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Analysis of Lipizzaner horse breeding in Serbia and Bosnia and Herzegovina

ŠTRBAC, Ljuba[1]* – ROGIĆ, Biljana[2] – BABAN, Mirjana[3] – TRIVUNOVIĆ, Snežana[1]

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]University of Banja Luka, Faculty of Agriculture, Bulevar vojvode P. Bojovića 1a, 78000 Banja Luka, Bosnia & Herzegovina
[3]Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1 HR-31000 Osijek, Croatia
*correspondent author: ljuba.strbac@stocarstvo.edu.rs

Abstract

The aim of this paper is to analyse the breeding of Lipizzaner horses in Serbia and Bosnia and Herzegovina with special reference to the population size and implementation of breeding programs. Lipizzaner is one of the most popular breeds for horse breeding in these countries. Today, stud farms (one state and three private) and individual breeders (about three hundred) breed Lipizzaners in the Republic of Serbia, and the total population is 692 horses. In Bosnia and Herzegovina, breeding is organized primarily at the state stud farm Vučijak, where 105 horses of all categories are breeding. The work on genetic improvement and preservation of Lipizzaners is defined through various laws and bylaws, but above all through national breeding programs. The implementation of breeding programs in both countries is at a five-year level while the selection program mostly refers to the improvement of the horse's exterior, and less to the working ability exam.

Keywords: Lipizzaner, population size, breeding program

Introduction

Lipizzaner is one of the most popular breeds for horse breeding in Serbia and Bosnia and Herzegovina. This breed has a centuries-old tradition of breeding in Serbia. The first official stud farm, Gladnoš, was founded in 1920 on Fruška Gora. During the Second World War, the stud farm was disbanded, and after the end of the war, in 1946, it was renewed as the state stud farm. Also, in 1921, Karađorđevo became a state stud farm, which in that period used Lipizzaners from private breeding as a riding horse. With own breeding of Lipizzaner horses Karađorđevo started in 1929-1930, using stallions and mares from other state stud farms. Great importance for the breeding of Lipizzaner horses in Serbia has Kelebija Stud Farm which formed an initial breeding herd from Karađorđevo Stud Farm, individual Lipizzaner breeders and owners mostly from Serbia and from other state stud farms (Lipica, Đakovo, Vučijak and Lipik). In Serbia, a very important role in preserving the tradition of Lipizzaner breeding is played by individual breeders which are previously organized through equestrian cooperatives and today through equestrian clubs. The oldest equestrian cooperative was founded in 1924 in

Novi Slankamen. The members of this cooperatives rented stallions from state stud farms and already owned an enviable number of purebred mares. In Bosnia and Herzegovina the national stud farm Vučijak was founded in 1946 in Prnjavor municipality with primary goal to improve the existing horse population in North Bosnia. Initial breeding herd was formed from the Croatian state stud farms (Đakovo and Lipik) and from private breeders and owners in Croatian. Lipizzaner is one of the oldest horse breeds is breeding in many countries of the world. The largest part of the population is in Europe, but Lipizzaner is also breeding in USA, Australia and South Africa. The total number of Lipizzaner horses is 12.286 (LIF, 2021). According to the data of this organization, on state stud farm the total number of Lipizzaner is 2.114, while in private breeding total number is 10.172. The nowadays national stud farms are considered as traditional studs and they represent important centres for the preservation of the Lipizzaner horse. Each state stud farm or country has its own national breeding program, and according to the breeding goal we have two different types of Lipizzaner horses: driving and classic types which differ in body dimensions and conformation.

Morphological characteristics are very important in the breeding of horses and for a more accurate assessment of working ability, it is necessary to regularly measure breeding horses until the end of development and growth. The Lipizzaner is a very expressive horse, with a noble posture, harmonious conformation, and the frames of the body are more rectangular than squared, which can be differ according by morphological characteristics from one country to another, or from the stud farm to stud farm. ZECHNER et al., 2002 notes that the breeding goals of the stud farms have been different and are partly changing over time. According to ROGIC et al., 2018 the primary goal of the Austrian stud farm is to provide horses for classical dressage, the Hungarian stud farm has specialized in breeding of top horses for carriage driving, the Slovenian, Slovakian and Croatian stud farms are breeding riding horses, while the Romanian studs providing stallions for improvement of the local farm horse population. ČAČIĆ and ČURIK, 2015 notes that Croatian breeding of Lipizzan horses is consist of two types (two breeding goals), driving type in Đakovo stud farm and in private breeding, and classic type in Lipik stud farm. According to the LIF, 2010 the breeding goal is to preserve the purebred Lipizzaner according to the traditional breeding regulations for this type of baroque parade horse which have withers height between 153 cm and 158 cm. Research on the morphological characteristics of Lipizzaner has been conducted by many authors: ZECHNER et al., 2001; RASTIJA et al., 2004; BABAN et al., 2006; ŠTRBAC and TRIVUNOVIĆ, 2014; PALLOTINO et al., 2015; DRUML et al., 2016; VAŽIĆ et al., 2016; ROGIĆ et al., 2018, 2019, 2021.

The aim of this paper is to analyse the breeding of Lipizzaner horses in Serbia and Bosnia and Herzegovina with special reference to the population size and implementation of breeding programs.

Materials and methods

For this research, the database of the Department of Animal Science, Faculty of Agriculture in Novi Sad and database of Vučijak Stud Farm was used. Also, the national breeding programs and breeding program of Vučijak Stud Farm were used for analysis of breeding goal and selection program. The Department of Animal Science, as the Central Breeding Organization for Lipizzaner, has kept Stud Book records (central database), issued pedigrees and coordinated the implementation of the breeding program in Serbia (private breeders and Karađorđevo Stud Farm). Stud farm Vučijak implements a breeding program only on its own breeding herd while private breeders are organized through the association that does not yet have approval to implement breeding programs.

Results and discussions

Population size

According to the data from the selection reviews in 2021, stud farms (one state and three private) and individual breeders (about three hundred) breeding Lipizzaners in the Republic of Serbia, and the total population is 692 horses. Only 14 purebred Lipizzaner horses are breeding at the Karađorđevo Stud Farm and 678 at private breeders farms. The number of Lipizzaner by categories is shown in Table 1.

Table 1: Number of Lipizzaners in Serbia, 2021

Category	Karađorđevo	Private breeders	Total
Stallions for breeding	1	21	22
Stallions	3	83	86
Mares	5	293	298
Colts and fillies	5	201	206
Foals	-	80	80
Total	14	678	692

In Bosnia and Herzegovina, breeding is organized primarily at the state stud farm Vučijak, where 105 horses of all categories are breeding (Table 2). In 2022, private breeders from Bosnia and Herzegovina formed the Association of Lipizzaner Breeders, which currently has 20 members who breeding about 80 Lipizzaner horses. The plan of this association is to get the status of a breeding organization in the next period and submit an application for membership in LIF.

Table 2: Number of Lipizzaners at Vučijak Stud Farm, 2021

Category	Number of horses
Stallions for breeding	8
Stallions	6
Mares	32
Geldings	3
Colts and fillies	48
Foals	8
Total	105

If we compare the data presented in tables 1 and 2 with the data on the number of Lipizzaners from other LIF member countries (LIF, 2021), we see that Serbia is on the seventh place in the total population of Lipizzaner horses (about 6 % of total population). Hungary has the largest total population size of Lipizzaner horses (2.554), than is Croatia (2.218) and on third place is Slovenia (1.204). According to the number of Lipizzaners from private breeders, Serbia is on the fifth place (about 7 % of population), and the largest number of Lipizzaner horses is again in Hungary (2.261), then Croatia (1.982), and on the third place is the USA (919). Lipizzaner is breeding on 11 state stud farms (2.114 Lipizzaners i.e. about 17 % of total population) and according to the size of the population Karađjorđevo is on the last place (less than 1 % of stud farms and total population), and Vučijak on the ninth place (about 5 % of stud farms population and less than 1 % of total population). The largest number of Lipizzaner is breeding on the

Lipica Stud Farm (369), than on the Piber Stud Farm (368) and on the third place is the Szilvásvárad Stud Farm (293).

Implementation of breeding program

The work on genetic improvement and preservation of Lipizzaners is defined through various laws and bylaws, but above all through national breeding programs. In Serbia and Bosnia and Herzegovina there are Main Breeding program for Lipizzaner in Republic of Serbia, Breeding program of Lipizzaner horse breed in Republic of Srpska for the period 2020-2024 years and Breeding program of Lipizzaner horse breed Vučijak Stud Farm.

The implementation of breeding programs in Serbia is organized at several levels that are interconnected. The first level constitutes breeders of breeding animals, and they can be registered as stud farm (private or state/national) or as private/individual breeders. Every breeder has the right to become a member of the primary breeding organization (second level) if they agree to participate in the implementation of the breeding program. The breeder confirms their consent by applying to be listed on the register of breeders of quality breeding animals and concluding the agreement on the application of the main breeding program (so-called triple agreement) with the primary and regional breeding organizations (third level). The breeder reports to the primary breeding organization all changes on their farm (births, mating, deaths, sales etc.) in the manner defined by the breeding program. In each specified region, the regional breeding organization conducts a selection review, i.e., morphological measurement and evaluation of animals. Once a year, the primary and regional breeding organizations report on the implemented activities to the central breeding organization (fourth level). The main activity of the central breeding organization is the adoption of the main breeding programs, registration of animals and keeping the Stud Book records, as well as coordinating the work of other entities that participate in the implementation of breeding programs. In the previous period, two five-year cycles of implementation of breeding programs were realized and the third one was started 2020 year.

Currently in Bosnia and Herzegovina, only the Vučijak Stud Farm is registered as a breeding organization that implements a breeding program on its own herd. Also, the Vučijak Stud Farm is authorized to keep the Stud Book in cooperation with the Faculty of Agriculture in Banja Luka and issue pedigrees for its horses. For private breeders, the registration of the breeding organization is in progress.

Breeding goal

The most important aspect of Lipizzaner breeding is organized selection work aimed at realization the following goal: breeding horses of harmonious, elegant and noble build with characteristic breed traits of the exterior, regular and generous gaits suitable for achieving top results in driving and riding, and which are useful for equestrian tourism, therapeutic riding, recreation, etc. (ŠTRBAC et al., 2019). According to PRAČEK, 1999 breeding goal of founding stud farm Vučijak was to create a horse with smaller body frame suitable for driving and carrying. ZECHNER et al., 2001 were made morphological descriptions of Lipizzaner horse populations from seven Europe countries (eight national stud farms): Lipica, Piber, Đakovo, Lipik, Fagaras, Monterotondo, Szilvásvárad and Topol'cianky. ROGIĆ et al., 2022 compare the results for the height at withers we can conclude that the stallions from Karađorđevo are the smallest in relation to the European population of Lipizzaner stallions, while the stallions from Kelebija are higher than stallions from Lipica, Monterotondo, Piber and Topol'cianky. Only mares from Piber were not smaller then mares from Karađorđevo. Mares from Kelebija are higher than mares from Beclan, Lipica, Monterotondo, Piber, Szilvásvárad and Topol'cianky.

Selection program

Selection program mostly refers to the improvement of the horse's exterior, and less to the working ability exam. Horse evaluation and selection for breeding can be conducted individually on the breeder's farm, or publicly, at previously defined places in order to evaluate a larger number of animals of the same category. Individual assessment is conducted by a commission composed of representatives of regional and primary breeding organizations who have been trained to assess the exterior of Lipizzaners. In the case of public evaluation, the commission is appointed by the central breeding organization, where in addition to the representatives of breeding organizations, international evaluators who have a LIF license can also be appointed as members of the commission.

Conclusions and recommendations

The implementation of breeding programs in both countries is at a five-year level while the selection program mostly refers to the improvement of the horse's exterior, and less to the working ability exam. For exterior assessment a linear scoring system should be considered because that score can be included in genetic analysis to estimating genetic parameters and breeding values. Also, introducing SNPs for parenting testing that could be used for other Lipizzaner genome research should be considered.

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Dalmatian Bušak horse

LISTEŠ, Ema[1] – MARGETA, Polona[2] – PRVANOVIĆ BABIĆ, Nikica[3] – LISTEŠ, Eddy[4] – MAURIĆ MALJKOVIĆ, Maja[3]*

[1]Association of Dalmatian bušak lovers and breeders, Gundulićeva 6, 21000 Split, Croatia
[2]Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, 31000 Osijek
[3]Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia
[4]Regional Institute Split, Croatian Veterinary Institute, Poljička cesta 33, 21000 Split, Croatia
*Corresponding author: mmauric@vef.unizg.hr

Abstract

One of the Croatian autochthonous breeds in the Mediterranean region that has been classified as uncategorized or unknown is the Dalmatian Bušak horse. It has been noted that in Dalmatia a small number of individuals are still bred and body measurement (as part of the exterior description) and genotyping research were carried out. Nine measurements were taken from 25 horses. Hair samples were taken from 23 horses for preliminary genotyping at 17 microsatellite loci. Lydtin stick was used to measure the height at withers (129.88 ± 3.94 cm), height at croup (129.62 ± 4.33 cm), body length (136.11 ± 7.04 cm), and chest depth (58.14 ± 4.48 cm); ribbon to measure the chest (156.81 ± 10.6 cm) and cannon bone circumference (17.57 ± 1.29 cm); and caliper measure the chest width (28.57 ± 2.62 cm), croup width (41 ± 2.76 cm), and croup length (43.98 ± 2.65 cm). Microsatellite analysis showed that each locus had between 4 and 12 different alleles, the average observed heterozygosity was 0.74 and expected 0.76, and, consecutively, the inbreeding coefficient (F_{IS}) was 0.02. The polymorphic information content (PIC) revealed that 16 out of 17 markers were highly informative. The results indicated that the Dalmatian Bušak horses are a relatively homogeneous, but not inbred, population and that the next steps leading to the official recognition of this autochthonous breed in Croatia should be taken.

Keywords: Dalmatian Bušak horse, autochthonous breed, exterior, microsatellite

Introduction

Small horses known as the Dalmatian Bušak horse have been observed in the Mediterranean part of Croatia over the past ten years. Researching various historical sources, it was established that they have been found in that area for more than two centuries. Despite the breed currently being classified as not evaluated by the Food and Agriculture Organization (FAO) and as insufficiently known by the categorization of the International Union for Conservation of Nature (IUCN) (BARAĆ et al., 2011), there is still a small population of horses in Dalmatia whose conformation corresponds to the Dalmatian Bušak breed.

The first mention of small horses in Dalmatia can be found in the report of the Split district from 1822, compiled by district chief Enrico Reha. The horses were described as miserable,

poorly groomed, and shod, and they bred in herds on pasture without shelter. They were used exclusively as pack horses, sometimes for riding, and were not suitable for military purposes (REHA, 2016). The next written document was from the 1830s, where governor Lilienberg, in his description of the Habsburg province of Dalmatia, also mentioned very small horses that were often poorly kept, fed, and shod (TROGRLIĆ and CLEWING, 2015). As in the previous report, the author noted that horses of this breed were not used for any kind of pulling but as pack and/or riding animals.

The name Dalmatian Bušak horse was mentioned for the first time in the "Report of the National Economic Administration of the Kingdom of Croatia and Slavonia for the period 1896-1905", where Prof. dr. Oton Frangeš, in the chapter on horse breeding, wrote about small horses, stating that certain regions (Dalmatia) do not have good conditions for horse breeding (BARAĆ et al., 2011). Beautiful horses like the Posavina horse did not develop in these areas, but horses of a smaller build, with heavy heads, short necks, long backs, large abdomen, steep hindquarters, thin legs with dry muscles, and solid hooves, whose height at withers ranged from 110 to 140 cm. He also mentioned that they could safely carry heavy loads or riders through steep and rocky roads in all kinds of weather (storm and rain).
Along with the historical records, quite a large number of postcards and photos of the Dalmatian Bušak horse were found (Figures 1 and 2). Although the horses in the photos are in different positions and, in some of them, they are not even fully visible, it is visible that they have quite a similar exterior.

Figure 1. Postcard from webpage Forgotten Dalmatia (www.zaboravljenadalmacija.hr), Split surroundings, 1899.

Figure 2. Photo from 1980s from "Slobodna Dalmacija" by Feđa Klarić: "A man and a horse on Split riva".

The next important record was Giacomo Gospodnetich Martinovich's booklet "Libretto delle cavalle" from 1935 (given by his great-grandson). It is a notebook in which he entered data about bought mares: name, origin, age, time of arrival at his property in Dol on the island of Brač, height at withers (average height was 126.71 cm), time of foaling, whether the foal was a mule or a horse, and the picture of the mare. Another interesting record is the documentary film "Autumn on the island of Brač" (Croatia film, 1957), which talks about life, people, and customs on the island of Brač, and it is also possible to see horses in the Dalmatian Bušak type. In this study, we presented the exterior body measurements of the Dalmatian Bušak horse and the preliminary genotyping data (research is still ongoing).

Materials and Methods

A total of 38 horses (11 males and 27 females; Figure 3) aged 6 months to 16 years, situated on 10 farms in the Split-Dalmatia and Dubrovnik-Neretva counties, were included in this study.
Nine body measurements were taken from 25 horses, as most of them were not used to any kind of human manipulation. Lydtin/measuring stick was used to measure the height at withers, height at croup, body length, and chest depth; ribbon was used to measure the chest and cannon bone circumference; and caliper was used to measure chest with, croup width, and croup length. Hair samples, 10-50 hairs from the main region, were taken from 23 horses. DNA was isolated using NucleoSpin Tissue (Macherey-Nagel, Germany) following the supplier's manual. Seventeen (17) horse-specific, ISAG recommended microsatellite (MS) loci were amplified using StockMarks™ for Horses 17-Plex Genotyping Kit (Thermo Fisher Scientific, Finland) according to supplier's protocol instructions: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1, and CA425. MS PCR products were analysed using genescan™ 500 LIZ® Size Standard (Applied Biosystems) on ABI Prism™ 3130 Genetic Analyzer. Determination of the size of the amplified fragments was performed using GeneMapper 4.0 (Applied Biosystems).
Descriptive statistics of the acquired measurements were calculated with Statistica v.14 (TIBCO SOFTWARE INC., 2020). Allele frequency, polymorphic information content (PIC), observed (H_O), and expected heterozygosity (H_E) were analysed with Cervus v.3.0.7 (MARSHALL et al., 1998; KALINOWSKI et al., 2007) while the fixation index (F_{IS}) and testing for the deviation from the Hardy-Weinberg equilibrium with R software (R CORE TEAM, 2021) adegenet package (JOMBART and AHMED, 2011). All loci were analysed together except LEX3, located on the X chromosome, which was analysed separately only in mares (N = 16).

