sicht nützlich sein. Mitochondriale DNS-Studien können sogar mit großem Erfolg auf Fossilien angewendet werden. Darüber hinaus eignen sich auch Studien über retrovirale Integrationen (CHESSA et al., 2009), das Y-Chromosom (MEADOWS et al., 2004) und mtDNS-Mutationen für genetische Studien.

Struktur des mitochondrialen Genoms und Perspektiven für die Forschung

Eukaryoten verfügen über zwei Arten von Erbmaterial. Der größte Teil besteht aus der Kern-DNS (nukleäre DNS), das sind etwa 3,3 x 10^9 Basenpaare genetischer Information beim Menschen, die in Chromosomen verpackt sind. Ein kleinerer Teil des Erbguts befindet sich außerhalb des Zellkerns (extranukleäre DNS), in den Mitochondrien, den Kraftwerken der Zelle. Das mitochondriale Genom (Mitogenom) hat eine zirkuläre Struktur, ist wie die DNS im Zellkern doppelsträngig und speichert etwa 16500 Basenpaare an Informationen. Sie sind im Laufe der Evolution die späten Nachkommen freilebender Bakterien, die durch Endosymbiose in eukaryontische Zellen eingeschleust wurden (CUMMINS; 1998). Die mitochondriale DNS wird vom Plasma der Eizelle an die Nachkommen weitergegeben, so dass die Vererbung der mitochondrialen DNS ausschließlich klonal, d.h. mütterlicherseits erfolgt.
Eines der vielversprechendsten Forschungsgebiete ist die Untersuchung genetischer Störungen mitochondrialen Ursprungs, da mitochondriale Funktionsstörungen mit vorzeitiger Alterung, bestimmten neurodegenerativen Erkrankungen und Krebs sowie mit einer Reihe von Stoffwechselstörungen in Verbindung gebracht werden (POLYAK et al, 1998). Dies ist unter anderem auf die hohen Mengen an ROS (Reactive Oxygen Species) zurückzuführen, die bei der Energieerzeugung in den

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Mitochondrien entstehen, wodurch die Zahl der Mutationen gesteigert, und der Zellstoffwechsel gestört wird (www.umdf.org).
Eine weitere wichtige Rolle spielt die mitochondriale DNS bei der Erhaltung der genetischen Vielfalt und damit dem genetischen Schutz gefährdeter Tierarten. Das Mitogenom eignet sich ideal für die Kartierung evolutionärer Abstammungslinien zwischen verschiedenen Arten sowie zur Veranschaulichung mikroevolutiver Veränderungen bei Haustierarten und des Ursprungs moderner Rassen (KIM et al., 2020). Heutzutage werden mitochondriale DNS-Tests in großem Umfang eingesetzt, um den mütterlichen Hintergrund von Individuen zu ermitteln, d. h. zu welcher mütterlichen mitochondrialen Abstammungslinie die Nachkommen gehören.

Das mitochondriale Genom und diejenige von seinen Genen, die bei der Erhaltung von Genen eine besondere Rolle spielen

Das Mitogenom zeichnet sich durch eine sehr hohe Mutationsrate aus, die etwa 100-mal so hoch ist wie die des Zellkerngenoms. Dies führt nicht nur zu einer Vielfalt an mitochondrialer DNS innerhalb eines bestimmten sich teilenden Mitochondriums, sondern auch dazu, dass sich im Laufe der Zeit immer mehr unterschiedliche Mitochondrien innerhalb der Zellen bilden. Wenn sich eine tierische Zelle teilt, werden ihre Mitochondrien nach dem Zufallsprinzip auf die Nachkommenzellen übertragen, und sie können die gleichen oder sogar unterschiedliche genetische Informationen tragen. Es ist daher schwierig, beim Austausch genetischer Informationen während der Mitose und Meiose den genauen Überblick zu behalten, ganz zu schweigen von der Tatsache, dass immer wieder Fehler und spontane Mutationen auftreten können, was die Arbeit am Mitogenom zusätzlich erschwert. Doch gerade diese hohe Mutationsrate bildet die Grundlage für phylogenetische Untersuchungen und molekularbiologische Datierungen (GÁSPÁRDY, 2021).
Das Mitogenom enthält hoch konservierte Sequenzen und einen geringen Prozentsatz nicht kodierende Region (3 %) im Vergleich zur Zellkern-DNS (93 %). Diese nicht kodierende Region, die eine prominente Kontrollregion (CR oder Displacement loop – D-Loop) in der Abstammungsforschung darstellt, ist jedoch hypervariabel und wird von stark konservierten Sequenzen flankiert. Die Kontrollregion ist eine spezielle Sequenz, die in hohem Maße an der Initiierung von Transkription und Translation beteiligt ist (CUMMINS, 1998). Bei Schafen wurden ihre Sequenz und Länge (1189 bp) von ZARDOYA et al. (1995) bestimmt.
Regionen mit hoher Variabilität (Mutations-Hotspots) sind durch hohe Mutationsraten gekennzeichnet und bieten eine hervorragende Grundlage für die Rückverfolgung der Geschichte einer Population über einen kurzen Zeitraum.
Die andere kodierende Region, das als phylogenetischer Marker verwendet werden kann, ist Cytochrom b Gen (Cyt b), welches ebenfalls variabel ist und sich ideal für die Kartierung der Verwandtschaft zwischen verschiedenen Schafrassen eignet.

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Anhand dieser beiden mitogenomischen Regionen können bei Schafen fünf Haplotyp-Gruppen unterschieden werden (MEADOWS et al., 2011).