Figure 3. Dalmatian Bušak stallion, about 3-year-old

Results and Discussion

The results of the assessed body measurements are given in Table 1. The obtained values indicate the relative homogeneity of the population. The most variable measurement was chest circumference, due to it being the most affected by horse weight variations (measured horses varied from underfed to overweight). Determined heights at withers and croup of 130 cm, body length of 136 cm, and cannon bone circumference of 17.6 cm indicate that the population belongs to a group of smaller horses and that their body conformation is rectangular (a little bit longer than taller).

Table 1 Mean values of the taken body measurements in cm (N = 25). Results are presented as arithmetic mean ($\bar{X}$), standard deviation (SD), minimal (min), and maximal (max) values.

Measurement	$\bar{X}$	SD	min	max
Height at withers	129.88	3.94	122	136
Height at croup	129.62	4.33	123	137
Body length	136.11	7.04	124.50	148
Chest circumference	156.81	10.60	140	180
Cannon bone circumference	17.57	1.29	15	20
Chest width	28.57	2.62	23	34.50
Croup width	41	2.76	34	44
Croup length	43.98	2.65	38	49
Chest depth	58.14	4.48	50	65

Considering the limited availability of literature data on the Dalmatian Bušak horse, the measurements from this research could have been compared only with the measurements from "Libretto delle cavalle" and IVANKOVIĆ et al. (2012). When comparing the height at withers of the mares in this study to the mean height at withers value from "Libretto delle cavalle", it was found that it was higher for 3.82 cm (the average height for mares is 130.5 cm). In relation to IVANKOVIĆ et al. (2012), the horses measured in this research were 2.9 cm higher at withers, 1.72 cm at croup, and had a 1.1 cm longer body, 4.8 cm larger chest, and 1.6 cm cannon bone circumference. Slightly lower values were recorded for chest width (3.23 cm), croup width (4.4 cm), croup length (1.82 cm) and chest depth (0.66 cm). The conclusion is that these are small deviations, which are partly possible due to differences in the way of taking measurements from person to person (personal communication with other researchers), and that the horses are very similar in appearance.

In Table 2. are statistics of MS loci (N = 23) analysed presented: locus name, number of alleles found, range allele sizes in base pairs (bp), observed (H_O) heterozygosity, expected heterozygosity (H_E), fixation index (F_{IS}), polymorphic information content (PIC), Hardy-Weinberg (H-W) equilibrium (P-value), chromosome on which the locus is found.

Table 2. Statistics of microsatellites investigated

Locus	No of alleles	Range allele sizes (bp)	H_O	H_E	F_{IS}	PIC	H-W (P)	Chro-mo-some
VHL20	9	83-102	0.83	0.84	0.01	0.80	0.93	30
HTG4	5	116-137	0.52	0.44	-0.19	0.40	0.98	9
AHT4	8	140-166	0.83	0.83	0.00	0.79	0.79	24
HMS7	7	167-187	0.74	0.79	0.06	0.74	0.28	1
HTG6	6	74-103	0.39	0.68	0.43	0.62	<0.01	15
AHT5	6	126-147	0.74	0.74	0.00	0.69	0.19	8
HMS6	6	154-170	0.74	0.77	0.04	0.71	0.34	4
ASB23	7	176-212	0.61	0.83	0.27	0.79	<0.01	3
ASB2	9	237-268	0.83	0.85	0.03	0.81	0.04	15
HTG10	10	83-110	0.96	0.83	-0.15	0.79	0.32	21
HTG7	4	114-128	0.83	0.63	-0.23	0.61	0.11	4
HMS3	7	146-170	0.65	0.74	0.13	0.70	0.46	9
HMS2	8	215-236	0.83	0.83	0.00	0.78	0.23	10
ASB17	12	104-106	0.87	0.86	-0.01	0.82	0.06	2
HMS1	6	166-178	0.61	0.72	0.16	0.66	0.16	15
CA425	6	224-247	0.96	0.82	-0.17	0.77	0.91	17
$\bar{X} \pm SD$	7.25 ± 2.02		0.74 ± 0.15	0.76 ± 0.11	0.02 ± 0.17	0.72 ± 0.11		

The Dalmatian Bušak horse measurements differ greatly from the measurements of all other recognized Croatian autochthonous horse breeds (Croatian Coldblood, Croatian Posavina horse, and Međimurje horse; LISTEŠ et al., 2019), which is not surprising given that they belong to the group of cold-blooded animals, and the Dalmatian Bušak belongs to a group of smaller horses.

The preliminary genotyping data (research still ongoing) showed that all investigated MS loci were polymorphic and at least 4 different alleles were determined per locus. The total number of alleles found on autosomal loci was 116 (Table 2), and an additional 9 for the LEX3 locus (Table 3). The presence of a large number of alleles in different microsatellites is a good indicator of the genetic variability of the population.

Table 3. Statistics of locus LEX3 (N = 16)

Locus	No of alleles	Range allele sizes (bp)	H_O	H_E	F_{IS}	PIC	H-W (P)	Chromosome
LEX3	9	141-161	0.75	0.88	0.15	0.84	0.33	X

The average H_O was 0.74 and H_E 0.76. Consequently, F_{IS} was slightly positive (0.02), meaning a slight deficit in heterozygosity. The relatively high H_E indicates that the population has retained the presence of several alleles. The observed heterozygosity being not significantly different from the expected (0.74 and 0.76, respectively) is in accordance with the fact that the population has a random-mating structure as there is no organized selection scheme systematically applied. Five out of 17 MS loci had negative F_{IS}, nine had positive F_{IS} and three had equal H_O and H_E with F_{IS} being 0 (Table 2). Even from the nine positive F_{IS}, four had F_{IS} lower than 0.06. Looking at the overall F_{IS}, and the ones separately for each locus, it indicates a low level of inbreeding. These results confirm the relatively high heterozygosity test and indicate that there are variations between the individuals within the population. Of 17 investigated loci, 16 showed highly informative PIC (BOTSTEIN et al., 1980), which could be attributed to a large number of alleles or heterozygosity. Despite a small population, almost all observed loci were in genetic equilibrium according to the Hardy–Weinberg law.

Conclusions

According to the results of this study, the Dalmatian Bušak horse population is quite homogeneous with a low level of inbreeding. The relatively high expected heterozygosity and PIC, along with a quite low inbreeding coefficient, imply a higher amount of genetic variability. The aforementioned can be exploited and used in planning breeding strategies, particularly in populations of small size. The next steps for the Dalmatian Bušak horse are focused on increasing the number of individuals, popularization, and receiving formal recognition in Croatia.

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Conformation traits of Hungarian Fallow Donkey mares according to their basic colour

HARMAT, Levente[1]* – KUNCICKY, Ariane[1] – LÉNÁRT, Zoltán[1] – ERNST, Mihály[2] – NAGY, János[3] – GÁSPÁRDY, András[1]

[1]Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine Budapest, István utca 2, 1078 Budapest, Hungary
[2]Hungarian Donkey Breeders Association, Rákóczi utca 46, 7477 Szenna, Hungary
[3]Game Management Landscape Centre, Kaposvár Campus, Hungarian University of Agriculture and Life Sciences Malom utca 3, 7475 Bőszénfa,
*corresponding author: harmat.levente@univet.hu

Abstract

The breed reconstruction program of Hungarian Fallow Donkey has begun in 2021 in order to unify the domestic donkey population. It has several elements, e.g., according to the breed standard, the selection of individuals to be entered in the nucleus part of stud book based on, among other things, their conformation and constitutional characteristics. Currently, the number of registered mares exceeds 1000 individuals. The purpose of this combined processing is to compare the colour variants of the registered donkeys based on some major body measurements and body indices. Trait investigated were corrected for age 7 years, and then the effect of colour variants, time of recording, and location as a fixed effect, as well as the year of birth as a covariate, were examined using a linear model. No differences in colour variants were detected for either body measurements or indices. In addition, the population investigated is about 117 cm high at the withers, it has a relatively shallow chest and long trunk for its withers height. However, location had a significant effect on body measurement and index values in all cases ($P < 0.001$). SEM values remained low, except for the most complexly calculated body capacity index, which characterizes a population of uniform shape. These results contribute to the establishment of the Hungarian Fallow Donkey seed stock. A larger national population of that breed is expected to play a specific segment in the food chain as a favourite animal for climate change and ecological livestock farming.

Keywords: Hungarian Fallow Donkey, body measurements, body indices

Introduction

The Hungarian Donkey Breeders' Association (MSzE) was established on 13 April 2002 in Rudabánya with the participation of some active donkey keepers and breeders, as well as supporters trying to improve the situation of donkey breeding in Hungary. The Association has faced and still faces difficult tasks today: in addition to mapping the scattered, almost invisible donkey population in Hungary, strengthening a hitherto non-existent donkey breed, since in Hungary we could only talk about the donkey species (ERNST, 2004). The aim of MSzE is that this history-related animal species can finally integrate into consciously bred domestic animals

in Hungary in a worthy way as is the case in several European countries, where donkey breeding associations can look back on decades.

The MSzE want to like and spread this breed, which is still unjustly neglected, and whose characteristics, calm nature, adaptability and intelligence make it perfectly suitable both for use as a family hobby animal and for small and large-scale work on farms. Currently, the number of registered mares exceeds 1000 individuals, although the Hungarian donkey population is about of 3000-4000.
The Association has achieved the following results in recent years:

2003. Recognition of the Hungarian Fallow Donkey breed and the recognition of its breeding association, the MSzE.
2004. Amendment of the Livestock (1993 CXIV. law on animal husbandry; prior to the law in force today) Act and extension of its scope to donkeys.
2005. Declaration of the protected native breed of the Hungarian Fallow Donkey.
2021. Adoption of the breed-reconstruction program and open nucleus stock.

The breed reconstruction program of Hungarian Fallow Donkey has successfully begun in order to unify the domestic donkey population. It has several elements, e.g., according to the breed standard, the selection of individuals to be entered in the nucleus part of stud book based on, among other things (e.g. microsatellite diversity, mtDNA descendance), their conformation (ZÁBORSZKY, 2005) and constitutional characteristics (constitutional faults included, LÉNÁRT et al. 2017). Only and exclusively individuals of known origin (molecular parentage control) that meet the breed standard and achieve satisfactory results in terms of appearance, behaviour and movement, results of a complex judging, may enter the nucleus stock in both sexes. The creation of the nucleus stock first started in the Game Management Landscape Centre, Bőszénfa.
The purpose of this combined processing is to compare the colour variants of the registered donkeys based on some major body measurements and body indices by what we would like to contribute to the enlargement of the Hungarian Fallow Donkey seed stock.

Material and method

The Hungarian Donkey Breeders' Association (MSzE) provided us with the availability of the livestock owners. The recording of body measurements of a total of 208 mares was conducted in two periods of time (2016-2017 and 2020-2021) on 16 farms in Hungary (the average number of “jennets” per farm was 13).
The following body measurements were taken: height at withers (the highest point of the withers measured from the ground), chest depth (distance between the sternum and vertebra behind withers), heart-girth (the circumference of the chest, measured just behind the elbow), and diagonal trunk length (distance between the greater tubercle of humerus and the ischial tuberosity). To measure the donkeys measuring tape and measuring stick were used. From the investigated parameters the height at withers, chest depth, and diagonal trunk length were measured by measuring stick. The heart-girth was recorded by tape measure.
In the current study four body indices were calculated determined by body measurements and body weight according to the Table 1. The larger the index value, the more pronounced is the index property at the animal.

Table 1. Applied calculations of body indices

Stubbiness index =	(greater trunk length/height at withers)*100
Index of long-leggedness =	(height at withers-chest depth)/chest depth*100
Strength back index =	(rump with I/shoulder width)*100
Body capacity index =	(shoulder width+rump with I)/2*chest depth*diagonal trunk length/1000

Sqrt – Square root; rump with I – distance between coxal tubers of left and right ilium; shoulder width – distance between greater tubercles of left and right humerus

The merged dataset was used for a combined processing. At the beginning, all values were adjusted for age at 7 years by linear regression. The age of donkeys was known from date of birth registered in their 'equine passport' or stud book, but several times the microchip number was used for retrievement of birth date. The average age of investigated mare population was seven years (from a range of 1-21 years).
For the investigated body measurements and indices the (LSM - Least Square Means) and its error (SEM – Standard Error of Mean) were computed by a General Linear Model (TIBCO Software Inc., 2020) with fixed effects of time of recording, basic colour of the animal and farm, and year of birth as covariate (a covariate is a variable that is possibly predictive of the outcome under study; in this study LSM was computed for 2014 from a range of 1995-2019).
The most common basic hair colours are the brown, black, and grey in their shade variants. Donkeys usually have “swallow belly” in each colours. The end of the legs is often lighter than the shade of the body. However, there are also entirely black exemplars. Individuals carry often a “cross” made of the dorsal strip and the stripes running down each of their shoulders. But shade and colour variant were not considered when separating colour groups. Results (LSM and SEM) are presented in this paper for colour groups only, while the significance levels (P-value) are shown for all three effects.

Results and discussion

As it is presented in Table 1 and Table 2, no differences in colour variants were detected for either body measurements or indices. Further on, SEM values remained low, except for the most complexly calculated body capacity index, which characterizes a population of uniform shape.
According to a former experience (LÉNÁRT et al, 2017), the colour of the animal could determine the size. A possible explanation can be found in the origin of the donkeys. It can be supposed that the brow individuals are the descendants of a large framed Italian donkey, Martina Franca (which was used in creation of mule in Hungary in the past, KUGLER et al., 2008), while the grey individuals stem from smaller sized Balkan donkeys. However, VLAEVA et al. (2016) published data about donkey eco-types in Bulgarian which were nearly the same as our ones (119.8 and 121.0, respectively). MATIUTI et al. (2011) informed us about much less values (105.0 cm) of donkey population in Banat (Romania). Sadly, the pedigree information is incomplete currently.

In addition, the mare population investigated is about 117 cm high at the withers (Table 2). It has about 51 cm deep chest, 138 cm large heart-girth, and 94 cm long greater trunk length.

Table 2. Body measurements adjusted for 7 years of age and birth year 2014 (cm)

Effect	Hight at withers		Chest depth		Heart-girth		Diagonal trunk length	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
Time of recording, P-value	<0.001		0.015		0.155		0.004	
Colour group, P-value	0.664		0.145		0.412		0.496	
brown (n=86)	117.0	0.892	51.6	0.605	138.6	1.273	94.5	0.687
black (n=26)	117.0	1.378	52.0	0.935	139.1	1.967	94.0	1.062
grey (n=74)	116.1	0.881	50.4	0.598	136.8	1.259	93.5	0.679
Farm, P-value	<0.001		<0.001		<0.001		<0.001	
Year of birth, P-value	0.494		0.111		0.704		0.535	

The mean value stubbiness index is about 81% (Table 3). A value being below 100 % is to mean that the trunk of the body is longer than the height at withers; so the shape of the Hungarian Fallow Donkey from side view is rather elongated than rectangular.
The average value of long-leggedness index is about 130%. This can be considered as a high value, which indicates that the Hungarian Fallow Donkey is a long legged animal, it has relatively long fore limbs for its chest depth. None of the effects investigated were influencing the index of long-leggedness.
Based on strength back index (which is about 140% on average), the donkey mares have a remarkable wider pelvis region than their chest.
we can state that the mares have a relatively shallow chest and long trunk for its withers height.

Table 3. Body indices adjusted for 7 years of age and birth year 2014 (cm)

Effect	Stubbiness index		Index of long-leggedness		Strength back index		Body capacity index	
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
Time of recording, P-value	0.575		0.882		0.099		<0.001	
Colour group, P-value	0.553		0.241		0.645		0.115	
brown (n=86)	80.7	0.910	129.4	2.071	140.7	1.975	154.9	3.197
black (n=26)	81.2	1.381	127.7	3.142	142.7	3.052	155.1	4.941
grey (n=74)	81.8	0.883	132.6	2.008	139.7	1.952	147.3	3.161
Farm, P-value	<0.001		<0.001		<0.001		<0.001	
Year of birth, P-value	0.146		0.154		0.020		0.518	

As the colour variant, the year of birth did not show significant impact on the body measurement and body indices.
However, farm had a significant effect on body measurement and index values in all cases (P <0.001). SEM values remained low which characterizes a population of uniform shape. Except for the most complexly calculated body capacity index, where a SEM of 4.0 corresponds to a coefficient of variation of approximately 18%.

Time of recording appeared to be of significant effect in four cases. To our mind, the significant effect of recording can be argued by size preference in the different farms, which is manifested in keeping of larger or smaller bodied individuals. There were two farms in overlap and four individuals in the combined processing which were recorded in both occasions. The statistical comparison of them did not revealed significant impact of time of recording for the four traits in question. So, it is concluded our recordings in two different times were satisfactorily carried out.

Conclusion and recommendation

These results contribute to the establishment of the Hungarian Fallow Donkey seed stock. A larger national population of that breed is expected to play a specific segment in the food chain as a favourite animal for climate change and ecological livestock farming.

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Evaluation of the conservation status of the Slavonian-Syrmian Podolian cattle based on pedigree data

IVANKOVIĆ, Ante[1]– PEĆINA, Mateja[1]* – KONJAČIĆ, Miljenko[1] – RAMLJAK, Jelena[1] – KELAVA UGARKOVIĆ, Nikolina[1] – IVKIĆ, Zdenko[2]

[1] Faculty of Agriculture University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia
[2] Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, 31000, Osijek, Croatia
*corresponding author: matejapecina@agr.hr

Abstract

Slavonian-Syrmian Podolian cattle is one of the three autochthonous breeds in Croatia. After three decades of implementation of conservation measures, it is still in a critically endangered status. The objective of this work is to determine the population parameters based on pedigree records. The ENDOG v4.8 software package was used to estimate the population parameters. The mean number of complete and equivalent generations is relatively low (1.72; 2.71). The average inbreeding coefficient is relatively high (5.13%), while the mean average relatedness coefficient is of concern in terms of population sustainability (14.56%). The effective number of founders and ancestors of the reference population is low (9, 8) and their ratio (1.12) confirms the bottleneck that the population went through three decades ago. The average generation interval is 6.34 years and ranges from 5.84 (father - son) to 6.76 years (mother - son). The mean age of parents at the birth of offspring was 6.92 years. The population indicators calculated on the basis of pedigree records indicate the vulnerability of the Slavonian-Syrmian Podolian cattle. It is necessary to reduce the level of inbreeding, preserve existing lines and partially refresh the genetic potential of the breed.

Keywords: Slavonian-Syrmian Podolian cattle, pedigree, conservation.

Introduction

Slavonian-Syrmian Podolian cattle (SSPC) is one of three Croatian local cattle breeds. It is a longhorn, resilient breed with a moderate-sized frame (withers height from 125 to 140 cm, body mass from 400 to 600 kg) and a light to dark grey body colour (Figure 1). Until the middle of the 20th century, it was used mainly for field work and as a draft animal, and to a smaller extent for milk and meat production. Due to the modernization of agricultural production and the introduction of allochthonous breeds, local cattle breeds were marginalized and almost became extinct (some cattle breeds are extinct in Croatia). In the nineties of the 20th century, a program was started to protect the SSPC breed. The original population for the protection of the Slavonian-Syrmian Podolian cattle included eighteen adult animals (RAMLJAK et al., 2011). The current SSCP population includes approximately three hundred adult animals (15 bulls and 294 cows) housed on 35 farms (CAAF, 2022). Considering the population size, the SSPC is a critically endangered breed. Local cattle breeds are recognized as an important part of

biodiversity and genetic heritage. Of the total number of breeds of mammalian species used in agriculture, cattle breeds account for 22.90% (FAO, 2019). Although the number of extinct cattle breeds is relatively low (12.8%), a large number of breeds are endangered (21.1%), and for most cattle breeds the endangerment status is unknown (53.8%) (FAO, 2019). Therefore, it is very important to maintain the genetic diversity of the remaining cattle breeds, mostly captured in unselected autochthonous breeds (MEDUGORAC et al., 2009).

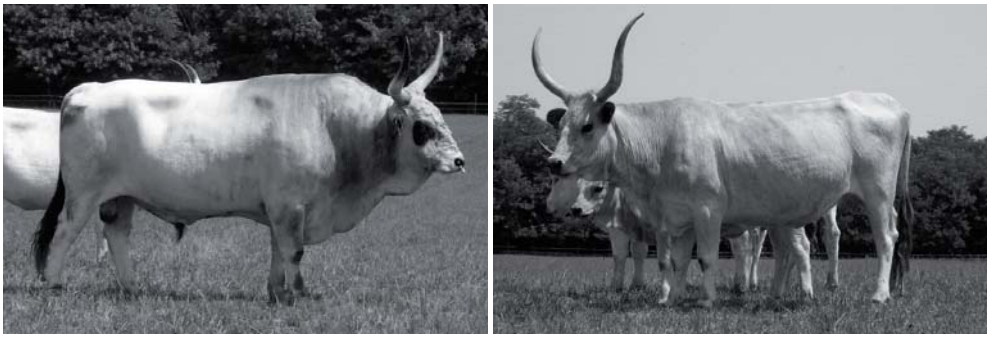

Figure 1. Bull and cows of the Slavonian Syrmian Podolian breed

Small natural populations raise several problems when faced with their conservation: they have lost most of their economic value, they usually show a high inbreeding level which threatens their long-term maintenance, and the conservation of biodiversity that they represent makes them unsuitable for the introduction of individuals in other populations (DUNNER et al., 1998). Monitoring and preservation of genetic diversity is the basis for effective selection and/or conservation programmes (GUTIÉRREZ et al., 2003). Pedigree information is a useful source for conservation programs to analyse the breeding status of a population (JARNECKA et al., 2021), and is important tool to describe genetic variability and its evolution across generations (GUTIÉRREZ et al., 2003). Therefore, the objective of this study was to describe the conservation status of the Slavonian Syrmian Podolian cattle through pedigree analysis.

Materials and methods

The database of Slavonian-Syrmian Podolian cattle includes 1,836 animals born between 1984 and 2022. For each animal in the database, information such as identification number, date of birth, sex, sire, dam, and herd was recorded. In this study, active (alive) animals of both sexes were selected as the reference population (REF 01). Demographic and population parameters are presented for the last two and a half decades. Pedigree analysis was performed using ENDOG v4.8 software (GUTIÉRREZ and GOYACHE, 2005). Pedigree completeness was determined by the mean number of complete generations, the mean number of maximum generations, and the mean number of equivalent generations (BOICHARD et al., 1997). Parameters related to inbreeding (mean inbreeding coefficient (F), mean average relatedness coefficient (AR), effective population size (Ne)), probability of gene origin (effective number of founders (f_e), effective number of ancestors (f_a), effective number of founder herds (f_h)), and generation interval (GI) were assessed. The average relatedness coefficients and individual inbreeding coefficients were calculated based on GUTIÉRREZ and GOYACHE (2005).

Results and discussion

The demographic trend of the Slavonian-Syrmian Podolian cattle population was positive in the last two decades (Figure 2). During the period of the initial implementation of the SSPC protection program (1997 to 2004), all animals were kept in one herd, which limited the development of the population. After 2004, the redistribution of the protected population to several herds was initiated, which resulted in a positive development of the population (annual population growth of 8.79%). The diversity of interests of several breeders (herds) makes the sustainability of breeding endangered breeds more secure. Breeding of SSPC where the population is concentrated in a smaller number of owners or in a narrower breeding area represents a risk for sustainability, due to the potential absence of interest in breeding the local breed (if it is not economically well-affirmed), or because of certain infectious diseases or natural disasters.

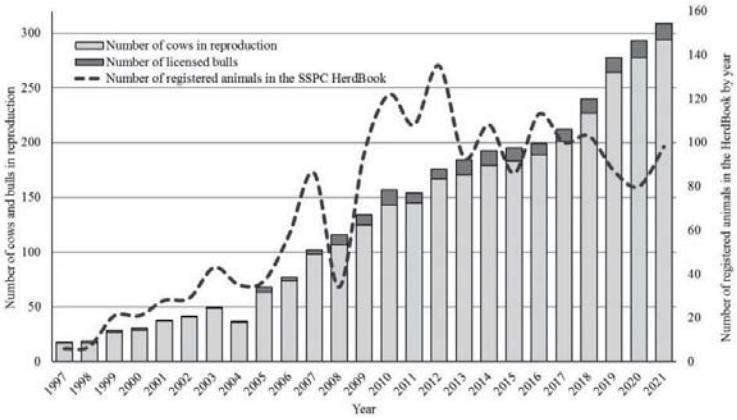

Figure 2. Population trends in the Slavonian-Syrmian Podolian cattle population in the period from 1997 to 2021 (number of licensed and new registered animals in the SSPC HerdBook)

Table 1. Demographic and genetic indices of the Slavonian-Syrmian Podolian cattle

Item	Total
Original dataset (TP; total population)	1,836
Base population (BP; one or more unknow parents)	107
Reference population 1 (REF 01; live animals)	573
Mean inbreeding coefficient (*F*)	5.13
Mean Average Relatedness coefficient (*AR*)	14.55
Ne via increase in inbreeding by equivalent generation (N_e)	18.85
Mean number of complete generations (*MCG*)	1.72
Mean number of equivalent generations (*MEG*)	2.71
Mean number of maximum generations (*MMG*)	4.94
Number of founders contributing to reference population	48
Number of ancestors contributing to reference population	46
Effective number of founders for the reference population (f_e)	9
Effective number of ancestors for the reference population (f_a)	8
Effective number of founder herds for the reference population (f_h)	1.9
Effective number of founders/effective number of ancestors (f_e/f_a)	1.12

An analysis of all pedigree entries revealed 1,729 animals for which both parents were known. The active living population consisted of 573 animals (Table 1). Mean number of complete/equivalent/ maximum generations (1.72 / 2.71 / 4.94) is relatively low, but sufficient for estimation and monitoring of the conservation status of the SSPC population.

A similar value of the mean number of complete/equivalent/maximum generations (1.79 / 2.99 / 5.81) was observed in the population of Istrian cattle (IVANKOVIĆ et al., 2022), because the official protection program was started at the same time. Compared to the values in the SSPC population, the mean number of maximum generations (5.81 vs. 4.94) is higher in the population of Istrian cattle (IC), because the informal IC protection program was started earlier (in 1989 the Breeders association for the protection of Istrian cattle was established), and previously established Istrian cattle HerdBook.

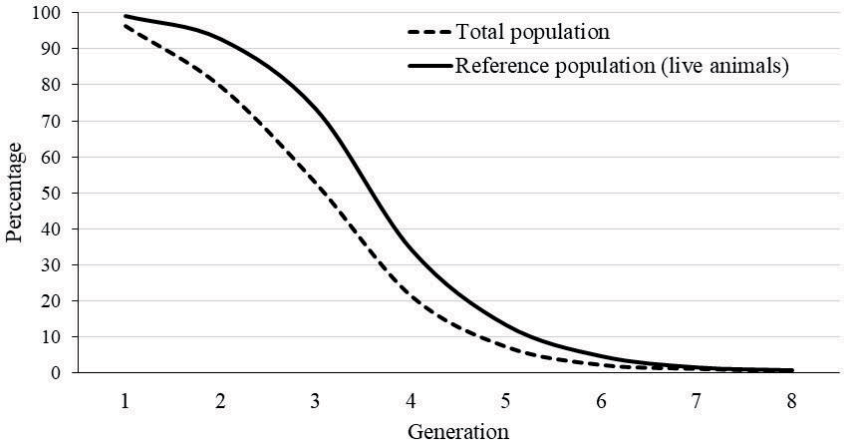

Figure 3. Completeness of total and REF 01 population (in %) in the Slavonian-Syrmian Podolian cattle from 2000 to 2020

The SSCP reference population (REF 01) had a completeness value in the first three generations (99.04%, 92.63%, and 96.66%), whereas completeness dropped to 34.26% in the fourth generation (Figure 3). The entire SSCP population had a lower completeness value in the first four generations (96.33%, 79.51%, 52.61%, and 21.32%). After the ninth generation, the completeness was close to zero.

The mean inbreeding coefficient in the whole population was 5.13%, and the stagnation of the *F* level during the last decade is noticeable (Figure 4). The *AR* coefficient in the population was relatively higher (14.55%) and continues to increase, indicating the need for more careful monitoring of the breed. Lower *F* and *AR* values and more favourable stagnation tendencies were observed in the population of Istrian cattle (IVANKOVIĆ et al., 2022), which puts the Istrian cattle population in a more favourable position in terms of long-term sustainability compared to the SSPC population.

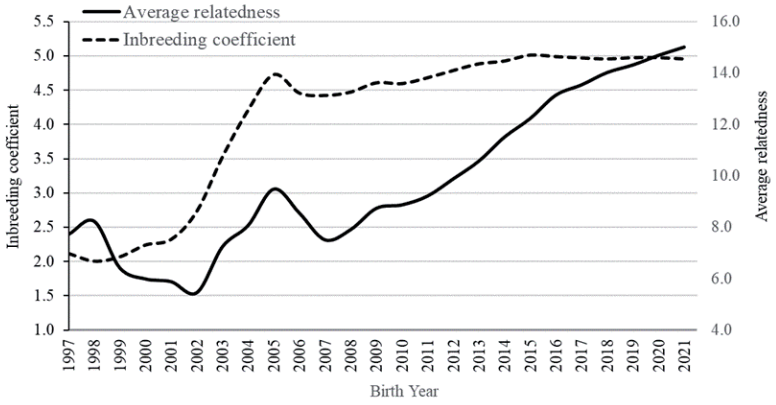

Figure 4. Level of inbreeding coefficient and average relatedness (in %) in the Slavonian-Syrmian Podolian cattle from 2000 to 2020

The number of founders in the SSPC was 48 while the effective number of founders was 9 or 18.75%. The effective number of ancestors was 8 or 17.39% of the total ancestors (46). The f_e/f_a ratio of 1.12 indicates a balanced use of breeding animals. Observed value of the f_e/f_a ratio in the population of Istrian cattle was lower (1.03; IVANKOVIĆ et al., 2022). BOICHARD et al. (1997) observe that f_e/f_a ratio close to 1 indicates lack of *bottlenecks* in the population.

The average generation interval was 6.34 ± 3.12 years and ranged from 5.84 ± 3.43 (sire-son) to 6.76 ± 2.98 (dam-son), with a longer average generation interval for dams than for sires (Table 2). CAROLINO and GAMA (2008) observed mean generation intervals of 6.0 and 6.8 years for the sire and dam pathways, respectively, in the "Alentejana" cattle breed in Portugal. In the Mertolenga cattle breed, the generation interval was 6.4 years for the sires and 7.1 years for the dams (CAROLINO et al., 2020). In the Spanish local cattle breeds "Berrenda en Negro" and "Berrenda en Colorado" *GI* were 6.50 and 6.92 years, respectively (GONZÁLEZ-CANO et al., 2022). The estimates of the average age of the parents at the birth of their offspring were generally higher in the maternal pathway (7.32 ± 4.00, dam - son ; 6.99 ± 3.86, dam - daugthter) compared to the paternal pathway (6.81 ± 3.19, sire - son; 6.58 ± 2.98, sire - daugthter).

Table 2. Average age of parents at the birth of their offspring and generation interval (years) for the Slavonian Syrmian Podolian cattle

Pathway	Average age		Generation interval	
	n	Mean ± SD	n	Mean ± SD
Sire — son	830	6.81 ± 3.186 ^{a}	28	5.84 ± 3.427
Sire — daughter	900	6.58 ± 2.981 ^{A}	363	6.05 ± 2.545^{a}
Dam — son	869	7.32 ± 3.999 ^{B,b}	29	6.76 ± 2.979
Dam — daughter	938	6.99 ± 3.865	382	6.63 ± 3.551^{b}
Overall	3537	6.92 ± 3.549	802	6.34± 3.118

a, A, b, B - different superscript letters in the columns show significant differences (lowercase letters $p < 0.05$; capitals $p < 0.01$)

Considering all the pathways, the average age of the parents at the birth of their offspring was equal to 6.92 ± 3.55 years. Significant differences were found in the generation interval on the '*sire – daughter* vs. *dam – daughter*' ($p < 0.05$), and in the average age of parents at the birth of offspring on the pathways '*sire – son* vs. *dam – son*' ($p < 0.05$) and '*sire – daughter* vs. *dam – son*' ($p < 0.01$).

As for the number of animals, the trend of the population is positive, as well as the increase in the number of herds to 35 (in 2021) and the stabilization of the average size of herds to nine adult animals per farm. The sex ratio in the population (cows/bulls) is 17.1 : 1 and is slightly higher than in the population of Istrian cattle.

Conclusion and recommendation

The Slavonian-Syrmian Podolian cattle population is subject to a systematic conservation program. In the last decade, the level of inbreeding stagnated in the last five years. Although BOICHARD et al. (1997) indicated that the *F* trend has some limitations, especially when pedigree completeness is low, the observed level of inbreeding requires careful monitoring of the population. The f_e/f_a ratio in the population indicates that there is no obvious bottleneck in the population. We assume that this is a consequence of the higher proportion of natural mattings in practice. The shallow depth, i.e., number of generations, of the SSPC pedigree is real, but all information on relationships among breeding animals is essential for optimizing conservation strategies. These results can be used as a guide for further implementation of effective conservation programs for endangered SSPC breed.

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Cryopreservation of *in vitro* produced blastocysts from endangered Pinzgau cattle breed

OLEXIKOVÁ, Lucia[1]* – DUJÍČKOVÁ, Linda[1, 2] – CHRENEK, Peter[1, 3] – MAKAREVICH, Alexander V.[1]

[1]Agricultural and Food Centre (NPPC) - Research Institute for Animal Production Nitra, Slovak Republic, 95141, Lužianky, Hlohovecká 2
[2]Constantine the Philosopher University Nitra, Department of Botany and Genetics, Nitra, Slovak Republic, 94901, Tr. A. Hlinku 1
[3]Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Nitra, Slovak Republic, 94976, Tr. A. Hlinku 2
*corresponding author: lucia.olexikova@nppc.sk

Abstract
Pinzgau cattle is a traditional livestock breed in Slovakia suitable for sub-mountain and mountain areas. Numbers of these animals have been declining. The Pinzgau Cattle was registered at the UN FAO as endangered by extinction. As a contribution to the *ex situ* strategies of conservation of Pinzgau cattle breed, our goal was to produce blastocysts of the Pinzgau cattle breed *in vitro* and to preserve them in the Gene Bank of Animal Genetic Resources. The ovaries from slaughtered Pinzgau cow were transported to the laboratory. The oocytes, isolated by aspiration of ovarian follicles, were matured *in vitro* and subsequently fertilized using a frozen sperm of a Pinzgau bull. Presumably fertilized zygotes were cultured for 6-8 days until the blastocyst stage. Totally, 20 blastocysts have been produced so far. Of them, 17 good quality blastocysts were vitrified using an ultra-rapid cooling method. Cryopreserved blastocysts were placed for storage in the Gene Bank of Animal Genetic Resources and entered into the CryoWeb database.

Keywords: Pinzgau cattle, IVF, blastocyst, cryopreservation

Introduction

Slovak Pinzgau Cattle is an undemanding, healthy and hardy breed with good ranging ability, suitable for sub-mountain and mountain areas. The beginning of breeding of the Pinzgau breed in our region dates from 1870 to 1880. In 1994, the Pinzgau Cattle was registered at the UN FAO as endangered by extinction. Pinzgau cattle in Slovakia have a long tradition, but their numbers have been declining for a long time. At present, there are less than 11000 animals. For comparison, while in 1989 more than 90,000 cows of this breed were bred in Slovakia, in 2014 there were about 2,700 purebred cows registered in the studbook of Pinzgau cattle, now it is only 2,024 cows. Therefore, this breed is among the endangered in our country. In our laboratory, we decided to contribute to the *ex situ* strategies of conservation, by creating embryos of this breed *in vitro* and their preservation in the Gene bank of animal genetic resources.