Haplotypen und Haplogruppen von Schafen

Das Schaf (*Ovies aries*) wurde zwischen 9000 und 7000 v. Chr. im fruchtbaren Halbmond domestiziert, was es zu einer der ältesten mit uns lebenden Tierarten macht und ihm eine herausragende Rolle im Leben früherer prähistorischer und historischer Gesellschaften verlieh. Für die phylogenetische Untersuchung von Schafen, die Verbesserung von Schafsrassen und die Erhaltung seltener einheimischer Varianten ist die Kenntnis der mtDNS-Diversität ein wichtiger Ausgangspunkt. Von den beiden oben erwähnten Allelen wird Cyt b für den Vergleich von Hausschafen mit wilden Vorfahren empfohlen, während es für die Trennung von Haplogruppen unter Hausschafen relevanter ist, zusätzlich zu Cyt b die hypervariablen Sequenzen der Kontrollregion zu berücksichtigen, da PEDROSA et al. (2005) herausgefunden haben, dass die Zeit bis zur Segregation von Haplogruppen bei Schafen viel früher anzusetzen ist, wenn hypervariable CR berücksichtigt werden.
Die Varianten der Mitogenom-Kontrollregion und des Cytochrom-B-Gens können in Haplotypen unterteilt werden. Unter Haplotypen versteht man Allele, die von einem Elternteil an die Nachkommen vererbt werden. Solche Genvarianten befinden sich in verschiedenen Chromosomenregionen, sind eng miteinander verbunden und werden in der Regel gemeinsam vererbt. Wir untersuchen Mutationen (SNPs), die in ausgewählten Sequenzen dieser Allele auftreten, welche mit größerer Wahrscheinlichkeit in einer charakteristischen Region einer bestimmten Gensequenz vorkommen. Solche Mutationscluster werden zur Klassifizierung von Haplotypen in Haplogruppen (HG) verwendet, die auf eine einzige Abstammungslinie hindeuten, da sie auf Mutationen in einem gemeinsamen Vorläufer-SNP zurückzuführen sind.
Verschiedene Schafrassen können sich auch in ihrer genetischen Zusammensetzung auf der Grundlage von mt-DNS-Haplotypen unterscheiden. Die Anwesenheit mehrerer Mutationen deutet auf das Vorhandensein verschiedener Gründervorfahren in den jeweiligen Schafpopulationen hin. Wenn wir die Geschichte der Rasse und insbesondere der Schafpopulation in dem Gebiet kennen, können wir die Genetik nutzen, um die genetische Vielfalt und die Rolle anderer Schafrassen und Unterarten bei der Entwicklung der Rassen zu untersuchen.
Bei Schafen gibt es 5 Haplogruppen: A (HGA), B (HGB), C (HGC), D (HGD), E (HGE). Nach GUO et al. und MEADOWS et al. (2005) dominiert bei asiatischen Schafen eher die Haplogruppe A, während in Europa die Haplogruppe B vorherrscht. Dies wird auch durch die mütterliche Herkunft der Schwäbischen Cikta Schafe bestätigt, die mehrheitlich der Haplogruppe B angehören (KOVÁCS et al., 2020a).

DOI: https://doi.org/10.59913/dagr.2024.16486

Die Haplogruppe C hat jedoch eine sehr weite geografische Verbreitung (CHEN et al., 2006). Interessanterweise gehört nur eine kaukasische Rasse zur Haplogruppe D (TAPIO et al.; 2006). MEADOWS et al. (2011) fanden den größten phylogenetischen Abstand zwischen den Haplogruppen B und C, während die Abstände zwischen den Haplogruppen A und B sowie zwischen den Haplogruppen C und E geringer waren.

Bei der Analyse von Fossilien aus Anatolien fanden DEMIRCI et al. (2013) zeitabhängige Variationen in Bezug auf die Häufigkeit und den Anteil der Haplotypen. Diese Proben zeigten das Vorhandensein der Haplogruppe E (3 %) in der Bronzezeit und der Haplogruppe C (6 %) in der hellenistischen Zeit, während die Haplogruppen A und B durchgängig vorhanden waren (jeweils fast 50 %). DYMOVA et al. (2017) führten eine mtDNS-CR-Analyse an Jahrtausende alten Knochenresten von Schafen aus dem Altai durch. Sie konnten alle zuvor definierten Haplogruppen (Linien A, B, C, D und E) identifizieren. Diese hohe Diversität deutet darauf hin, dass das Altai-Gebiet in der Vergangenheit ein Migrationsgebiet für viele ethnische Gruppen und ihre Tiere darstellte.

Eine Studie von HORSBURGH und RHINES (2010), in der Überreste von Schafen aus einer neolithischen Höhle in Südafrika ausgewertet wurden, ergab, dass sie zur Haplogruppe B gehören.

Die Ursprünge vieler Rassen sind unklar und widersprüchliche Informationen kommen aufgrund der wissenschaftlichen Dokumentation häufig vor. KUSZA et al. (2015) verglichen das phänotypisch sehr ähnliche Gyimeser Racka, das in Ungarn wiedereingeführt wurde, mit dem rumänischen Turcana-Schaf. Ihre Studie zeigte eine sehr geringe genetische Segregation zwischen den beiden Rassen, so dass es sich tatsächlich um eine in zwei verschiedenen Ländern gezüchtete Rasse handelt.