Such an approach seeks to be applied in the *ex situ* protection of several species and breeds of endangered animals. *In vitro* fertilization is used not only in cows, where this methodology is best developed. Similar methodologies have been adapted and implemented for different endangered species of livestock, domestic and wild animals. Methods of *in vitro* embryo production are described also in buffaloes (SUTEEVUN et al., 2006), horses (RODRIGUES and RODRIGUES, 2006), camel (KHATIR et al., 2004), antelope (MAHESH et al., 2011), roe deer (COMIZZOLI et al., 2021), in canine and cats (VANSOOM et al., 2014), as well as in other animal species (ANDRABI and MAXWELL, 2007).
These results show that it is possible to preserve the valuable genetic resources from a declining population and also to contribute to the improvement of genetic diversity by creating new individuals from new parent pairs *post-mortem*. Especially in combination with the use of cryopreserved male insemination doses, it is possible to produce their progeny *in vitro* for long period *post-mortem*. To enhance the genetic progress, both by selection of the most genetically valuable individuals and by increased number of offspring from these individuals is the aim of many further improvements in these reproductive technologies (SJUNNESSON, 2020).
Our laboratory participates in cooperation with several Pinzgau cattle breeders in Slovakia. Based on the agreement, the breeders provide us with information about planned slaughters of cows. After slaughter, the ovaries of such a cow are then transported to the laboratory. The oocytes obtained from them are matured *in vitro* and fertilized using an insemination dose of a Pinzgau bull registered in a Breeding book of Pinzgau Cattle. The aim of this work was to produce blastocysts *in vitro* as a gene resource of Pinzgau cattle breed and to preserve them in the Gene Bank of Animal Genetic Resources.

Material and methods

All the chemicals used in this study were purchased from Sigma-Aldrich Inc. (Saint-Louis, Missouri, USA), unless otherwise indicated.

Biological material
The ovaries were collected at slaughtering of one Pinzgau cow at the age of about 8 years and 6 lactations, kept at the Agricultural farm "PD Trstenik" (Trstena, Slovak Republic). Oocytes from an undefined population of cows slaughtered in a local slaughterhouse served as a control group.

Oocyte retrieval and in vitro maturation (IVM)
The ovaries were transported to the laboratory, washed with 70 % ethanol and then with sterile saline. The oocytes were recovered from antral follicles (2-8 mm) by the aspiration of follicular fluid using sterile 5 mL syringe with a needle (18 gauge). Cumulus-oocyte complexes (COCs) were collected into a Petri dish with a holding medium (M199-HEPES with 10% foetal bovine serum - FBS) and only COCs with several layers of cumulus cells and homogeneous ooplasm were selected for IVM. COCs were matured for 23 h in a maturation medium containing M 199 (Gibco), sodium pyruvate (0.25 mmol.L^{-1}), gentamycin (50 mg. mL^{-1}), 10% FBS and FSH/LH (1/ 1 I.U., Pluset) at 38.5°C and 5% CO2.

In vitro fertilization (IVF) of oocytes and embryo culture
Morphologically good-looking oocytes after IVM were washed in IVF-TALP medium (Tyrode-Albumin-Lactate-Pyruvate solution with 10 mg. mL^{-1} heparin, 50 mg. mL^{-1} gentamycin) and put into 100 µml droplets of IVF-TALP medium under a mineral oil, where the sperm (at $2x10^6$ per mL) and PHE solution (20 mM penicillamine, 10 mM hypotaurine,

1 mM epinephrine) were previously added, and incubated for 18 h at 38.5°C in 5% CO_2. Following IVF, presumptive zygotes were vortexed in an Eppendorf tube containing 0.5 mL of a holding medium for 30 s to remove residual cumulus cells. Denuded zygotes were transferred to the dish with the BRL-1 cell (Rat epithelial cell line, ECACC) confluent monolayer in a culture medium (prepared according to the Menezo B2 composition) with 10% FBS. Developmental ability was determined by cleavage (Day 2) and blastocyst (Days 7 to 9) rates.

Cryopreservation of blastocysts

For cryopreservation of *in vitro* produced embryos, ultra-rapid cooling technique in a minimum volume on electron microscopy grid was used (OLEXIKOVA et al. 2020). Selected embryos were washed in M199-HEPES medium with 10 % FBS. Then they were placed into equilibration solution (ES): 7.5 % ethylene glycol (EG), 7.5 % dimethyl sulfoxide (DMSO) in M199-HEPES, supplemented with 20 % FBS for 3 min. Following equilibration, the embryos were transferred to vitrification solution (VS): 16.5 % EG, 16.5 % DMSO and 0.5 M sucrose in M199-HEPES with 20% FBS at room temperature (RT) for 30 s. The embryos (2 - 4) in a small drop were transferred onto 300 mesh nickel electron microscopy grids with a glass micropipette. An excessive medium was removed by a filtration paper and then the oocytes were immediately plunged into liquid nitrogen for storage.

Results and Discussion

We present here a preliminary data, as to date only one experiment with two ovaries of a Pinzgau cow was performed (Table 1). From these ovaries, 62 cumulus-oocyte complexes (COCs) morphologically suitable for *in vitro* maturation were recovered by follicular fluid aspiration. All of these COCs following IVM were fertilized in vitro and 61.29% of them started cleavage. The cleavage rate of Pinzgau oocytes was slightly lower ($p<0.05$) than that of the oocytes derived from an undefined population of cows (control group). However, the blastocyst rate did not differ significantly between groups (Table 1).

Table 1. Preimplantation embryo development after IVF of cow oocytes

Group	IVF oocytes, n	Cleavage rate, n (%)	Blastocyst D6, n	Blastocyst D7, n	Blastocyst D8, n	Total blastocyst rate, n (%)
Ovaries	62	38 (61.29)	3	14	3	20 (32.26)
Control*	60	45 (75.00)	7	8	7	22 (36.66)

* slaughterhouse-derived ovaries from an undefined cow population

Currently, an average blastocyst rate in group culture systems is usually in the range of 30−40% from all IVF oocytes (CATTEEUV et al., 2017). Therefore, the blastocyst percentage obtained in our work (32.26%) is comparable to above reported results, what supports a validity of the protocol of in vitro embryo production in our study.

The in vitro fertilization method and embryo production are important reproduction tools in cattle breeding worldwide. According to the annual International Embryo Technology Society (IETS) statistics, in the last five years, an average of almost 470,000 bovine embryos produced *in vitro* were transferred worldwide annually (FERRÉ et al., 2020). However, most of them were directly transferred fresh without cryopreservation. It was probably caused by their lower cryotolerance compared to their *in vivo* counterparts. An important aspect of applied *in vitro* embryo production system is the use of somatic cell co-culture systems, because under this co-

culture system the embryos were more suitable for freezing, in comparison to other (cell-free) culture systems (HASLER et al., 1997). Good cryotolerance of this embryos is important for high survival rate after vitrification and high pregnancy rates after embryo transfer (FERRÉ et al., 2020).
Taking into account a reported average pregnancy rate after transfer of vitrified/thawed blastocyst in the range of 40-56% (XU et al., 2006; ZHANG et al., 2015), our yield of 17 cryopreserved blastocysts represents future potential of 6-9 new individuals of the Pinzgau breed of known origin.

Conclusion and recommendation

The described methodology of *in vitro* production of bovine embryos allowed us to extent the reproductive potential of a cow from Pinzgau breed after culling by preserving blastocysts in the Gene Bank of Animal Genetic Resources.

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Can we make our agriculture more sustainable by reintroduction of horse traction and broader reliance on the on-farm produced fertilizers?

GANTNER, Ranko[1]* – ZIMMER, Domagoj[1] – DEL VECHIO, Igor[2] – SCHLECHTER, Pit[3] – HERMAN, Goran[1]

[1]Josip Juraj Strossmayer University in Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, HR-31000 Osijek, Croatia
[2]Croatian Federation of Heavy Draft Horse Breeders Association, Jelengradska 13, HR-44317 Popovača, Croatia
[3]FECTU asbl. (The European Draught Horse Federation), rue principale 21, L-7475 Schoos, Luxembourg
*corresponding author: ranko.gantner@fazos.hr

Abstract

Soil compaction and rise of fuel and mineral fertilizers prices are probably the most important problems that modern arable farmers face. Modern soil compaction is mainly induced by the use of heavy machinery, extensive use of mineral fertilizers and lack of organic fertilizers like farmyard manure (FYM) and green manures (GM). Soil compaction is associated with poorer water soaking capacity and more pronounced drought effects. Luckily, a research conducted in Ohio (USA) has revealed that traditional Amish horse-powered farm has soil with greater water infiltration rate than neighbouring no-till cultivated soil. Considering the use of mineral fertilizers, their more than a three-fold rise of prices during the last year may push the farmers to search for a cheaper alternative, like on-farm produced fertilizers (FYM and GM). The contemporary circumstances can give rise to a question if biologically improved traditional agriculture can offer solutions to the presented problems. In the sense of this work, traditional agriculture means agrotechnical measures powered by animal traction, while the biological improvement means broader use of green manuring and cover cropping.

Key words
agriculture, sustainability, animal traction, green manure, farmyard manure

Introduction

Sustainable development of humanity and agricultural production which supports us have come into a focus of our concerns. Among the 17 sustainable development goals of UN, responsible consumption and production of food falls into SDG #12, while transitioning to affordable and clean fuels falls into SDG #7. Prospective exhaustion of distant and finite sources of fossil fuels and phosphorus fertilizers additionally stresses the importance of change of our farming into more sustainable way. In this work we try to elucidate if reintroduction of animal power in arable farming and broader reliance onto on-farm produced fertilizers can improve the sustainability of modern agriculture.

Material and methods

Research is based on the relevant literature review.

Results and discussion

Responsible and sustainable food production should be based on farming practices that conserve, or even better, improve soil quality and fertility. However, high mechanical load from modern farm machinery, especially when combined with low soil organic matter, leads to soil compaction which furtherly decreases soil porosity, aggregate stability, soil hydraulic conductivity and nutrient availability (NOOR SHAH et al., 2017). Mentioned effects in their combination negatively affect the overall soil fertility. Luckily, a research conducted in Ohio (USA), that compared soil quality under traditional horse-powered cultivation by Amish farmers and modern no-till production has revealed more favourable soil properties in Amish farm (JACKSON, 1988). Namely, the Amish farm exhibited significantly higher rates of rainfall infiltration, more organic matter content, higher levels of alkaline phosphatase activity, and lower bulk density of soil than the no-till farm that produced corn and alfalfa. Lesser soil compaction by much lighter animals and implements in Amish farm than tractors and implements in modern no-till farm is likely to be among the reasons for better soil properties in the Amish farm. What is furtherly interesting in the mentioned research, is that both, Amish and no-till farm produced corn yields well above the state average, thus showing that traditional horse-powered cultivation does not limit the achievement of modern high yields. Recent rise of fossil fuels prices is likely to give an additional moment to consideration of horse-powered agriculture as a more sustainable option since it uses locally produced fodder as a biofuel (GANTNER et al., 2017). Thus, the animal traction (figures 1 to 4), contemporarily considered as only of historical importance, may become a mean for modern agriculture improvement, at least for small farms.

Use of green manures for building the soil fertility is currently very rare in majority of conventional farms in Croatia and worldwide. But a recent dramatic rise of mineral fertilizers prices, along with previously known their detrimental effects to environment (GUTIÉRREZ, 2012), and together with modern knowledge on the benefits of cover-cropping and green manuring (COUËDEL et al., 2018; ZHANG et al., 2019) are likely to encourage the modern farmers to increasingly rely on the on-farm produced fertilizers, like green-manures and farmyard manure. However, green manuring is necessary associated with additional agricultural operations and energy consumption for the establishment of green manure crop and its either termination or incorporation into a soil (AMBRUŠEC et al., 2021), depending on the cropping system (no-till or conventional). In the contemporary circumstances of steadily rising fossil fuels prices, additional operations associated with green manuring can act discouraging. Anyway, if a farmer takes animal traction into a consideration for powering his crop production, rise of fossil fuel prices becomes less important since the on-farm produced fodder acts as a biofuel, which has already been estimated as a cost-effective alternative to diesel fuel (GANTNER et al., 2017).

Among the on-farm produced fertilizers, farmyard manure is also of great importance since it provides plant nutrients and high-quality organic matter for feeding the soil microbiome, and thereafter improving the soil quality. In a field research of BENBI et al. (1998) there was proved that application of farmyard manure increases the soil organic carbon content, water infiltration rate, water retention, soil aggregation and soil aggregate stability in water. Also, farmyard manure application enables the achievement of high maize grain yields (8 to 9.8 t/ha in Ontario,

Canada, MA et al., 1999) and high maize herbage yields (18.8 to 21 tons of dry matter per ha in Michigan, USA, BASSO and RITCHIE, 2005).

Conclusions

Presented findings indicate that reintroduction of horse traction into a field crop production and a broader reliance on green manuring and use of farmyard manure can much contribute to the sustainability of agriculture in the contemporary circumstances of steady growth of purchase prices of mineral fertilizers and fossil fuels. Rising consciousness of needs for soil and environment conservation and improvement can give additional moment to the broader acceptance of animal traction and on-farm produced fertilizers.

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The wool quality of Jezersko-Solčava sheep in Slovenia

BIZJAK, Marko – ŽAN, Metka* – BOJKOVSKI, Danijela – SIMČIČ, Mojca

Department of Animal Science, University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Ljubljana, Slovenia
*corresponding author: metka.zan@bf.uni-lj.si

Abstract

The Jezersko-solčava sheep is one of five indigenous Slovenian sheep breeds. It is a dual-purpose breed known for the lamb and wool production. However, some breeders process all the produced fleeces at home, and quality of the wool is subjectively estimated by touch and appearance. Therefore, the study was conducted to evaluate the wool quality in young rams of Jezersko-Solčava sheep that finished the performance test for growth traits in the period 2018 - 2020 in two test stations: 447 rams from Logatec and 91 rams from Jezersko. Wool samples were classified into four quality classes according to the fibre diameter. The majority of rams (44.80%) had coarse (26.01-30.00 µm) and 47.58 %) very coarse wool (>30.00 µm). We found that most wool quality traits as well as raw fleece weight were significantly affected by the year of shearing, the month of shearing, test station, age of the ram and flock of origin.

Keywords: sheep, Jezersko-Solčava sheep, wool, quality

Introduction

Jezersko-Solčava sheep is the indigenous sheep breed in Slovenia with the highest population size (Register, 2021) of about 5,564 animals and is classified as a vulnerable breed according to the FAO criteria. The Jezersko-Solčava sheep is well adapted to the local agroclimatic and geophysical conditions and is geographically widespread over the entire territory of Slovenia. The areas of origin of Jezersko-Solčava sheep are Jezersko and Solčava in the northern part of Slovenia. There, the breed has been preserved as it first appeared centuries ago and has been a well-known and valued breed since the second half of the 19th century.
Jezersko-Solčava sheep was created by improving the white domestic sheep of the eastern Alps with Bergamasca and Paduan sheep (KOMPAN et al., 1996). It has a typical convex nose profile, which is inherited from Bergamasca sheep, and good-quality wool, which is inherited from Paduan sheep (Figure 1). Jezersko-Solčava sheep has the highest wool quality compared to other indigenous Slovenian sheep breeds (SIMČIČ et al., 2019).

Figure 1. Wool products from Jezersko-Solčava sheep

In Slovenia, there are two test stations for rams: the test station in Logatec and the test station in Jezersko, where is one of two original areas of Jezersko-Solčava sheep. On the test station Logatec the feed ratio consists of hay *ad libitum* and concentrates up to 1 kg, while on the test station Jezersko the main feed ratio presents grazing on the pastures around the test station.
Wool is a natural fibre with a unique combination of properties used in the clothing industry. The wool industry, especially the production of fine wool, plays an important role in the world trade, and the price of wool depends on its quality (TAYE et al., 2021). Sheep wool is a natural animal fibre composed of 60% protein fibres, 15% moisture, 10% fat, 10% sheep sweat and 5% impurities (PARLATO et al., 2022).
Key wool characteristics include fibre diameter, comfort factor, fibre curvature, spinning fineness, staple length, staple strength and clean fleece yield (ANDERSON et al., 2009). Fibre diameter refers to the average width of a single cross-section of wool fibre and is widely recognised as the most important wool characteristic in evaluating wool quality and value (HOLMAN and MALAU-ADULI, 2012).
There are several factors that influence the parameters of wool quality. Environment and genetics are the most important factors affecting the quality and quantity of sheep wool. Breed or genotype is one of the most important genetic factors affecting the product and productivity as well as the quality of sheep wool, i.e. the fleece of different breeds differs in its physical and chemical characteristics (TAYE et al., 2021).
Scientific knowledge about the quality characteristics of Jezersko-Solčava sheep wool was very limited. Therefore, the aim of this study was to determine in which quality classes the wool of young rams could be classified and which factors affect the wool traits (fibre diameter, comfort factor, spinning fineness, fibre length and fibre curvature) and the raw fleece weight.

Material and methods

Data for Jezersko-Solčava rams were obtained from the Slovenian National Breeding Program for small ruminants recorded, according to the ICAR standards. Data included ID number, the flock of origin, date of birth, test station, shearing date, fleece weight, and the results of laboratory analysis of wool samples.
The study was conducted to evaluate the wool quality in young rams of Jezersko-Solčava sheep that finished the performance test for growth traits in the period 2018 - 2020 in two test stations: 447 rams from Logatec and 91 rams from Jezersko. Rams originated from 50 different flocks which are included in Slovenian National Breeding Program for Jezersko-Solčava sheep. On the test stations where rams were housed, there were uniform farming conditions which could influence quality traits of wool as well. The average age of rams at shearing was 266.69 ± 36.68 days. Rams were sheared according to Bowen's method. After shearing, the fleece of each ram was weighed and the wool sample was taken for laboratory analyses. Wool sample was taken from the left side of ram, in the middle of body, on an area between 30th and 31th cut according

to Bowen method (Figure 2). All wool samples were individually packed in plastic bags, marked and sent to the laboratory Art of fibre (https://artoffibre.com/) to Finland or Great Britain, respectively. The chosen laboratory has implemented and certificated method of determination of wool quality traits. These traits are fibre diameter, comfort factor, spinning fineness, fibre length, and fibre curvature.

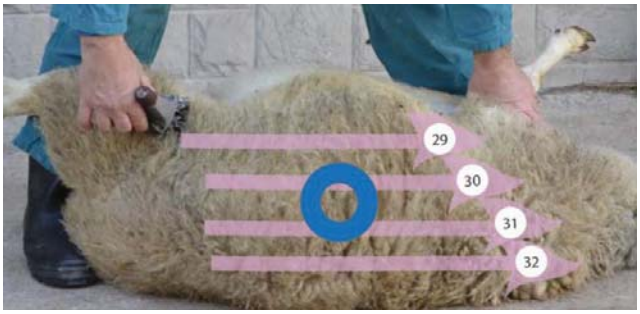

Figure 2. Location of fleece sampling on the body of the ram

Data were analysed with the MIXED procedure using SAS/STAT® Software Version 9.4 (SAS/STAT, 2016). The statistical model for wool quality traits included test station, year of shearing, and month of shearing as fixed effects while age at shearing was included as a linear covariate. In the model, there was included a random effect of flock of origin as well.

Results and discussion

The descriptive statistics for wool characteristics of young Jezersko-Solčava rams are presented in Table 1. The average raw fleece weight per ram was 1.58 ± 0.44 kg (Table 1). The average fibre diameter was 29.96 ± 2.72 µm and the average spinning fineness was 29.56 ± 2.84 µm. The average comfort factor was 57.81 ± 15.63%. On average, the wool fibres were 80.90 ± 20.10 mm long and the average fibre curvature was 48.70 ± 8.36 degrees/mm.

Table 1. Descriptive statistics for wool traits of young rams of Jezersko-Solčava breed

Wool characteristics	Number of samples	Average	Standard deviation	Min	Max
Fleece weight (kg)	505	1.58	0.44	0.64	3.00
Fibre diameter (µm)	538	29.96	2.72	22.90	43.70
Comfort factor (the proportion of fibres < 30 µm, %)	538	57.81	15.63	7.70	93.60
Spinning fineness (µm)	488	29.56	2.84	22.60	42.50
Fibre length (mm)	538	80.90	20.10	30.00	140.00
Fibre curvature (degrees/mm)	538	48.70	8.36	28.40	86.50

Min – minimum, Max – maximum

Based on the fibre diameter, the wool (fleece) of each ram was classified into different quality classes. The Art of Fibre laboratory (https://artoffibre.com) has introduced six quality classes of wool: ultra-fine, super fine, fine, medium, coarse and very coarse (Table 2).

Table 2. Wool quality classes according to the fibre diameter

Colour code	Wool quality classes	Fibre diameter (μm)
Yellow	Ultra-fine	< 18,01
Blue	Super fine	18,01 – 20,00
Pink	Fine	20,01 – 23,00
Red	Medium	23,01 – 26,00
Green	Coarse	26,01 – 30,00
Purple	Very coarse	> 30,00

For the production of wool products, the fineness of the wool fibre is a very important characteristic, that could not be accurately estimated only by the touch.
Fibre diameter is the width of the cross-section of the fibre, assuming that fibres are approximately cylindrical (SCOBIE et al., 2015). It is the single most important characteristic of wool that determines its price and largely the products for which it is used. Wool with a smaller fiber diameter (23 μm or less) can be processed into finer yarn.

Wool samples from rams of Jezersko-Solčava sheep were classified into four quality classes according to the fibre diameter. One ram (0.19%) had fine wool (20.01-23.00 μm), 40 rams (7.43%) had medium wool (23.01-26.00 μm), 241 rams (44.80%) had coarse wool (26.01-30.00 μm) and 256 rams (47.58%) had very coarse wool (>30.00 μm) (Figure 3). Wool of medium quality was the best quality class of Jezersko-Solčava sheep (ram with fine wool was an exception).

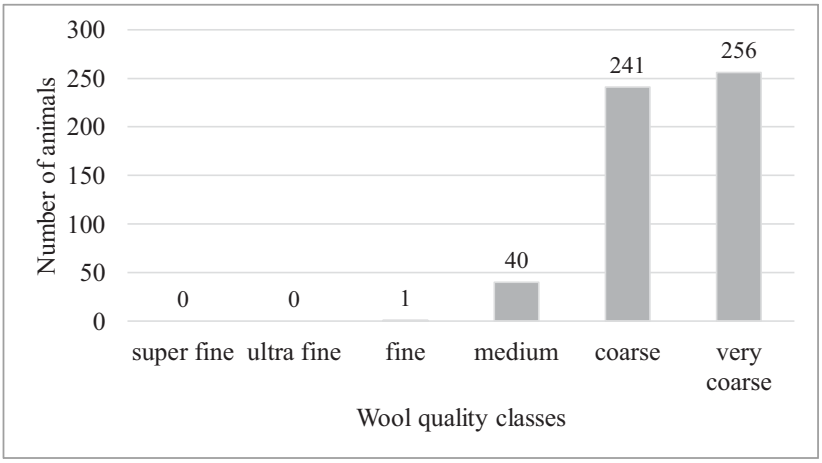

Figure 3. Number of rams of Jezersko-Solčava sheep classified in quality classes according to the fibre diameter

Wool quality is influenced by several management factors namely management, feeding, collection period, season of shearing and shearing methods which can affect the amount of clean fleece that can be obtained from sheep and its quality (TAYE et al., 2021). In general, many physiological (e.g. reproduction, sex), and environmental factors (production system, feeding, housing etc.) influence the quality and quantity of wool produced by sheep farms. These factors must be managed to optimize wool production and quality. Among the management strategies; shearing period, disease control, slaughter timing, feeding, pasture

management and reproduction management are the factors that need to be improved for better wool quality. In Table 3 are presented factors that significantly affected individual characteristics of wool quality of rams of Jezersko-Solčava sheep: test station, shearing period (year, month), age of the ram at shearing and the origin of the flock.

Table 3. Factors affecting wool quality parameters

Trait	Factor				
	Test station	Year of shearing	Month of shearing	Age of ram at shearing	Flock of origin
Fleece weight (kg)	√	√	√	√	√
Fibre diameter (μm)	√	√	√		√
Comfort factor (the proportion of fibres thinner than 30 μm %)	√	√	√		√
Spinning fineness (μm)	√	√	√		√
Fibre length (mm)	√		√	√	√
Fibre curvature (degrees/mm)		√	√		√

It is evident that the month of shearing and flock of origin affected all wool quality parameters as well as fleece weight. Test station affected fleece weight and all wool quality parameters with an exception of fibre curvature while the year of shearing affected fleece weight and all wool quality parameters with an exception of fibre length. On the other hand, the age of ram at shearing affected just fleece weight and fibre length. A strong correlation was found between fibre diameter and comfort factor, between fibre diameter and spinning fineness, and between comfort factor and spinning fineness.

The flock of origin was also included in the analysis as a random effect because we predicted differences between flocks of breeders using wool for products and flocks of breeders not using wool for products. Previously, the subjective evaluation of wool from Jezersko-Solčava sheep was introduced, so the differences were expected.

Table 4. Proportion of phenotypic variance explained by random influence of the flock of origin

Trait	Proportion of explained variance
Fleece weight (kg)	0.16
Fibre diameter (μm)	0.09
Comfort factor (%)	0.11
Spinning fineness (μm)	0.05
Fibre length (mm)	0.09
Fibre curvature (degrees /mm)	0.12

The highest proportion of explained variance with the flock of origin was for fleece weight (0.16), followed by fibre curvature (0.12) and comfort factor (0.11). Fibre diameter and fibre length had the same proportion of explained variance with the flock of origin (0.09). The lowest proportion of explained variance with the flock of origin was for spinning fineness (0.05; Table 4).

Conclusion and recommendation

The wool quality traits of Jezersko-Solčava sheep are highly variable. Therefore, breeders have decided to include the trait fibre diameter as a breeding objective in the breeding programme. In addition, in the areas of origin of the Jezersko-Solčava sheep, in the Jezersko and Solčava areas, there is a strong association called Bicka, which processes wool and sells various products such as knickers, clothing, bags, toys, jewellery and various other products.
Knowing the quality characteristics of the wool can help to control the final products, consumer comfort and processing intensity. Therefore, it is important to understand the factors that affect the physical and chemical properties of wool in order to improve the quality of wool through genetic and management conditions.

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Vergleich zweier Nachfolgerrassen des Zaupel-Schafs, Cikta und Waldschaf, basierend auf ihrer mtDNS Kontrollregion

FOGARASSY, Leonie[1] – BERGER, Beate[2] – ZENKE, Petra[1] – KOVÁCS, Endre[1] – SÁFÁR, László[3] – BECSKEI, Zsolt[4] – MARÓTI-AGÓTS, Ákos[1] – GÁSPÁRDY, András[1]*

[1]Institut für Tierzucht, Tierernährung und Labortierkunde, Veterinärmedizinische Universität Budapest, István Str. 2, 1078 Budapest, Ungarn
[2]Institut für Biologische Landwirtschaft und Biodiversität der Nutztiere, Austraße 10, 4601 Wels-Thalheim, Österreich
[3]Verband für Ungarische Schaf- und Ziegenzüchter, Lőportár Str. 16, 1134 Budapest, Ungarn
[4]Lehrstuhl für Tierzucht und Genetik, Tierärztliche Fakultät, Belgrad Universität, Bulevar Oslobođenja 18, 11000 Belgrad, Serbien
*Korrespondenzautor-E-Mail: gaspardy.andras@univet.hu

Zusammenfassung

Das Ziel dieser Arbeit ist es, den genauen genetischen Hintergrund und die dazugehörige Abstammung von heimischen Schafrassen zu erforschen, erhalten und in einer genetischen Datenbank zu archivieren. In dieser Studie liegt der Schwerpunkt auf dem direkten Vergleich des Cikta und des Waldschafes. Für diesen Zweck wurde das Erbgut der beiden Rassen mit der Kontrollregion in der mtDNS verglichen. Die biologischen Proben für diese Studie wurden zwischen den Jahren 2015 und 2017 aus insgesamt 97 Individuen entnommen, 70 aus Cikta (Ungarn) und 27 aus Waldschaf (Österreich).
Im Vergleich von Cikta und Waldschaf betrug die Gesamtzahl der polymorphen Stellen 125; die Anzahl der gemeinsamen Mutationen betrug 51, was eine bedeutende genetische Identität zwischen den Rassen zeigt. Das Ergebnis des D-Tests von Tajima war -0,47672 und zeigte keine Signifikanz (P>0,10) sowie der Wert der Fs-Statistik von FU und LIs (0,76212), der ebenfalls als nicht signifikant gewertet wird. Diese befinden sich weiterhin im genetischen Gleichgewicht und eine Abweichung davon gefährdet die untersuchte Gemeinschaft der Zaupel-Derivate nicht. Diese Studie bestätigt die gemeinsame Abstammung von Cikta und Waldschaf, welche für ihre Zuchtgeschichte bekannt sind.

Schlüsselwörter: Zaupel, Cikta, Waldschaf, mtDNS Kontrollregion

Einleitung

Das Bewahren einer genetischen Vielfalt ist einer der wichtigsten Aspekte, um gesunde, aber auch resistente Tiere hervorzubringen. Um diese Anforderung zu erfüllen, versucht man heimische Rassen wiederherzustellen, Standardrassen einzukreuzen und so Gesundheit, aber auch Widerstandsfähigkeit zu gewährleisten und die zukünftige Reproduktion zu erhöhen.
Mit diesem Anliegen haben wir Blutproben von mehreren Individuen zweier Rassen von Schafen untersucht, von denen angenommen wurde, dass sie mit dem ausgestorbenen

Zaupelschaf verwandt sind. Bei dem ausgestorbenen Zaupel gibt es einen gewissen Radius heute noch lebender Rassen, welche als engste Nachkommen anerkannt sind. Es umfasst das Cikta-Schaf, das Bovec-Schaf, das Bergschaf und das Bayerische Waldschaf. Konkret basierte unsere durchgeführte umfassende Untersuchung auf dem Vergleich der mutierten nuklearen (Mikrosatelliten, KOVÁCS et al., 2019) und mitochondrialen (Zytochrom b Gen, KOVÁCS et al., 2020a; Kontrollregion, KOVÁCS et al., 2020b) Material innerhalb der Rasse Cikta. Daher ist an dieser Stelle der Studie der Vergleich innerhalb der Rassen essenziell. Die phänotypischen Gemeinsamkeiten aber auch die Veränderungen in der DNS gaben uns die Möglichkeit, die gemeinsame Abstammung zu studieren und anschließend die Entwicklung der Schafrassen nachzuverfolgen. HEINZ et al. (2021) waren die ersten, die Cikta und Bovec Rassen basierend auf der Kontrollregion verglichen. In unserem Fall wurde das autochthone Cikta-Schaf mit dem Waldschaf als Nachfolgerrassen des ausgestorbenen Zaupel-Schafs verwendet. Das Zaupelschaf (*Ovis aries germanicus rusticus*) ist eine Mehrzweckrasse, welche im Mittelalter aktiv zur Gewinnung von Fleisch, Wolle und Milch genutzt wurde. Bis das Merinoschaf das Zaupel aufgrund einer angenehmeren Wolle ersetzte, waren Zaupel-Schafe die am weitesten verbreitete Rasse Europas. Unser Ziel dieser Studie ist es, andere Nachkomme der Zaupel-Schafe zunächst anhand ihrer phänotypischen Merkmale zu erkennen und daraufhin zu extrahieren, sie dann aber anhand ihrer genetischen Komponenten zu verifizieren. Dazu wird mit Hilfe modernster molekulargenetischen Technik gleiche Abstammung nachgewiesen, um auf eine gemeinsame Herkunft zu schließen. Um dies umzusetzen, haben wir die Unterschiede im mütterlichen Erbgut von zwei der drei bekannten Zaupel-Nachkommen untersucht: Cikta und bayrisches Waldschaf. Dazu wurde die Reihenfolge der Sequenzen der variablen Kontrollregion (CR) der mtDNS untersucht und anschließend genauer ausgewertet. Durch unsere erzielten Ergebnisse möchten wir die gleiche genetische Abstammung der verschiedenen historischen Rassen nachweisen und belegen. Jedoch ist es auch unser Ziel, eine Grundlage für die weitere Untersuchung und Forschungen der genetischen Eigenschaften der Zaupel-Derivate zu schaffen, um in Zukunft in diesem Bereich noch intensiver forschen zu können.

Materialen und Methoden

Im Jahr 2015 wurde aus drei Herden des Cikta-Schafes eine bestimmte Anzahl von Individuen ausgewählt, welche Teil dieser Studie wurden. Der erste Privatbetrieb wird von Herrn T. Nagy in Pénzesgyőr (Ungarn) geführt, der 11 Schaffamilien besitzt. Der zweite private Bauernhof gehört Herrn J. Jánosi in Szécsénke (Ungarn), dieser besitzt 5 Schaffamilien. Die dritte Farm ist der staatliche Duna-Dráva-Nationalpark in Nagydorog mit einem Besitz von 20 Schaffamilien. Jedes Tier wurde sorgfältig nach Mutterschaft beziehungsweise der mütterlichen Linie ausgewählt. Der Probensammlung ging die Verarbeitung des gesamten Stammbuches voraus (POSTA et al., 2019), dann wurde das „Gründer-Sampling“ verwendet (MARÓTI-AGÓTS et al., 2008), das die Zugehörigkeit der beprobten Individuen basierend auf der mütterlichen Abstammung berücksichtigt.
Die Schafe besitzen eine 6-5-4 Ahnengeneration, welche auf die 36 ältesten Schaffamilien zurückführbar sind. Aus jeder Urfamilie von Schafen wurden, in fast allen Fällen zwei Vertreter für die Probensammlung erwählt, sodass sich eine Gesamtzahl von 70 Exemplaren ergab. Das Sammeln der Proben begann im Herbst 2015. Das Blut wurde aus der Jugularvene (*vena jugularis*) entnommen und in einem EDTA Blutröhrchen (Ethylendiamintetraazetat) aufbewahrt (RABE, 2017).
Die zweite, in dieser Studie untersuchte Vergleichsrasse war das Waldschaf. Für gemeinsame Auswertung stellte ÖNGENE (Österreich) 27 aufgereinigte individuelle DNS-Proben dieser

Rasse zur Verfügung. Die Individuen, die die Probe abgegeben haben, waren nicht eng miteinander verwandt.
Den Anweisungen des Herstellers folgend wurde die mtDNS unter Verwendung des SIGMA GenElute Blood Genomic DNA Kits isoliert. Der Kontrollregion wurde zwei von HIENDLEDER et al. (1998) und GÁSPÁRDY et al. (2021) entworfenen Primerpaare zugewiesen. Ein programmierbares Thermal Cycler 2720 PCR-Instrument (Applied Biosystem) wurde verwendet, um das DNS-Segment zu amplifizieren. Die Sanger-Sequenzierung der Kontrollregion wurde in beide Richtungen durchgeführt, was zur Bewertung einer 1180-bp-Region (15437–16616) aus 97 Probe führte. Die resultierenden Sequenzen wurden mit MEGAX abgeglichen (KUMAR et al., 2018).
Mit der Software DnaSP v6.0 konnten wir die Anzahl der polymorphen Stellen in der gesamten Probe bestimmen und die mittlere Nukleotiddifferenz innerhalb und zwischen den Herden berechnen (ROZAS et al., 2017). Wir verwendeten zur weiteren statistischen Verarbeitung den von FU und LI vorgeschlagenen Test (FU und LI, 1993), die JUKES und CANTOR-Methode (JUKES und CANTOR, 1969) sowie der TAJIMA-Test (TAJIMA, 1989). Die Rassenverteilung der CR-Haplotypen wurde mit der Software Network 10.2.00 (fluxus-engineering.com; BANDELT et al., 1999) aufgezeichnet.

Ergebnisse und Auswertung

Tabelle 1 zeigt die bewerteten Individuen und deren Mutationen in der jeweiligen Rasse.

Tabelle 1. Individuen und Mutationen pro Rasse

Parameter	Cikta	Waldschaf
Anzahl der ausgewerteten Sequenzen	70	27
Anzahl polymorpher Stellen (Mutationen)	108	68

Insgesamt wurde eine Anzahl von 97 Sequenzen/Individuum zur genaueren Untersuchung genutzt. Bezüglich der Gesamtpopulation, unter Ausschluss von Alignment-Lücken, beträgt die Anzahl der ausgewerteten Stellen im CR-Genom 1179. Im Vergleich dazu betrug die Anzahl der polymorphen (mutierten) Basen 125 (davon 10 Singleton-Sites und 115 Sparsamkeits-Informationssites). 1054 der untersuchten Basen waren monomorph. Die Anzahl der geteilten Mutationen betrug 51. Daher kann die genetische Identität zwischen den beiden Rassen als signifikant angesehen werden.
Tabelle 2 zeigt, dass jede der Indikatoren Aufschluss über den Diversität innerhalb einer Population gibt. Aufgelistet werden hier die Anzahl der Nukleoiden Unterschiede (k) und die Nukleoide Diversität (π).