Praktische Anwendungen der mitochondrialen DNS – Die mütterlichen Linien

Die Kartierung der genetischen Vielfalt und die Umsetzung dieses Wissens in die Praxis ist ein wichtiges züchterisches Anliegen. Insbesondere bei seltenen Rassen geht es darum, die allelische Variation der Rasse oder des Bestandes kennen zu lernen. Es ist eine kardinale Zukunftsaufgabe, Tiere mit seltenen Allelvarianten zu schützen und zu züchten, auch wenn das Aussehen und die Produktionsmerkmale des einzelnen Tieres dies auf den ersten Blick nicht zu rechtfertigen scheinen. Diese weiblichen Vertreter der mütterlichen Linien der einheimischen Rassen sind ein zuverlässiger Indikator für die genetische Vielfalt. Leider nimmt jedoch die Zahl der Gründerfamilien von Generation zu Generation ab, so dass man sich bemühen muss, aus jeder Familie ein repräsentatives Individuum zu erhalten. Um die Gene so genau wie möglich zu erhalten, ist es unerlässlich, Stammbaumdaten korrekt zu verarbeiten, um die weiblichen Gründer zu identifizieren und die Ahnenfamilien zu ermitteln, die von ihnen abgeleitet werden können (Founder-Sampling-Methode).

DOI: https://doi.org/10.59913/dagr.2024.16486

Um ein vollständiges Bild der genetischen Vielfalt zu erhalten, müssen für die DNS-Probenahme Nachkommen ausgewählt werden, die noch lebende Vertreter der ältesten Familien sind (ANNUS et al., 2015a; POSTA et al., 2019). Je mehr Generationen die Familie gelebt hat, desto besser, aber im Allgemeinen sollten mindestens 7-9 Generationen repräsentativer Individuen aus den Familien für die statistische Auswertung der Kontrollregion und von Cyt b ausgewählt werden. Die Auswertung umfasst die Anzahl der Mutationen, die Anzahl der polymorphen Stellen, die Anzahl der Haplotypen und die Anzahl der Nukleotiddivergenzen. Anhand der Analyse der Kontrollregion der ungarischen Zigaya-Schafe haben ANNUS et al. (2015b) gezeigt, dass sie über eine gemeinsame Abstammungslinie mit europäischen Rassen verfügen und hauptsächlich der Haplogruppe B angehören, während nur 6 % der Haplogruppe A vertreten sind. KOVÁCS et al. (2020b) analysierten das Cyt b-Gen der mtDNS von Cikta Schafen und wiesen eine genetische Verengung in Bezug auf die Nukleotid- und Haplotypenvielfalt (Bottleneck effect, Engpasseffekt) nach, aber die durchschnittliche Anzahl der Nukleotidpaarunterschiede war ziemlich hoch, was auf unterschiedliche genetische Merkmale der bestehenden Familien hindeutet.

Die erzielten Ergebnisse eignen sich für weitere Studien, da sie in das Netzwerk anderer Rassen (GenBank-Sequenz) integriert werden können, wie dies von TULLY et al. (2023) für eine Schafspopulation mit einer kleinen Anzahl von Überlebenden in den südlichen Regionen Ungarns getan wurde.

Schlussfolgerungen

Wir können unsere seltenen und gefährdeten Haustierarten als nationale Schätze betrachten, zusätzlich können sie Naturlandschaften im Sinne der Nachhaltigkeit bewahren und bewirtschaften. Sie haben historische und kulturelle Bedeutung, da mit ihnen uraltes Wissen verbunden ist und besondere Produkte der Nachwelt erhalten bleiben, wenn wir sie vor dem Aussterben bewahren. Diese lebenden Reliquien können in sich wertvolle und seltene Gene tragen, die einerseits die Widerstandskraft der nächsten Generation erhöhen können, andererseits ändern sich die Marktbedürfnisse ständig, und man weiß nie, wann in Zukunft ein Bedarf an Rassen, die sich leicht in die extensive Umgebung integrieren lassen, besteht. Wenn wir neben der entsprechenden Individuenzahl auch ihre genetische Vielfalt erhalten, kann damit auch der genetische Schwund anderer Rassen ausgeglichen werden. In der heutigen Welt können somit moderne wissenschaftliche Ergebnisse auch unter anderem in den Dienst des Genschutzes gestellt werden. Dabei gewinnt die Forschung auf Basis der mitochondrialen DNS immer mehr an Bedeutung. Das Mitogenom wird mütterlicherseits vererbt, daher finden mtDNS-Mutationen auch entlang der mütterlichen Abstammungslinie statt. In der Viehzucht gibt es mehr Weibchen und sie bleiben in der Zucht, was Weibchen zu idealen Objekten für die

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Genforschung macht. Anhand einer vorläufigen Stammbaumanalyse lässt sich die Zahl der Gründerfamilien ermitteln, die von Generation zu Generation abnimmt. Während der Zucht ist es immer notwendig, zu versuchen, einen lebenden Vertreter jeder Familie für weitere Forschungen zu behalten. Unter den mitochondrialen DNS-Genen spielen die Kontrollregion und Cytochrom b eine herausragende Rolle bei der Untersuchung des genetischen Hintergrunds. Um die genetischen Unterschiede zwischen Wild- und Hausschafen zu verstehen, lohnt es sich, das weniger mutagene Cyt b zu untersuchen. Für die Untersuchung von Haplogruppenbeziehungen zwischen Hausschafen jedoch ist die Verarbeitung des gemeinsamen Datensatzes beider Gene die empfohlene Methode. Die hypervariablen Sequenzen der Kontrollregion scheinen am zuverlässigsten zu sein, um die Haplotypen möglichst genau voneinander zu unterscheiden. Mithilfe dieser lässt sich eine bestimmte Tierrasse genetisch gut beschreiben und lassen sich die Individuen ihrer Population mit dem genetischen Material anderer Rassen vergleichen (GenBank-Sequenz). All dies dient dazu, die Verwandtschaftsverhältnisse der verschiedenen Rassen zu beleuchten und ihre Herkunft zu klären. Darüber hinaus können auch territoriale Veränderungen von Schafrassen durchgehend verfolgt werden (Ausbreitung vom Zentrum der Domestizierung, Bewegungen während der Kolonisierungsaktivitäten). Abschließend sei darauf hingewiesen, dass die heutigen molekularen Methoden auch die Generhaltung in hohem Maße unterstützen, die nicht nur quantitativ (ausreichende Individuenzahl), sondern auch qualitativ (genetische Vielfalt) realisiert werden muss.