Tabelle 2: Werte von k und π pro Rasse

Parameter	Cikta	Waldschaf
Durchschnittliche Anzahl von Nukleotidunterschieden, k	21,251	18,661
Nukleotiddiversität, π	0,01802	0,01583
Durchschnittliche Nukleotiddiversität nach Jukes und Cantor, π (JC)	0,01837	0,01616
Standardabweichung von π (JC)	0,00186	0,00308

Die Nukleotiddiversität (π) ist die Anzahl verschiedener Nukleotide an einer bestimmten Basenstelle in zwei zufälligen Chromosomen einer Population. Es ist sichtbar, dass die Cikta-Herde als die Population mit der größeren Diversität und höhere Anzahl durchschnittlicher Nukleotidunterschieden (21,251) und einer Nukleotiddiversität (0,01802) gekennzeichnet ist. Im Vergleich dazu waren die Werte der Waldschaf Population mit einer durchschnittlichen Nukleotiddifferenz von 18,661 und einer Nukleotiddiversität von 0,01583 eindeutig niedriger. Die Überprüfung der durchschnittlichen Anzahl von Nukleotidunterschieden (k) und der Nukleotiddiversität (π) ist besonders nützlich bei der Beurteilung verschiedener Populationen und bei einem direkten Vergleich der untersuchten Rassen.

Tabelle 3 zeigt die Ergebnisse der durchschnittlichen Zählung der Nukleotidunterschieden (k_{xy}) und Nukleotid-Substitutionen beim direkten Vergleich der beiden Rassen.

Tabelle 3. Ergebnisse von k_{xy} und D_{xy} im Durchschnitt und Nukleotid-Substitutionen im Rassevergleich

Parameter	Cikta und Waldschaf
Durchschnittliche Anzahl der Nukleotidunterschieden, k_{xy}	20,635
Durchschnittliche Anzahl von Nukleotid-Substitutionen pro Seite zwischen den Rassen, D_{xy}	0,0175
Durchschnittliche Anzahl von Nukleotid-Substitutionen pro Seite zwischen den Rassen nach Jukes und Cantor, D_{xy} (JC)	0,01784
Standardabweichung von D_{xy} (JC)	0,00237
Paarweise genetische Differenzierung, k_{st}	0,01324

In voller Vereinigung von Cikta und Waldschaf betrug die durchschnittliche Anzahl der gepaarten Nukleotidunterschiede (k_{xy}) 20,635 und die durchschnittliche Anzahl von Nukleotid-Substitutionen pro Seite zwischen den Rassen (D_{xy}) 0,0175.
Bei den korrigierten Werten nach JUKES und CANTOR (D_{xy} (JC)) beträgt die durchschnittliche Nukleotiddiversität 0,01784, mit einer Standardabweichung von 0,00237.
Die Schätzung der paarweisen genetischen Differenzierung (K_{st}), die unter Verwendung der sequenzbasierten Statistik für CR berechnet wurde, betrug 0,01324 (P > 0,05); somit zeigten diese keine genetische Differenzierung in beiden Sequenzen zwischen Cikta und Waldschaf.

Tajima D-Test ergabt -0,47672, was statistisch nicht signifikant ist (P>0,10). Wir können daraus schließen, dass die Rassen als Mitglieder derselben gemeinsamen Population betrachtet werden können, welche im Gleichgewicht ist. Ein signifikant negatives Ergebnis wäre ein Indikator für kürzlich aufgetretene selektive Mutationen, die auf eine Expansion der Population nach einem kürzlich erfolgten Engpass schließt (Flaschenhalseffekt/Gendrift). Wohingegen ein signifikant positives Ergebnis ein Indikator für eine genetische Verengung und/oder eine Fragmentierung in Subpopulationen ist.
Die Ergebnisse des FU & LI D*-Tests ergaben den Wert 1,45676, der mit P>0,05 als unbedeutend angesehen wird. Das Ergebnis des F*-Test von FU und LIs war 0,76212, was als nicht signifikant (P>0,10) in der Gesamtpopulation gewertet wird. Ein signifikant negativer Wert ist ein Hinweis auf eine übermäßige Anzahl von Allelen, der in einer kürzlichen Populationsexpansion oder einem genetischen Drift zu erwarten wäre. Hingegen ist ein positiver Wert ein Beweis für einen Mangel an Allelen, der sich durch einen kürzlich aufgetretenen Flaschenhalseffekt in einer Population anzeigt. Fu´s F-Statistik: -0.387 (P>0.05)

Die Gesamtzahl der Haplotypen (*h*) betrug 38. Davon wurden 29 im Cikta und 11 beim Waldschaf nachgewiesen. Die Haplotyp-Diversität (h_d) betrug 0,96478, während die gesamte genetische Struktur der vereinigten Population (0,02458) auch unter Verwendung der H_{st}-Schätzung nicht signifikant war.

Abbildung 1 zeigt die CR-Haplotypen der Cikta-Schafe (gelb) und der Waldschafe (grün) basierend auf deren informativen Mutationen. Die Größe der Kreise hängt von der Anzahl der Individuen ab, die einen speziellen Haplotypen repräsentieren. Es ist gut sichtbar, dass die Cikta-Haplotypen hauptsächlich nahe beieinander liegen, gleichzeitig jedoch auch nahe der Haplogruppe B der GenBank aufweisen. Die Haplotypen der untersuchten Waldschaf Gruppe bilden eine andere, jedoch eng verwandte Gruppe bezüglich der Vertreter der Haplogruppe B der GenBank. Ausnahmen sind Individuen der Haplogruppe A und insbesondere der Haplogruppe C. Die Haplotypen von Urial (schwarz) und Argali (schwarz) liegen weit entfernt von Waldschaf und Cikta, was eine sehr distanzierte Verbindung zueinander zeigt. Das Mufflon (schwarz) ist in der Nähe der Haplogruppe B der GenBank abgebildet, was seine engere Verwandtschaft mit den Hausschafen zeigt.

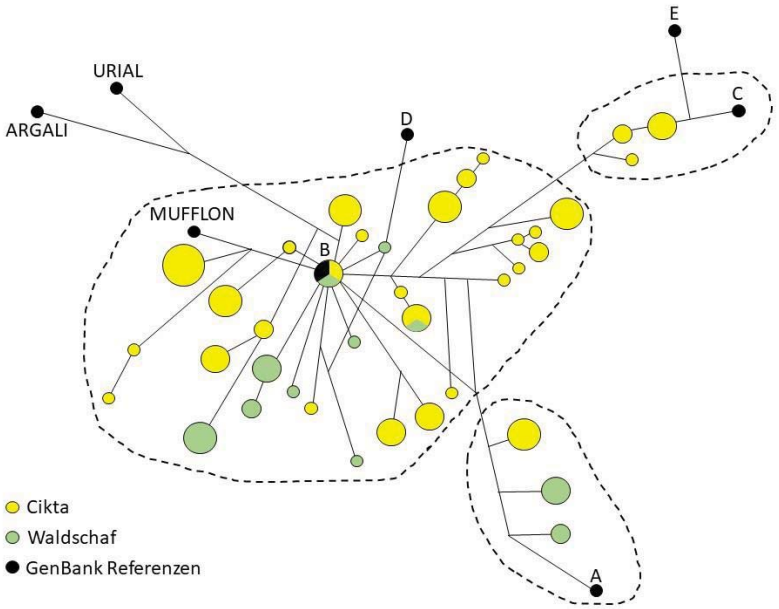

Abbildung 1. Beziehung zwischen der untersuchten CR Haplotypen und der Referenz-CR Haplogruppen (schwarz; A-HM236174, B-HM236176, C-HM236178, D-HM236180, E-HM236182 MEADOWS et al., 2005 und O. musimon Mufflon HM236184, O. ammon Argali HM236188, O. vignei Urial HM236186 HIENDLEDER et al., 2002) als Median-Joining Network (siehe Legende im Text)

WASSMUTH et al. (2002) fanden heraus, dass alle Steinschaf-Individuen zur Haplogruppe B gehören. Beim Waldschaf wurde die Haplogruppe A jedoch bei mehr als der Hälfte der Tiere gefunden. Sie zeigt, dass nicht alle Nachkommen der Zaupelschafe auf denselben domestizierten Vorfahren (Torfschaf oder Ovis aries palustris) zurückgeführt werden können. Vielmehr bestätigt die vorliegende Studie, dass Waldschaf wahrscheinlich auch Mitglieder der Haplogruppe B sind.

Schlussfolgerung und Empfehlungen

Diese Studie soll helfen, die Gene von verschiedener Schafrassen nicht nur zu erhalten, sondern auch zu rekonstruieren mit dem Ziel, die Urschafe so ähnlich wie möglich den historischen Dokumentationen nahezukommen beziehungsweise nachzuziehen. Abschließend wurden im Rahmen unserer Studie mitochondriale Teilsequenzen von 70 Individuen der Cikta-Schafe aus Ungarn und 27 Waldschafen aus Österreich untersucht. Die Zahlen zeigen, dass die Populationen von Cikta und Waldschaf 51 (41%) polymorphe Punkte teilen. Hinsichtlich der Nukleotid- und Haplotyp-Diversität beim Waldschaf ist eine moderate genetische Verdichtung zu erkennen. Diese Auswirkung ist auf einen möglichen Flaschenhalseffekt zurückzuführen, der in der Vergangenheit aufgetreten sein musste. Das Ergebnis des D-Tests von Tajima war -0,47672 und zeigte keine Signifikanz (P>0,10) sowie der Wert der Fs-Statistik von FU und LIs (0,76212), der ebenfalls als nicht signifikant gewertet wird. Diese befinden sich weiterhin im genetischen Gleichgewicht. Eine Abweichung davon gefährdet die untersuchte Gemeinschaft der Zaupel-Derivate nicht. Diese Studie bestätigt die gemeinsame Abstammung von Cikta und Waldschaf, welche für ihre Zuchtgeschichte bekannt sind.
Die paarweise genetische Differenzierung (K_{st}) ist jedoch überhaupt nicht signifikant. Daraus ist zu schließen, dass es in den einzelnen Rassen Unterschiede ergeben sich nicht. Für unsere Züchter ist es nach wie vor von großer Bedeutung, auch in Zukunft gemeinsame Gene innerhalb der Zaupel-Nachfolger zu finden. Dieses Ziel bedarf jedoch weiterer Forschung.

Danksagung

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Abstract

The aim of this work is to research, preserve and archive the exact genetic background and the associated ancestry of native sheep breeds in a genetic database. In this study, the focus is on the direct comparison of the Cikta and the Forest Sheep (Waldschaf). For this purpose, the genomes of both breeds were compared with the control region in the mtDNA. The biological samples for this study were collected between 2015 and 2017 from a total of 97 individuals, 70 from Cikta (Hungary) and 27 from Forest Sheep (Austria).
The total number of polymorphic sites was 125; the number of shared mutations was 51, showing significant genetic identity between the breeds. The result of Tajima's D-test was -0.47672 and showed no significance (P>0.10) as well as the value of the Fs statistic of FU and LIs (0.76212), which is also considered not significant. These are still in the genetic equilibrium. A deviation from it does not endanger the examined community of Zaupel derivatives. This study confirms the common ancestry of Cikta and Forest Sheep, which are known for their breeding history.

Key words: Zaupel sheep, Cikta, Forest Sheep, mtDNA control region

Biodiversity of sheep population in Vojvodina - Serbia

GRBA, Jovana – DRAGIN, Saša – PIHLER, Ivan, – KUČEVIĆ, Denis – ČOBANOVIĆ, Ksenija – BJEDOV, Siniša

University of Novi Sad, Faculty of Agriculture, Republic of Serbia 21000, Novi Sad, Trg Dositeja Obradovića 8
*corresponding author: jovana.grba.94@gmail.com

Abstract

On the territory of AP Vojvodina, there are several autochthonous breeds of sheep: Tsigai, Chokan Tsigai, Horn-twisted Zuja and various strains of Pramenka. The creation of autochthonous races was influenced by specific climatic factors, so they are adapted to bad environmental conditions and are characterized by good resistance to diseases and a long lifespan. Productivity of autochthonous breeds is low, and they are suppressed in modern production that leads to a reduction in genetic variability. In this study, we explored how the racial composition and number of sheep in the area of Vojvodina changed in the period from 2010 to 2020. Data were collected and processed within main breeding organization of the Department of Animal Husbandry of the Faculty of Agriculture in Novi Sad, Serbia. The research included all sheep that are registered and whose records are kept. Based on the analysis, of sheep number by breed, an assessment of the current state, as well as the prospects for preservation and development of autochthonous sheep breeds were given. The population of Tsigai in the period of 2010 was 1757 animals, while in 2020 it was recorded at 5161 individuals. The population of Chokan Tsigai in the period of 2010 was 351, while in 2020 it was recorded at 2092 sheep. The population of Horn-twisted Zuja in 2010 was 145, while in 2020 there were 1056 sheep of that breed.

Keywords: biodiversity, sheep, autochthonous, Vojvodina region

Introduction

Process of degradation of natural habitats is very pronounced through various types of human activities (RADOVIĆ and PETROV, 2001). In recent decades, almost all domestic animals have experienced a significant reduction in genetic diversity (STANČIĆ and STANČIĆ, 2013). Preservation of biodiversity in domestic animals is in the interest for the world community survival (PRENTICE and ANZAR, 2011). Biodiversity conservation is a process of genetic conservation through the restoration of degraded ecosystems and natural habitats with autochthonous animal breeds (STANIVUK et al., 2017). Sheep breeds are mainly bred in economically underdeveloped regions with modest food sources (ŽUJOVIĆ et al., 2011). These genotypes are an important element of regional agro-biodiversity with impact on agro-ecosystems that determine the cultural heritage of a given region (JOVANOVIĆ et al., 2011). The analysis of autochthones breeds population size, in the Republic of Serbia, shows that

many of them are endangered and may disappear (STOJANOVIĆ, 2003). The structure and racial composition of sheep on the territory of AP Vojvodina is quite diverse. Expert report of the work performed in order to control the implementation of breeding programs in AP Vojvodina in 2020 shows greater interest of people in sheep breeding.
Autochthonous sheep breeds differ in their characteristics, but with the help of new technologies, the animal population is characterized in an appropriate way, both phenotypic and genotypic (DRAGIN et al., 2016). Preservation of indigenous breeds both in situ or ex situ would preserve gene pool, gene variability and biodiversity (DRAGIN et al., 2017).

Material and methods

According to the data of the Republic Bureau of Statistics, on the territory of AP Vojvodina for 2020, 286000 sheep of all breeds and categories are bred at the moment. 15 different breeds of sheep are entered into the Main Registry Book of sheep for the territory of Vojvodina (Table 1). According to the data of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, the genetic resources of endangered autochthonous breeds of sheep, bred on the territory of Serbia, include three breeds of sheep: Chokan Tsigai, Horn-twisted Zuja and Pramenka with different strains. In this research, we collected and processed data from the main breeding organization of the Department of Animal Husbandry of the Faculty of Agriculture in Novi Sad, Serbia, on the racial composition of registered sheep herd in the territory of AP Vojvodina in the period from 2010 to 2020.

Results and discussion

Table 1 show that there is a slight increase in the stock of sheep in the period from 2010 to 2020 in the territory of Vojvodina. The number of sheep sheds increased from 13034 in 2010 to 167426 in 2020. Such a rapid increase in the number of heads in the breeding stock of sheep in AP Vojvodina is primarily a consequence of the state's subsidy policy. In 2010, there were 5.11% of sheep in the breeding stock of sheep of AP Vojvodina, out of the total number of sheep in AP Vojvodina, while in 2020 there were 51.69% of sheep in the breeding stock (Expert report and results of performed tasks of control of breeding programs in AP Vojvodina for 2020).

A continuous increase in the breeding stock is observed in the noble breeds of sheep, especially if we observe the two dominant breeds in this area, Württemberg and Ile d'France. The autochthonous breeds of Tsigai sheep, Chokan Tsigai and Horn-twisted Zuja, in the observed period, had oscillations in their number. The reason is the subsidy policy of the state and the fact that AP Vojvodina is a region with extremely intensive agricultural production suitable for stable system of sheep keeping, so farmers more often decided to breed noble breeds. Indigenous breeds of sheep have found their profitability through the exploitation of the few remaining natural pastures in AP Vojvodina.

Table 1. Population of registered sheep on the territory of Vojvodina in the period from 2010 to 2020

Breed	2010	2012	2014	2016	2018	2020
Bergamo sheep	111	351	599	1301	3209	2334
Tsigai	1757	1367	2054	3167	5580	5161
Chokan tsigai	351	632	632	346	1098	2092
Il de france	1681	2256	6614	10094	28455	44183
Suffolk	28	58	186	318	1210	1716
Charole	10	51	92	312	364	341
Texel	0	33	67	95	175	171
Racka sheep	145	219	288	62	331	1056
Vurttemberg	8951	11980	2143	31971	74460	10270
British milk sheep			33	327	206	121
Romanov				291	2135	5188
German Blackheaded						43
Pramenka					133	1860
German meatmerino						182
Lacon						269

In Figure 1, we can notice that the largest part of the sheep population in the territory of Vojvodina in 2020 consists of two noble breeds of combined abilities, Vurttemberg, with 61%, and Ile d'France, with 26% representation. While all other breeds occupy only 13% of the total sheep population.

Indigenous sheep breeds in AP Vojvodina make up less than 5% of the total sheep stock. At the selection review in 2020, 8309 sheep were recorded. The most represented breed was Tsigai, which had 5161 registered individuals. The incentive of the Ministry of Agriculture, Forestry and Water Management affects the number of registered breeding animals because funds are allocated to breeders for the preservation of genetic resources, regardless of whether they are registered in the Main register or not. Given that sheep breeding in Serbia is mainly based on

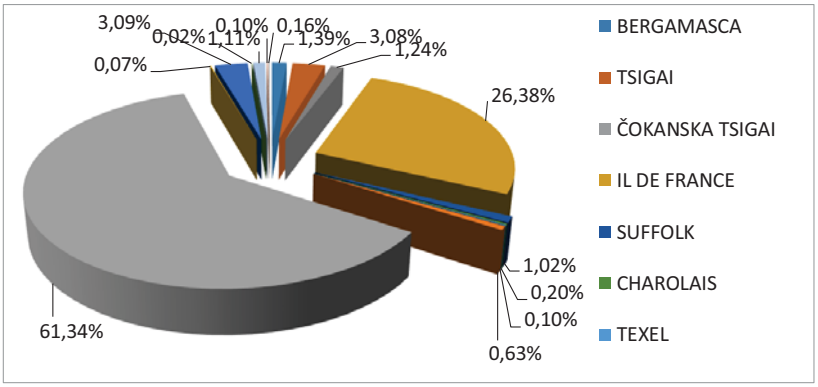

Figure 1. Schematic representation of the racial structure of sheep on the territory of Vojvodina in 2020

meat production, the breed structure does not support this. Sheep breeds selected for meat production, such as Suffolk, Charolais and Texel make up only 1.32% of the total breeding sheep population. Their number in 2020 was 2228 animals. One of the reasons is that sheep breeding in Serbia and on the territory of Vojvodina does not represent primary production, sheep are bred semi-intensively and extensively, and therefore the most represented breeds of sheep have combined abilities.