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Exploring the genetic diversity of sheep using mitochondrial DNA

Abstract

Under today's prevailing economic conditions, the keeping of world breeds predominates. These animals are capable of excellent performance and produce efficiently and profitably. The more these breeds spread, the more native breeds are pushed out of breeding and public awareness. In the latter case, in addition to the decline in the number of those, perhaps an even greater problem is that without appropriate mating plan, their allele diversity will also decline, and they will fall into risk. The mitochondrial DNA (mtDNA)-based investigation is playing an increasingly important role in the genetic conservation of endangered domestic breeds. The authors aim to give an overview of the possibilities offered by this, with a special focus on sheep. After a general description of the mtDNA and it genes and their functions the specificity of the maternal lineages is presented. In this way, they inform the practical breeders to the promising potential of mtDNA for controlling the genetic diversity of endangered livestock breeds. All this not only preserves an essential part of our culture and history, but also contributes to sustainability.

Key words: mtDNA, genetic diversity, gene conservation

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Cryopreserved Sperm Quality in Tsigai Sheep: Implications for Biodiversity Protection

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Abstract

The objective of our research was to conduct a comparative analysis of cryopreserved sperm quality parameters of Tsigai (TS) sheep breed. Ejaculates (n=12) from TS sheep rams (n = 2) were collected using electro-ejaculation. Samples were prepared from suitable ejaculates, ensuring at least 70% progressive motility. These samples were then equilibrated in Triladyl® diluent and subjected to automated freezing. Before freezing and after thawing, the sperm samples underwent assessments including motility (CASA), viability and apoptosis (DRAQ7/Yo-Pro-1), mitochondrial activity (MitoTracker), capacitation status (FLUO4), and acrosomal status (PNA). The results revealed significant differences ($P < 0.05$) in total motility between fresh (91.25±0.80 %) and frozen/thawed (F/T) (41.14±12.04 %) samples, as well as progressive motility between fresh (89.83±1.85 %) and frozen/thawed (33.57±8.25 %) groups. Moreover, significant differences ($P<0.05$) were also observed in the proportion of apoptotic spermatozoa (2.26±0.60 %), dead (7.40±1.72 %), and mitochondrial active spermatozoa (84.52±5.60 %) in fresh samples compared to F/T samples (12.34±0.80 %, 46.59±2.82 %, 47.71±4.04 %). Our findings indicate that the selected cryopreservation protocol is relatively sufficient for the cryoconservation of sperm from Tsigai sheep breeds, since up to 50% of F/T sperm were motile and live. This has significant implications for biodiversity protection and simplifies the establishment of an animal genetic resources gene bank.

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Keywords: Ram, spermatozoa, CASA, flow cytometry

Introduction

Protecting biodiversity is a critical challenge facing us today. Local animal breeds, representing a crucial component of cultural heritage, play a vital role in the biodiversity of individual countries and regions. Cryopreservation of sperm emerges as a powerful tool for conserving animal genetic resources through the establishment of gene banks housing long-term stored genetic material. However, the cryopreservation of ram sperm encounters various obstacles, including the potential impact of individuality and breed characteristics on susceptibility to damage induced by low temperatures. In our research, we focus on investigating these differences among individuals raised in Central Europe. Sperm cryopreservation is a crucial biotechnological tool for enhancing breeding programs in various species, including small ruminants. Recent efforts have been dedicated to optimizing the cryopreservation process for ram sperm to enhance reproductive performance (SILVA et al., 2011). While frozen-thawed ram sperm is valuable for genetic distribution, its application in artificial insemination often results in low pregnancy rates. Nevertheless, cryopreservation plays a vital role in the conservation of animal genetic resources and biodiversity (JIMÉNEZ-RABADÁN et al., 2016), enabling the long-term storage of biological materials for future use or research. Transporting genetic resources in a frozen state offers ethical and logistical advantages over live animal transport (RASPA et al., 2017).

Sheep farming plays a vital role in Slovak agriculture, with approximately 230,000 ewes in total, of which 105,000 are used for milking purposes. While the breeding programs for dairy sheep in Slovakia and other countries have historically focused on milk yield (ORAVCOVÁ et al., 2005; SMULDERS et al., 2007), there is a growing emphasis on functional traits in recent years. This shift is driven by increased production costs compared to milk prices, consumer demand for safe and high-quality food, and societal concerns for animal welfare (BARILLET, 2007). In addition to milk production, lamb production contributes significantly to the income of dairy sheep farmers, accounting for approximately 25 to 35% in Slovakia and 30 to 60% in Mediterranean countries (FAO, 2008). Therefore, enhancing ewe reproductive performance, lamb survival, and lamb growth is crucial for modern sheep breeding programs (LEGARRA et al., 2007).

The Tsigai (TS) sheep breed is a domesticated breed primarily found in Central and Eastern Europe, particularly in countries such as Hungary, Romania, Serbia, Slovakia, and Ukraine. The Tsigai breed is among the oldest native breeds, traditionally raised in regions situated between 500 to 800 meters above sea level, predominantly in semi-extensive mountain farming systems. breed is known for its multi-purpose characteristics. According to Performance Testing Slovakia (PS SR,

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2009), there are approximately 19,500 TS ewes, with 8,400 ewes from 25 breeding flocks, undergoing performance testing.
Predictions for climate change suggest a range of potential impacts, such as increasing global temperatures, changes in precipitation patterns, more frequent extreme weather events, and alterations in ecosystems and biodiversity. These changes are anticipated to profoundly affect human societies, economies, and the environment. Given these challenges, it is crucial to safeguard genetic material as a form of "insurance" in case the breed faces endangerment. Ultimately, the primary objective of this study was to evaluate and cryopreserve TS ram sperm for inclusion in an animal gene bank.

Material and methods

Clinically healthy, sexually mature males (n=2) and 6 years old of TS were used in this experiment. The sperm collection process was conducted using electro-ejaculation equipment (Electro-ejaculator, Minitube, Tiefenbach, Germany). Initially, a voltage of 0.5 V was applied, which was gradually increased in each series of pulses, separated by 2-second breaks, until reaching a maximum value of 7 V. Once the voltage reached 7 V, the pulses were maintained at this level until ejaculation was complete. To alleviate stress, rams were administered xylazine at a dosage of 0.2 mg/kg (Xylariem 2% average unit volume (a.u.v.), Riemser Arzneimittel GmbH, Greifswald, Germany) if deemed necessary. Following collection, the sperm samples were transported to the laboratory in a collection vessel submerged in a water bath maintained at 30 °C. Upon arrival at the laboratory, semen was promptly evaluated for volume, concentration, motility (initial assessment). Heterospermic samples were prepared by blending suitable ejaculates, ensuring at least 70 % progressive motility (PM).

Diluent Preparation and Freezing Process
Dilution of Triladyl® was performed on the day of collection. Raw Triladyl® was mixed with deionized water in a 1:3 ratio, followed by the addition of egg yolk to achieve a final concentration of 10 % v/v. The mixture was filtered, heterospermic sample was diluted with the prepared extender at a ratio of 1:10 (semen:extender). Dilution was carried out slowly with gentle rotations at room temperature (RT). The diluted semen was then transferred to 250 µL straws, sealed, and equilibrated in a refrigerator at 4 °C for 6 hours.
After equilibration, straws were placed in an automated freezing box (IceCube, Minitube) pre-cooled to +4 °C. The freezing program initiated automatically upon closing the lid, subjecting the samples to a temperature profile of −10 °C (120 s), −80 °C (450 s), −120 °C (100 s), and −140 °C (180 s) plunged into liquid nitrogen

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(−196 °C). After one week, samples were thawed in a water bath at 42 °C for 15 seconds and then transferred for sperm analysis.

Sperm motility

The motility and sperm movement were analysed by CASA (SpermVisionTM software, Minitube, Tiefenbach, Germany) with light microscope (at the 200× magnification; AxioScope A1, Carl Zeiss Slovakia, Bratislava, Slovakia) and Makler counting chamber (Microptic, Barcelona, Spain). Samples were diluted by saline (0.9% NaCl; Braun, Nuaille, Germany) at ratio 1:20 (v/v). A drop of diluted semen (10 µL) was transferred to a counting chamber and analysed with manufacturer's pre-set parameters for rams. We mainly focused on total (TM) and progressive motility (PM).

Flow cytometry

Viability and apoptosis

For the detection of apoptotic-like changes in ram spermatozoa, YO-PRO-1 nuclear green dye (Thermo Fisher Scientific, Waltham, MA, USA) was utilized. Semen samples containing 1 × 106 spermatozoa were diluted in 500 µL of phosphate-buffered saline (PBS) and then incubated with 0.5 µL of YO-PRO-1, achieving a final concentration of 100 nM. This incubation was carried out for 15 minutes in the dark at room temperature (RT). Subsequently, the samples were washed in PBS through centrifugation at 600× g for 5 minutes at 20 °C. Afterward, the samples were stained with ready-to-use DRAQ7 dye as previously described and analysed using a flow cytometer. The proportion of spermatozoa positive for YO-PRO-1, indicated by YO-PRO-1+/DRAQ7− and YO-PRO-1+/DRAQ7+, was considered as the proportion of apoptotic-like spermatozoa.

Acrosome status

To assess the integrity of the acrosome, PNA (peanut agglutinin) fluorescent probes was used.

One µL of PNA working solution (Alexa Fluor 488 conjugate; Thermo Fisher Scientific, Waltham, MA, USA) was incubated with 1 × 106 spermatozoa diluted in 200 µL of PBS for 15 minutes in the dark at RT. The PNA working solution was prepared at a concentration of 0.5 mg/mL by dissolving the protein (1 mg/mL) in 2 mL of deionized water. After the incubation period, the samples were washed by centrifugation at 600× g for 5 minutes at 20 °C, stained with ready-to-use DRAQ7 dye as previously described, and then analysed by flow cytometer. The proportion (%) of spermatozoa positive for PNA, indicated by PNA+/DRAQ7− and PNA+/DRAQ7+, was considered as the proportion of acrosome-damaged spermatozoa.