In Figure 2, we can see that of the total number of the sheep population in the territory of AP Vojvodina in 2020 (which was 167,426) , the largest part consists of two noble breeds of combined abilities, Württemberg with 102709 and Ile d'France with 44183 sheep. Breeders keep them for the production of mutton and lamb meat. Popularity that these breeds have risen expectation that the number of animals will grow in the coming years.

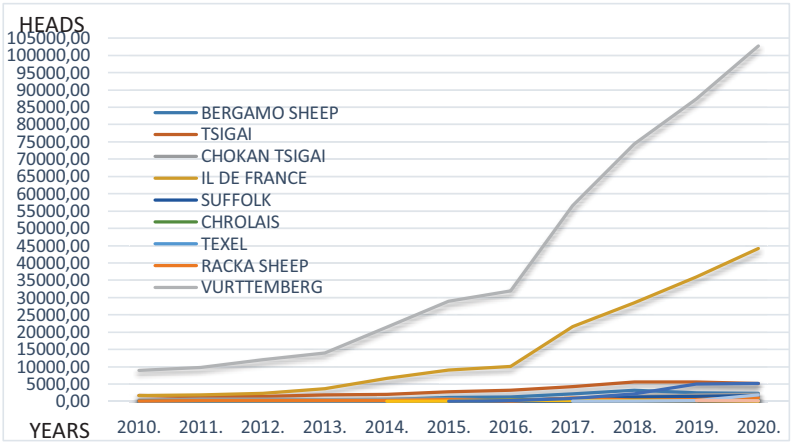

Figure 2. Schematic representation of the racial structure of sheep on the territory of Vojvodina in the period from 2010 to 2020

In Figure 3, our autochthonous breeds of sheep Tsigai, Chokan Tsigai and Horn-twisted Zuja are singled out. Their number in a period of 10 years is shown. Tsigai is our most productive breed of sheep, with combined production abilities in the direction of wool-meat-milk. Apart from Serbia, it is grown in Hungary, the Czech Republic, Slovakia, Russia, Romania and Bulgaria. The native population of the Tsigai breed on the territory of Vojvodina increased in the period from 2014 to 2019. Unfortunately, we notice that the number of Tsigai is decreasing from 5613 as it was in 2019, to 5161 in 2020. The reason for that may be that some animals did not meet the criteria for getting government subsidies determined by the breeding program in 2019, which was approved by the Provincial Secretariat for Agriculture, Water Management and Forestry. Due to the somewhat poorer quality of meat, Tsigai is less popular for the production of lamb, while their potential for milk production has almost never been used. Milk and dairy products are one of the ways to preserve this breed in the future because the market is open for these products. Chokan Tsigai represents our autochthonous breed of sheep, which is also a genetic resource in AP Vojvodina. It is used for meat production although, beside Tsigai, it is our best breed for milk production. If fed adequately, milk production is about 50 to 150 liters in lactation. From Graph 3, we can see that the number of this breed is relatively stagnant and slightly decreased in the period from 2010 to 2017. In the past few years, we have

noticed that that number was growing from 696, as it was in 2017, to 2092, in 2020. Today, its population size is stable.

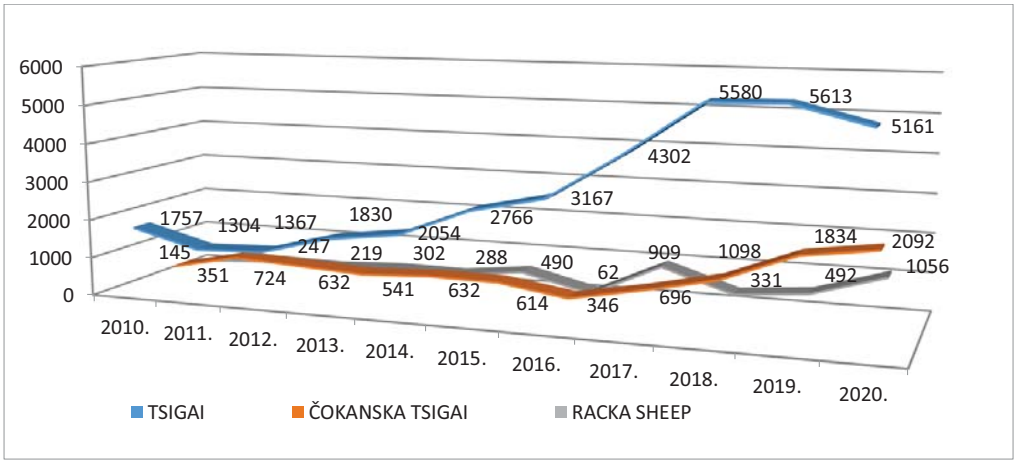

Figure 3. Schematic presentation population of autochthonous sheep on the territory of Vojvodina in the period from 2010 to 2020 in the period 2010 to 2020

The Horn-twisted Zuja is our autochthonous breed of sheep, which is also a genetic resource in AP Vojvodina. It is a breed of sheep with combined production characteristics, that has been bred in our area since ancient times and it is adapted to traditional housing conditions, which is mostly extensive. From the Figure 3, we can see that the breeding stock of Horn-twisted Zuja increased in the period of 10 years (their number was only 145 in 2012, while in 2020, 1056 were recorded). The reason for the decrease in the population in 2012, 2014, 2016 and 2018 is because the sheep were rejected if they did not meet the criteria for subsidies determine by the Main Breeding Program and the Law on animal husbandry (Official Gazette of RS 41/09, 93/12, 14/16). Today, the number of registered sheep is stable, which means that the conservation program is being successfully implemented and that efforts are made in order to preserve this breed. Subsidy measures of the state (Decree for genetic improvement of animal husbandry issued by the Ministry of Agriculture and Environmental Protection, as well as the Law on Incentives in Agriculture) contributed to the increase in the parent stock.

Conclusion

The subsidy policy of the state (Republic of Serbia) in the last 11 years has led to an explosion in the number of sheep in the registered parental herds of sheep in the territory of AP Vojvodina, but it has not led to a significant increase in the total number of sheep. In addition to the large increase in the number of noble breeds of sheep (in the number of registered parental herds) in AP Vojvodina, the number of autochthonous breeds also increased. We can conclude that the state policy aimed at genetic improvement in sheep breeding has achieved its first goal - a large number of heads are included in the registered breeding stock and their production capabilities are monitored. The next step is to increase the quality of the breeding stock. especially in autochthonous breeds, which (although their number has increased in the last 11 years) still make up less than 5% of the total number of registered heads in the breeding stock of sheep of AP Vojvodina

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Der Status der Homozygotie des Gyimeser Zackelschafes in Ungarn auf der Grundlage einer vorläufigen Analyse der Herdbuchdaten

KÁRPÁTI, Edina[1,3]* – KOVÁCS, Endre[1] – GÁSPÁRDY, András[1] – SÁFÁR, László[2] – GULYÁS, László[4]

[1]Institut für Tierzucht, Tierernährung und Labortierkunde, Veterinärmedizinische Universität, Budapest, István Straße 2, 1078 Budapest, Ungarn
[2]Ungarischer Verband der Schaf- und Ziegenzüchter, Lőportár Straße 16, 1134 Budapest, Ungarn
[3]Antal Wittmann Multidisziplinäre Doktorandenschule für Pflanzen-, Tier- und Lebensmittelwissenschaften, Egyetem Platz 1, 9026 Győr, Ungarn
[4]Institut für Tierwissenschaften, Fakultät für Agrar- und Lebensmittelwissenschaften, Széchenyi István Universität, Vár Platz 2, 9200 Mosonmagyaróvár, Ungarn
*Korrespondenzautor-E-Mail: edina.karpati@gmail.com

Zusammenfassung

Die Gyimes Racka (Gyimeser Zackelschaf oder Ţurcană) ist eine der in Siebenbürgen gezüchteten Varianten der Zackelschaf-Gruppe. Anfang der 1990er begann man wieder es in Ungarn zu züchten (vorerst in geringer Zahl) und in den folgenden Jahren nahm die heimische Population, dank einiger größerer Importe von Zuchttieren, zu. Heute besteht die Schafherde von Gyimeser Zackelschaf aus fast 1000 Mutterschafen.
Die Autoren schätzten den Wright'schen Inzuchtkoeffizienten (COI) mit Hilfe der Software Pedigree Viewer auf der Grundlage der vollständigen Stammbuchdaten (von 2005 bis 2020). Die durchschnittlichen Inzuchtkoeffizienten wurden sowohl auf der Grundlage der Basiswerte als auch der transformierten Werte geschätzt.
Der durchschnittliche COI für die gesamte Stammbuchpopulation (n=16947) lag bei 1,99 %, verglichen mit einem höheren Wert für die ingezüchtete Teilpopulation (n=3828, 8,81 %). Mit zunehmender Anzahl der mütterlichen Generationen stieg der COI in der gesamten Stammbuchpopulation signifikant an ($P<0,001$); in der achten Generation betrug er 10,72 %. Im Gegensatz dazu stagniert der COI in der Untergruppe der ingezüchteten Tiere im Bereich von 8 bis 10 %, obwohl ANOVA signifikante Unterschiede zwischen den Generationen bestätigt. Der korrigierte gewichtete Mittelwert und der durch Logarithmierung ermittelte geometrische Mittelwert weisen niedrigere Werte auf (1,43 % und 6,30 %). Wir sind der Ansicht, dass die letztgenannten Werte nicht nur günstiger sind, sondern auch zuverlässiger den Gruppenmittelwert repräsentieren, da sie aufgrund einer Datenbasis ermittelt wurden, die der Normalverteilung näherkommt.
Ausgehend von den berechneten COI-Werten ist der Status der Homozygotie des aktuellen Tierbestandes bedrohlich hoch. Deshalb ist eine sorgfältige Planung der Verpaarung in der Zukunft erforderlich.

Schlüsselwörter: Inzuchtkoeffizient, Paarungsplan, Abstammung durch mütterliche Generationen

Einleitung

Das Herdbuch ist die Grundlage für die Zucht von Nutztieren. Bei unseren einheimischen Rassen ist das Herdbuch in den meisten Fällen geschlossen. Das heißt, dass nur die Nachkommen der eingetragenen Eltern registriert werden. Unsere einheimischen Rassen sind in Bezug auf die Populationsgröße oft nahezu kritisch. Eine Population gilt als kritisch, wenn weniger als 100 Weibchen vorhanden sind und nur 5 Männchen zur Fortpflanzung der Rasse beitragen. Gibt es keine fortpflanzungsfähigen Individuen mehr, gilt die Rasse als ausgestorben (FRÖLICH und KOPTE, 2014). Lokale einheimische Tiere werden aufgrund ihrer minderwertigen Leistung aus der Produktion verdrängt und durch intensive Weltrassen ersetzt (MAIJALA, 1970). Leider entsprechen diese Tiere möglicherweise nicht den aktuellen Bedürfnissen von Industrie und Handel und sind daher nicht von Interesse (SAMBRAUS, 2016).
Darüber hinaus sind unsere alten Haustierrassen durch den Rückgang ihrer genetischen Vielfalt bedroht. Die Homozygotie einer Nutztierpopulation kann auf verschiedene Weise und mit unterschiedlichen Index ausgedrückt werden. Einer davon ist der Inzuchtkoeffizient, der auf der Grundlage des Herdbuches berechnet wird. Der Inzuchtkoeffizient beschreibt die Wahrscheinlichkeit, dass zwei Allele eines bestimmten Lokus identisch in der Abstammung ist. Von Inzucht spricht man, wenn Nachkommen aus der Paarung verwandter Eltern stammen. Auf der Grundlage direkter genomischer Informationen ist es nun möglich, die sogenannte molekulare Homozygotie (Autozygotie, ROH - Runs of Homozygosity) durch Auswertung mehrerer Mikrosatelliten (z. B. KOVÁCS et al.,) und tausender SNPs (FERENČAKOVIĆ et al., 2013) zu schätzen.

Die Vorfahren des Gyimeser Zackenschafes kamen aus Kleinasien in den südlichen Gebirgszügen der Karpaten. Das Gyimeser Zackelschaf (Ţurcană, sowieso Siebenbürgischer Zackel) ist einer der Vertreter Zackelschafgruppe mit der größten Körperaufbau. Reinrassige Vertreter sind heute nur noch an wenigen Orten zu finden (z. B. Gyimeser-Gebirge, Vrancea Region). Es hat ein charakteristisches gebogenes Horn, das bei Widdern länger ist als bei Mutterschafen (GÁSPÁRDY, 2011). Auch Hornlosigkeit ist keine Seltenheit. Sie haben eine gemischte Wolle mit gewellten Locken. Die Wolle ist schmutzig weiß und oft mit farbigen Flecken gesprenkelt. Der Kopf und die Spitzen der Beine sind meist schwarz. Der Fleck um das Auge ist oft schwarz, was als „Okular“ bezeichnet wird. Es ist eine echte Dreinutzungsrasse, deren Milch reich an Vitaminen und Mineralien ist. Und die Muskeln, die auf der Almen wachsen, sind die Grundlage für viele saftige Gerichte (KOPPÁNY, 2002).
Anfang der 1990er Jahre wurde die Rasse in Ungarn wieder eingeführt (damals allerdings nur in geringer Zahl), und in den folgenden Jahren nahm die einheimische Population dank einiger größerer Importe von Zuchttieren zu. Heute beläuft sich die Population der Gyimeser-Zackelschafe auf fast 1000 Muttertiere.

Ziel dieses Artikels ist es, den Inzuchtstatus der Gyimeser Zackelschafpopulation aufgrund der ungarischen Herdbuchdaten zu ermitteln und die Aufmerksamkeit der Züchter auf die Erhaltung der genetischen Vielfalt der Rasse zu lenken. Der Grad der Homozygotie der Rasse wurde auf zwei Arten bestimmt: durch das arithmetische Mittel der individuellen Inzuchtkoeffizienten und durch das geometrische Mittel der transformierten individuellen Koeffizienten.

Material und Methoden

Für die Bearbeitung des Herdbuches der Rasse Gyimeser Zackelachaf haben wir die Excel-Datenbank des Ungarischen Schaf- und Ziegenzuchtverbands (MJKSZ) verwendet, die das ganze Land abdeckt und die Zuchtdaten von 2005 bis 2020 enthält. Nach der Datenaufbereitung (Codierung der mütterlichen Abstammung, Erstellung der Pedigree Datei) haben wir den Wright'schen Inzuchtskoeffizient (Wright's Coefficient of Inbreeding - COI) mit Hilfe der Software Pedigree Viewer (KINGHORN und KINGHORN, 2010) berechnet. Die Herdbuchdatei ist eine Datentabelle, in der alle Individuen des Herdbuches in der ersten Spalte und ihre Eltern (Vater, Mutter) mit ihren Identifikationsnummern in der zweiten und dritten Spalte eingetragen sind. Die Abstammung der Gründerindividuen ist nicht bekannt, daher werden in diesen Zeilen die Eltern mit der Kennung 0 angegeben.

Zu Beginn der Verarbeitung führten wir einen Normalitätstest für COI mit Hilfe des Kolmogorov-Smirnov-Tests für eine Stichprobe und des Lilliefors-Tests für den gesamten Datensatz durch. Wenn die Werte dieser Tests signifikant sind, wird die Hypothese der Normalverteilung verworfen. In unserem Fall wurden signifikante Werte erzielt (K-S d = 0,4305, P < 0,01; Lilliefors P < 0,01), so dass die Verteilung des COI nicht normal war (Abbildung 1.a).
Um die Verteilung zu normalisieren, wurde eine natürliche Logarithmus-Transformation durchgeführt (COI → LogCOI), und die Normalitätstests wurden erneut an den normalisierten Daten durchgeführt, allerdings nur in der Teilpopulation der ingezüchteten Tiere (in welcher der COI größer als 0 ist). Die Verteilung war sichtbar normalisiert (Abbildung 1.b), aber die Tests haben dies nicht bestätigt (K-S d = 0,1032, P < 0,01; Lilliefors P < 0,01). Dennoch glauben wir, dass die statistische Verarbeitung der transformierten Werte zuverlässiger ist als die der nicht transformierten Werte. Wir haben jedoch eine statistische Verarbeitung sowohl des COI als auch des LogCOI durchgeführt, um die Ergebnisse zu vergleichen.

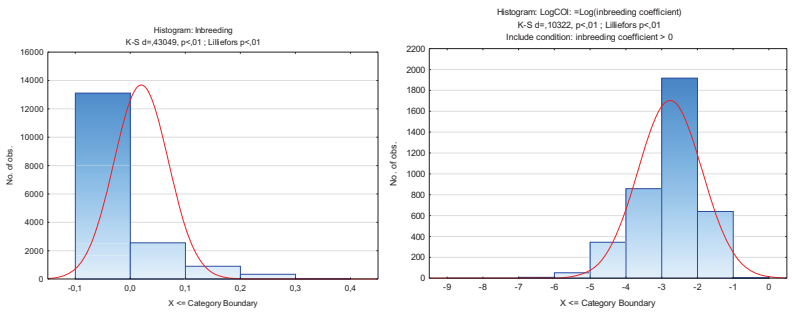

Abbildung 1.a: COI-Lauf und Normalitätstest in der Gesamtpopulation (links),
Abbildung 1.b: LogCOI-Lauf und Normalitätstest in der Inzucht-Subpopulation (rechts)

Die statistische Auswertung erfolgte mit der Software Statistica (TIBCO Software Inc., 2020). Zunächst wurden die arithmetischen Mittelwerte und 95 %-Konfidenzintervalle für die gesamte Herde und die ingezüchtete Teilpopulation im COI berechnet. Anschließend wurden die gleichen Werte für die ingezüchtete Teilpopulation in LogCOI ermittelt. Später wurden die Wirkungen des Geschlechtes (männlich, weiblich) und der Anzahl der mütterlichen Generationen (zwischen 1 bis 8) auf die Entwicklung der beiden Arten von Inzuchtkoeffizienten in einem einfachen ANOVA-Modell getestet. Das Signifikanzniveau der Effekte (P-Wert,

ANOVA) und das paarweise Signifikanzniveau ihrer Klassen (P-Wert, Post-Hoc-Test: Unequal N Tukey HSD - Honestly Significant Difference) wurden ebenfalls berechnet.
Nach der statistischen Aufbereitung wurden die LogCOI-Ergebnisse rücktransformiert, um einen interpretierbaren Mittelwert (geometrisches Mittel, $COI_{back} = e^{LogCOI}$) und dessen 95%-Konfidenzintervalle (unteres Konfidenzintervall = $e^{-95\%LogCOI}$ und oberes Konfidenzintervall = $e^{+95\%LogCOI}$) zu erhalten. Aufgrund unserer Erfahrung haben wir uns nicht mit den Werten der Standardabweichung befasst, da ihr rücktransformierter Wert irreführend hoch ist, während das geometrische Mittel durch das Konfidenzintervall klarer kommuniziert wird.
Schließlich haben wir der Vollständigkeit halber die korrigierten gewichteten Inzuchtwerte für die gesamte Herde berechnet, indem wir das geometrische Mittel COI_{back} mit den Elementnummern einbezogen haben.