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Sperm Capacitation Status
To assess the capacitation status of ram spermatozoa, FLUO-4 AM, a specific Ca2+ green fluorescent probe (FLUO-4; Thermo Fisher Scientific, Waltham, MA, USA), was utilized. FLUO-4 dye (at a final concentration of 100 nM) was incubated with 1 × 106 spermatozoa diluted in 500 µL of PBS for 20 minutes in the dark at 37 °C. After the incubation period, the samples were washed by centrifugation at 600× g for 5 minutes at 20 °C, stained with ready-to-use DRAQ7 dye as described previously, and then analysed by flow cytometer. The proportion (%) of spermatozoa positive for FLUO-4, indicated by FLUO-4+/DRAQ7− and FLUO-4+/DRAQ7+, was considered as the proportion of capacitated spermatozoa.

Mitochondrial Activity
The activity of mitochondria was assessed by MitoTracker® Green FM fluorescent dye (MT Green; Thermo Fisher Scientific, Waltham, MA, USA). In summary, 1 × 106 spermatozoa diluted in 500 µL of PBS were incubated with MT Green dye at a final concentration of 300 nM in the dark at 37 °C for 10 minutes. Following incubation, the samples were washed by centrifugation at 600× g for 5 minutes at 20 °C, stained with ready-to-use DRAQ7 dye as described earlier, and then analysed by flow cytometer. The proportion (%) of spermatozoa positive for MT Green, indicated by MT Green+/DRAQ7−, was considered as the proportion of spermatozoa with high mitochondrial membrane potential (MMP).
Stained sample aliquots were promptly analysed using flow cytometry employing a FACSCalibur instrument (BD Biosciences, San Jose, CA, USA). This instrument was equipped with a 488 nm argon ion laser and a red diode (635 nm) laser. The acquired fluorescent signals were processed by Cell Quest ProTM software (BD Biosciences, San Jose, CA, USA), utilizing a 530/30 nm bandpass filter for the green FL1 channel and a 670 nm long-pass filter for the red FL3 channel. Each sample underwent analysis for a minimum of 10,000 events (spermatozoa) activity.

Statistical Analysis
Experiments with cryopreservation of ram spermatozoa were performed four times. For data normality distribution, the Shapiro–Wilk test was used. Subsequently, acquired data were evaluated by one-way ANOVA (Tukey method) using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, CA, USA). The data are represented as means ± SD. Differences at $P < 0.05$ were considered statistically significant.

Results and discussion

Preserving the genetic diversity of farm animal species is a crucial endeavour to safeguard domestic biodiversity and ensure their adaptation to evolving

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environmental conditions, unforeseen breeding mishaps, or disease outbreaks (JOOST and BRUFORD, 2015). Many indigenous or endangered breeds with limited population sizes may preserve their genetic material through cryopreserved reproductive cells (RAKHA et al., 2016; SVORADOVÁ et al., 2018). However, cryopreservation of ram sperm faces challenges compared to other livestock species like bulls, rabbits, and horses, owing to its heightened susceptibility to freezing and thawing procedures and the sperm's low cryotolerance (GÁSPÁRDY et al., 2020). Moreover, variations in frozen sperm quality among small ruminants suggest breed-specific disparities in sperm vulnerability to cryopreservation methods (BARBAS and MASCARENHAS, 2009). Notably, native and purebred breeds are often deemed more robust and healthier than crossbreeds (KUMARESAN et al., 2021). Therefore, it's imperative to explore potential variances in the properties and quality of cryopreserved sperm across different breeds. Evaluating sperm characteristics is crucial for assessing the fertilization potential of male livestock. In our study, we noted a decrease in total motility after freezing and thawing. Similarly, progressive motility also showed a decline compared to the results obtained with fresh sperm (Table 1).

Table 1. Motility parameters of fresh and frozen/thawed Tsigai ram sperm

Parameter	Fresh	Frozen/thawed
Total Motility (%)	91.25±0.80	41.14±12.04*
Progressive motility (%)	89.83±1.85	33.57±8.25*

*Significant differences compared to the control at P < 0.05

GALARAZA et al. (2019), in their study utilizing three different protocols for automated freezing of Merino ram sperm, reported a decrease in total motility from 87.8 % in fresh to 61.4 % in thawed sperm and in progressive motility from 34.8 % in fresh to 27.2 % in thawed sperm. Although the decrease in progressive motility was slightly lower, it may be attributed to the initially lower value of progressive motility in fresh semen. In another investigation (PERIS-FRAU et al., 2019), Manchega ram semen was diluted with Biladyl® extender (Minitube) and frozen using a programmable biofreezer (Planer Kryo 10 Series III, Planer PLC, Sunbury-on-Thames, UK), resulting in a 49.1% relative decrease in total motility and 41.8 % in progressive motility. This indicates a similar obtained results compared to our findings, which may mean different breeds of rams used.
Sperm viability assessment utilized a range of fluorescent probes. Yo-Pro-1 was utilized to identify apoptotic-like ram sperm in conjunction with the DRAQ7 marker for dead sperm. FLUO4, a marker detecting sperm capacitation, was also evaluated. Additionally, PNA and MitoTracker were employed to assess acrosomal status and mitochondrial activity, respectively. To ensure high fertility levels, it's crucial to have a low percentage of damaged sperm, as only non-damaged ones can effectively

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fertilize an oocyte. All parameters evaluated through flow cytometry revealed higher significant $P < 0.05$ proportions of apoptotic and dead sperm and significant lower mitochondrial activity of sperm in frozen/thawed group compared to fresh (Table 2). However, no significant difference was found in the proportion of sperm with damaged acrosome in compared groups.

Table 2. Flow cytometric parameters of fresh and frozen/thawed Tsigai ram sperm

Parameter	Fresh	Frozen/thawed
Apoptotic spermatozoa (%)	2.26±0.60	12.34±0.80*
Dead spermatozoa (%)	7.40±1.72	46.59±2.82*
Capacitated spermatozoa (%)	1.42±0.60	3.23±0.06
Acrosome damaged spermatozoa (%)	3.52±1.30	4.55±2.95
Mitochondrial membrane potential (%)	84.52±5.60	47.71±4.04*

*Significant differences compared to the control at $P < 0.05$

Conclusion and recommendation

Our findings in the recent study indicate that the cryopreservation protocol of VOZAF et al. (2021) is also effective for preserving sperm from Tsigai ram breed. Parameters such as motility, viability, capacitation, acrosome status and mitochondrial activity significantly differ between fresh and frozen/thawed group. However, this decline is not dramatic, and the samples can be stored in a gene bank and used for artificial insemination when needed. These results have implications for safeguarding livestock biodiversity by potentially reintroducing native sheep breeds and storing male gametes as long-term animal genetic resources.

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In memoriam Professor Emeritus Dr. Imre Bodó (1932-2023)

Imre Bodó was born on October 4, 1932 in Budapest. His father, Imre Bodó Sr., was a military officer and teacher at the Riding Instructor Training Institute in Örkénytábor.
After his primary schooling in Budapest and Örkénytábor, he attended the Hunyadi Mátyás Military Secondary School in Kőszeg ("Cögerei") and the Piarist Gymnasium in Budapest (Gymnasium Scholarum Piarum); in the latter, he passed his matriculation examination with excellent results in 1951. During World War II, as a student, he was taken prisoner by the Americans in Germany, but – although severely frostbitten – he managed to get home safely.
He graduated with honors from the Gödöllő University of Agricultural Sciences in 1956. His excellent academic performance saved him from expulsion, because they wanted to expel him from the university because of his origin. During the revolution, he worked as an intern, away from the fighting, at the university's training farm Herceghalom. Despite this, during the 1957 reprisal, he was imprisoned for questioning on trumped-up charges, which were not without violence - from where he was released for lack of evidence and the proceedings were terminated, but he lost his job, was banned from the capital, and had no opportunity to find a job in the field of research or education.

Thus, he got a job in agricultural enterprises. From 1961, he worked at the Hortobágy State Farm, where he was initially assigned the position of livestock breeder responsible for cattle breeding. Eventually, he held the position of chief livestock breeder. On an area of 70 thousand hectares, he managed 4,000 cattle, 7,500 sheep, 800 horses (including the Nonius stud), 600 sows and their offspring, and the production of several million poultry (chickens, ducks, guinea fowl, geese). The Hungarian Socialist Workers' Party ordered in the late 1950s that the breeding of Hungarian Grey Cattle should be suspended; in this spirit, all bull calves were ordered to be sent to the slaughterhouse, and the cows were ordered to be crossed with the Soviet Kostroma breed. This would have meant the end of purebred breeding of Hungarian Grey Cattle. Imre Bodó did not carry out this order at the time, although sabotage was still considered a capital offense in the sixties. With the help of the goulash, among whom he quickly gained great respect due to his professional competence and humane attitude, he managed to hide eight bull calves, that is, save them for breeding. The anecdote is literally true that the illegally

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introduced bull named Morgó was still in breeding when the leader who ordered the Kostroma crossbreeding was no longer in office. When new winds finally began to blow in the party in the second half of the 1960s, they would have restarted the breeding of the Hungarian Grey breed in pure blood. At that time, at a meeting organized at the National Center of State Farms in Budapest, it turned out that not a single farm breeding Greys had bulls left. It was a dramatic scene when Imre Bodó stood up in the back row and announced that there were indeed eight more bulls in Hortobágy.
He managed to win the decision to keep a herd of 200 cows purebred. The Hungarian Grey herd, now considered a Hungarikum and numbering 10,000 animals, is the offspring of these eight bulls!

Imre Bodó changed jobs and positions in 1971 in order to make it easier for his seven children to attend school.
He first worked in the horse breeding department at the Animal Breeding Research Institute Herceghalom, then became involved in the work of starting beef cattle breeding in Hungary, and from 1978 he became a consultant to the joint venture of cattle breeding cooperatives called TAURINA.
At the invitation of Academician Artúr Horn, he joined the Department of Animal Husbandry at the University of Veterinary Medicine in 1975, where he was appointed a university professor in 1984. He was the head of the department until his retirement, and with his participation the teaching of animal husbandry in English was also launched. His university lectures were uniquely attractive not only because of his subject knowledge, but also because of his lived professional practice and experience, and his friendly and direct relationship with his students was authentic.
From 1998, he transferred his knowledge and experience to the Department of Animal Husbandry and Animal Nutrition of the University of Agricultural Sciences Debrecen, and his teaching field also covered general animal husbandry, cattle, horse and dog breeding, and gene conservation. He was the head of the doctoral school for ten years.
He obtained his university doctorate in 1967 with a dissertation on the issues of Hungarian Grey Cattle breeding. He received the title of candidate for the defense of his thesis on population genetic issues of horse breeding in 1977, and he defended his academic doctoral thesis in 1991 with the title Preservation of gene reserves in animal breeding. He was inaugurated as an honorary doctor at the University of Agricultural Sciences Debrecen in 1995.
His scientific interests, in addition to the breeding issues of various domestic animal species, were primarily the description and protection of genetic diversity. He was a lecturer and debater in English, French and German.

He has published a large number of publications in various languages (8 books, 10 book chapters, 40 journal articles, over 100 communications). His important works

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published in the authorship community are Genetic Conservation of Domestic Livestock (1992), Conservation Genetics of endangered horse breeds (2005), and Der Lipizzaner im Spiegel der Wissenschaft (2011). His international professional recognition is reflected in the fact that he was invited, among other things, to organize the discussion session on genetic resources and genetic reserves at the Fourth World Congress of Applied Genetics (Edinburgh, Scotland, 1990) and to give a lecture there. In 1995, he gave the introductory lecture at the international conference on a similar topic in Tsukuba (Japan). He was invited to co-author the report on Europe at the Fifth World Congress of Applied Genetics (Guelph, Canada). He was the initiator of a successful INCO-COPERNICUS project (in collaboration with Austrian, Slovenian and Swiss universities), in which the genetic diversity within the Lipizzaner breed was explored using animal breeding and molecular genetic parameters.
He maintained a constant living connection with practice. Imre Bodó is an indelible and most notable merit in saving our unique national value, the Hungarian Grey Cattle breed. As soon as the organization of domestic livestock breeding associations began in 1991, he became the founder and first president of the Hungarian Grey Cattle Breeders Association, and from 2012 its perpetual president. He was also the first president (honorary president from 2010) of the Furioso-North Star Horse Breeding Association.
He was a member of the board of the Hungarian Livestock Breeders Association (MÁSZ), and between 2008 and 2017, he was the president, then honorary president, of the MÁSZ Animal Breeding History Committee.
In addition to his domestic activities, he also actively participated in the work of various international organizations. From 1982 until its dissolution in 1992, he was a member of the FAO Expert Panel for Conservation of Animal Genetic Resources, which dealt with the global conservation of endangered domestic animal breeds. From 1985 to 2000, he was a member of the working group operating within the framework of the European Federation of Animal Science (EAAP), which aimed to register and preserve the genetic diversity existing on the European continent.
From 1986 to 1992 he was vice-president of the Horse Breeding Department of the European Livestock Breeders' Association. From 1985 to 1990 he headed the breeding committee of the International Lipizzaner Breeders' Association (LIF), and was elected its president for the period from 1990 to 1994.
From 1991 he was a board member of the Rare Breeds International NGO, founded in Budapest and active throughout the world.
In December 1997, he was elected Deputy Director and member of the Executive Committee of SAVE (Safeguard of Agricultural Varieties in Europe). This organization is dedicated to the discovery and preservation of rare and endangered domestic animal breeds, as well as plant and horticultural values in Europe.
He was the initiator of the establishment of the DAGENE (International Association for the Conservation of Animal Breeds in the Danube Region), an association that

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organizes cooperation between neighboring countries; since its foundation in 1989, he was its Vice President, and between 1998 and 2010, its President, and then its Honorary President.
He has received numerous awards: Imre Újhelyi Award, János Nagyváthy Memorial Medal, EAAP Distinguished Service Award, Pro Natura Memorial Medal, Artúr Horn Award, Knight's Cross of the Order of Merit of the Republic of Hungary, Pro Scientiis Agriculturae Debrecen, Academic Award, Konkoly-Thege Award, Életfa Award, Silver Grade of the Életfa Memorial Plaque, Andor Jánossy Award, Ferenc Kozma Award and posthumous Hungarian Heritage Award.

In our time, it is unprecedented that after nearly two decades of practical work in "rubber boots", anyone can be so successful in the scientific field, and at the same time not be separated from their former colleagues and the everyday worries of the profession for a moment. Without Imre Bodó's optimism, diligence, and work ethic, all this would have been unthinkable. What else can give strength to this than a strong faith and a loving family? Imre Bodó wrote the following in a previous issue of Új Ember. "*And this requires sacrifice. Many consider this concept to be outdated. Yet without it, it is impossible to imagine the development of society. Moreover, there is no question of a truly great sacrifice. The happiness of life is not given by money, but by joy. For parents, children mean greater joy than what can be obtained with money. Joy, happiness, is very important in earthly life. Everyone strives for this in some form. Of course, money can also bring joy, but this cannot be compared to what a child can give a parent. Unfortunately, many people only wake up to this truth late or never. Yet it is at this point that the true interests of the individual and the nation meet*."
On the occasion of his 90th birthday, we greeted Imre Bodó in a ceremonial setting at the University of Veterinary Medicine. He was unable to appear in person at the "festive meeting organized on the occasion of the 150th anniversary of the founding of the first independent animal husbandry department (1973-2023)" at the end of November. Professor Emeritus Imre Bodó, a renowned professor of the Department of Animal Husbandry and an outstanding animal husbandry expert, passed away on December 18, 2023, at the age of 92. His funeral took place in Budapest on January 11, 2024, as part of a Catholic mass. His loss is touched by the of Veterinary Medicine at a personal level. Rector Péter Sótonyi described Imre Bodó as an opinion-forming teacher, Walter Hecker as a cooperative friend, and the Franciscan priest who presented the funeral mass described him as a pillar that we can all look up to and rely on.
He was the man, a dear friend, a colleague, whom we always think of with fondness and respect and whose memory we will cherish forever.
May he rest in peace!

Dr. András Gáspárdy
President of DAGENE