Ergebnisse und Auswertung

Das Herdbuch der Gyimeser Zackelschafpopulation (2005-2020) enthält Daten zu insgesamt 16947 Individuen. Davon waren 3828 Individuen ingezüchtet, während 13052 nicht ingezüchtet waren. Bei der COI-Berechnung auf der Grundlage der Analyse des Herdbuches wird die Verwandtschaft zwischen Individuen mit unbekanntem elterlichem Hintergrund, d. h.

Tabelle 1: Inzuchtkoeffizienten für den gesamten Bestand und die verwandten Bestände sowie für die mütterlichen Generationen

Wirkung	Alle Tiere	Ingezüchtete Tiere	Verhältnis der ingezüchteten Tiere
	Inzuchtkoeffizient, -95%CI arithmetisches Mittel +95%CI	Inzuchtkoeffizient, -95%CI arithmetisches Mittel +95%CI	%
Gesamtbestand:			
	0,0191 0,0199 0,0206 (n=16947)	0,0859 0,0881 0,0903 (n=3828)	22,68
Geschlecht:	P<0,001	P=0,750	
Männchen	0,0225 0,0237 0,0250 (n=7149)	0,0853 0,0884 0,0915 (n=1901)	26,84
Weibchen	0,0163 0,0173 0,0182 (n=9798)	0,0846 0,0877 0,0908 (n=1927)	19,67
Mütterliche Generation:	P<0,001	P<0,001	
1	$0,0000^{a}$ (n=2255)	-	0,00
2	0,0003 $0,0006^{a}$ 0,0008 (n=6712)	0,1941 $0,2243^{d}$ 0,2544 (n=17)	0,25
3	0,0201 $0,0218^{b}$ 0,0235 (n=3934)	0,0933 $0,0982^{b,c}$ 0,1030 (n=874)	22,21
4	0,0472 $0,0501^{c}$ 0,0531 (n=2054)	0,0782 $0,0821^{a}$ 0,0860 (n=1255)	61,10
5	0,0594 $0,0633^{d}$ 0,0672 (n=1188)	0,0777 $0,0820^{a}$ 0,0864 (n=917)	76,94
6	0,0798 $0,0848^{e}$ 0,0897 (n=621)	0,0849 $0,0898^{a,b,c}$ 0,0947 (n=586)	94,36
7	0,0808 $0,0900^{e}$ 0,0991 (n=172)	0,0830 $0,0921^{a,b,c}$ 0,1013 (n=168)	97,67
8	0,0643 $0,1072^{d,e}$ 0,1501 (n=11)	0,0643 $0,1072^{a,c}$ 0,1501 (n=11)	100,00

^{abcde} – Durchschnitte mit unterschiedlichen Kennbuchstaben unterscheiden sich signifikant (P<0,05)

den Gründervorfahren, nicht berücksichtigt, und ihr Inzuchtkoeffizient wird auf 0 gesetzt. Die durchschnittliche Inzuchtrate für die gesamte Herde beträgt 1,99%, während die Inzuchtrate für ingezüchtete Tiere 8,81 % beträgt (Tabelle 1).

Wir untersuchten auch die Inzuchtkoeffizienten des gesamten Bestandes und die Inzuchtkoeffizienten der ingezüchteten Tiere nach Geschlecht. In der gesamten Herde betrug der Homozygotie bei den Widdern 2,37% und bei den Mutterschafen 1,73% ($P < 0,001$), wobei es bei den ingezüchteten Tieren keinen signifikanten Unterschied zwischen Widdern (8,84 %) und weiblichen Tieren (8,77%) gab ($P = 0,750$).
Bei der Verarbeitung wurden acht mütterliche Generationen unterschieden. Die Werte der Inzuchtkoeffizienten pro Generation (mütterliche Generation) sind ebenfalls in Tabelle 1 aufgeführt. Es ist zu beobachten, dass der COI ab der zweiten Generation in der gesamten Population stetig und signifikant ansteigt (von 0,00 % auf 10,72 %; $P < 0,001$). Innerhalb der Population der ingezüchteten Tiere stagniert der Inzuchtkoeffizient bei Werten zwischen 8-10% (praktisch $P<0,05$), mit Ausnahme der zweiten Generation mit einer kleinen Anzahl von Elementen (17 Individuen) mit 22,43 %.

Tabelle 2: COIback-korrigierte gewichtete Durchschnitte und geometrische Durchschnitte

Wirkung	Alle Tiere	Ingezüchtete Tiere
	COI_{back} Inzuchtkoeffizient, gewichteter Durchschnitt	COI_{back} Inzuchtkoeffizient, -95%CI geometrisches Mittel +95%CI
Gesamtbestand:		
	0,0143 (n=16947)	0,0613 0,0630 0,0649 (n=3828)
Geschlecht:		P=0,301
Männchen	0,0172 (n=7149)	0,0615 0,0640 0,0666 (n=1901)
Weibchen	0,0122 (n=9798)	0,0596 0,0621 0,0647 (n=1927)
Mütterliche Generation:		P<0,001
1	0,0000 (n=2255)	-
2	0,0005 (n=6712)	0,1738 $0,2124^{d}$ 0,2595 (n=17)
3	0,0157 (n=3934)	0,0665 $0,0706^{c}$ 0,0750 (n=874)
4	0,0348 (n=2054)	0,0542 $0,0569^{a}$ 0,0599 (n=1255)
5	0,0440 (n=1188)	0,0536 $0,0570^{a,b}$ 0,0605 (n=917)
6	0,0671 (n=621)	0,0668 $0,0711^{c}$ 0,0757 (n=586)
7	0,0719 (n=172)	0,0654 $0,0736^{a,b,c}$ 0,0829 (n=168)
8	0,0954 (n=11)	0,0693 $0,0954^{a,b,c,d}$ 0,1313 (n=11)

^{abcde} – Durchschnitte mit unterschiedlichen Kennbuchstaben unterscheiden sich signifikant ($P<0,05$)

Die Analyse nach Generationen zeigt, dass die Größe der Inzucht-Subpopulation von der zweiten Generation (n =17) bis zur vierten Generation (n = 1255, bei der Geburt als vollständig angesehen) zunimmt. Mit anderen Worten: Der Anteil (%) der Inzucht-Subpopulation an der Gesamtpopulation nimmt in den aufeinanderfolgenden Generationen drastisch zu.

Nach der statistischen Aufbereitung wurden die LogCOI-Ergebnisse rücktransformiert, um ein aussagekräftiges geometrisches Mittel über die jeweilige Teilpopulation und innerhalb dieser Subpopulation nach Geschlecht und mütterlicher Generation zu erhalten (Tabelle 2). In der zweiten Spalte von Tabelle 2 werden zur Information auch die korrigierten gewichteten Inzuchtwerte für die gesamte Herde angegeben.
Der geometrische Mittelwert der Inzuchtrate für den gesamten Zuchtbestand beträgt 1,43%, während die Inzuchtrate für verwandte Tiere 6,30% beträgt. In der gesamten Herde beträgt der Inzuchtanteil bei den Widdern 1,72% und bei den weiblichen Tieren 1,22%. Bei den Inzuchttieren gab es keinen signifikanten Unterschied zwischen Widdern (6,40%) und Weibchen (6,21%) (P = 0,301).
Bei den mütterlichen Generationen zeigt sich ebenfalls ein kontinuierlicher und signifikanter Anstieg des COI_{back} ab der zweiten Generation in der Gesamtpopulation (von 0,00% auf 9,54%). In der Inzucht-Subpopulation beginnt der COI_{back} nach einem anfänglichen Rückgang jedoch ab der fünften Generation zu steigen (von 5,69% auf 9,54%, $P<0,05$).

Schlussfolgerungen und Empfehlungen

In fast 15 Jahren wurden im Herdbuch des Gyimeser Zackelschafes beinahe 17.000 Tiere erfasst, von denen fast 4.000 ingezüchtet sind.

Vergleicht man die nicht transformierten Daten, so ist der durchschnittliche Inzuchtkoeffizient für die gesamte Herde niedrig (1,99 %), während er für die verwandten Individuen höher ist (8,81 %). Der Logarithmus-Transformation gewichtete Durchschnitt und das geometrische Mittel sind niedriger als diese (1,43% und 6,30%). Wir sind der Ansicht, dass die letztgenannten Werte nicht nur vielversprechender, sondern auch repräsentativer für den Gruppenmittelwert sind, da sie auf einer Datenbasis ermittelt wurden, die der Normalverteilung näherkommt.
In der Gesamtpopulation beträgt die Inzuchtrate bei den Widdern 2,37% und bei den weiblichen Tieren nachweislich 1,73% ($P < 0,001$), während bei den ingezüchteten Tieren kein signifikanter Unterschied ($P = 0,750$ und $P = 0,301$) zwischen den Widdern (8,84% und 6,4%) und den weiblichen Tieren (8,77% und 6,21%) nach einer der Berechnungsmethoden festzustellen ist. Dies deutet darauf hin, dass Inzucht bei der Entstehung von Inzucht keine Rolle spielt, da die Wahrscheinlichkeit, dass beide Geschlechter geboren werden, 50-50% betragen.
Es ist wichtig zu betonen, dass die transformierten Daten es ermöglichen, einen Anstieg des Inzuchtkoeffizienten der Teilpopulation der ingezüchteten Tiere pro mütterliche Generation (die letzten fünf) früher zu erkennen, während die nicht transformierten Daten es erlaubten, ihn später zu erkennen (die letzten drei Generationen).
Vergleicht man die Ergebnisse von nicht transformierten und transformierten Angaben, so ist festzustellen, dass die durch die letztgenannte mathematische Operation genauer gemacht wurden.
Schließlich halten wir es für wichtig, diese Ergebnisse den Züchtern mitzuteilen, um sie auf das Problem der fragilen Vielfalt aufmerksam zu machen. Leider ist es für einheimische Tiere in der heutigen schnelllebigen Welt schwierig, mit den intensiven Züchtungen mitzuhalten und zu konkurrieren, was auch der Grund für ihren bedrohten Status ist.

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Abstract

The Gyimes Racka (Ţurcană) is one of the Zackel-group variants bred in Transylvania. In the early 1990s it was re-introduced into Hungary (then only in small numbers), then in the following years the national population of the breed increased due to some major imports of breeding animals. The herd of the Gyimes Racka ewes consists of almost 1000 individuals today.

The authors estimated Wright's inbreeding coefficient (COI) using Pedigree Viewer software based on complete pedigree data (from 2005 to 2020).

The average COI of the total herd book population (n=16880) was 0.02, compared to the higher value of the subpopulation of inbred individuals (n=3828, 0.0881). As the number of maternal generations increases, the COI increases significantly ($P<0.001$) in the total herd book population; in the eighth generation it is already 0.1072. On the other hand, in the subset of inbred individuals, the COI is more stagnant in the range of 0.08 and 0.10, even though ANOVA confirms significant differences between generations. Based on the calculated COI values, the homozygosity status of today's Gyimes Racka stock is threateningly high, which draws attention to careful mating in the future.

Keywords: coefficient of inbreeding, planning mating, descendance by maternal generations

FEDO.HU – Online COI calculator of planned litters for mate selection in Kuvasz dog breed

FÜRLINGER, Dóra[1] – BÁN, Beáta[2] – PALLOS, Réka[3] – PISCHOF, Ferenc[4] – ZSÓRI, Zsolt[4] – KISS, Daniella Panna[1] – GÁSPÁRDY, András[1] – MARÓTI-AGÓTS, Ákos[1]*

[1]Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine Budapest, István u. 2, 1078 Budapest, Hungary
[2]Veterinary Diagnostic Directorate, National Food Chain Safety Office, Kitaibel Pál u. 4, 1024 Budapest, Hungary
[3]Institute of Biology, Faculty of Sciences, University of Pécs, Ifjúság u. 6, 7624 Pécs, Hungary
[4]Hungarian Kuvasz Breeder Association, Gervay u. 117/a, 1147 Budapest, Hungary
*corresponding author: maroti-agots.akos@univet.hu

Abstract

Nowadays, more than ever before, the breeding of purebred dogs is based on their appearance. However, using mating of close relatives in pure-bred breeding to maintain "aesthetic" traits drastically reduces the genetic diversity of the breed and increases the occurrence of genetic diseases. Inbreeding value of Kuvasz dogs registered in the pedigree book of Hungarian Kuvasz Breeder Association (MKFE) was determined using two different methods (pedigree calculations and molecular genetic identification). Molecular genetic analyses used for parentage control in NÉBIH-ÁDI matched the inbreeding values calculated from pedigree data. The inbreeding values (COI) of the indigenous Kuvasz dog breed are low. FEDO.HU is a transparent, public, attitude-shaping, scientifically reliable professional decision support system, which is available to all breeders of our indigenous dog breed.

Keywords: COI, molecular inbreeding coefficient, Kuvasz breeding, pure-bred dog breeds, online calculator

Introduction

Most dog breeds were created in the mid-1900s, which is when we can date the development of purebred breeding methods and the establishment of breed standards (WILCOX et al. 1995, PARKER et al., 2004). After the foundation, due to the closed registers, further gene flow was forbidden, the aim was to maintain the characteristics of the founding individuals of the breed. Strict selection for appearance traits can have serious consequences. It may involve the complete loss of some genes, thereby reducing genetic diversity, or an enrichment of another allele variant and so resulting unwanted health consequences (PEDERSEN et al., 2015; KEIJSER et al., 2018; LAMPI et al., 2020).
Thorough pedigrees are an important source of information on genetic diversity and population structure (GOLEMAN et al., 2021). The calculation of the inbreeding coefficient (COI) based

on pedigree data dates back nearly a hundred years (WRIGHT, 1922). Determination of inbreeding value by molecular genetic methods is for some decades in practice, using short tandem repeats (STRs). From the results of the molecular tests, in addition to the number of alleles, values used to characterise genetic balance can also be calculated: E.g., the fixation index (which is in fact equivalent to the COI) expresses the probability that two alleles of a locus are derived from a common ancestor (PÉNZES, 2011). Inbreeding can be calculated based on both expected (H_e) and observed heterozygosity (H_o) of alleles on a certain locus. A low H_o value indicates a decrease in the number of heterozygous individuals for a given locus, which can be seen, for example, in the French bulldog ($H_o = 0.376$). In an ideal population, in Hardy-Weinberg equilibrium, the frequency of allelic variants of a gene is constant, does not change from generation to generation, so that, among other things, the observed heterozygosity remains close to the expected value (DZITSIUK et al., 2017).

Material and methods

Pedigree data

FEDO.HU was inspired by the English Kennel Club - Mate Select open access system (JANES et al., 2020). The database of the Hungarian Kuvasz Breeders Association (MKFE) being looked after by the Hungarian Kennel Club (MEOESZ), currently contains the pedigree data of 7713 Kuvasz individuals. An algorithm written in C++ performs graph theoretical COI computations, thereby after selecting two individuals from the database, degree of relatedness of these two individuals can be analysed taking into account 7 generations of data. The algorithm only counts 7 generations because including additional ancestors has no substantial influence on the results, for the present purpose.

Molecular genetic testing

STR-test results of parentage control carried out by the Animal Genetics Laboratory in NÉBIH-ÁDI were used. NÉBIH has created a database containing 768 dogs from the test results of dogs registered by MKFE, including the following data: pedigree name, breed, sex, chip number, pedigree number, date of birth, type and laboratory number of the sample, as well as the mother's and father's pedigree name. The database also includes the 21 canine-STRs investigated and recommended by ISAG and the amelogenin marker for sex determination.

For genetic calculations Genetic Analyses in Excel (GenAlEx) 6.5 macro was used (PEAKALL et al., 2012), which was applied to perform allele frequency analyses (F-statistics, heterozygosity, fixation index (F_{is}) and deviations from Hardy-Weinberg equilibrium). The number of alleles for the whole population and their frequency at each locus and the relative genetic entropy were determined (GÁSPÁRDY et al., 2018).

For statistical comparison of the inbreeding coefficients obtained, a differential test of the ratios as a function of percentage and number of samples.

Results and discussion

FEDO.HU

From the database, we have created an online inbreeding calculator freely available on the internet. Kuvasz dogs born after 01.10.2011. are registered on FEDO.HU as breeding animals and the website lists a total of 7713 individuals. Based on the data, the average inbreeding coefficient (COI) of the population is 5.36%. The few higher COI values are typical of

individuals born in the last century, with a maximum of 25%. However, there are also some of the recently born individuals with COI around 20%.
The number of founding individuals is 517. Among the founder individuals, in the case of 232 animals we found a recorded date of birth. According to these data, the first founder was born on 04.08.1987 and the last one on 29.09.2000.
On the website, the COI of the planned progeny of two breeding animals can be calculated. Based on the traffic data so far, users tend to retrieve the data of several hundreds of possible litters per month. The calculator is available in English and Hungarian and the COI values are automatically categorised: below 3.125% low, between 3.125-6.25% mild, above 6.25% we would get very highly inbred pups from the given parents. These categories were defined by the Association's management in the previous year in accordance with the average COI of the breeding population, and are going to be updated every year on the basis of new data. In addition to the COI values, the pedigree data of the breeding animals are freely available (Figure).

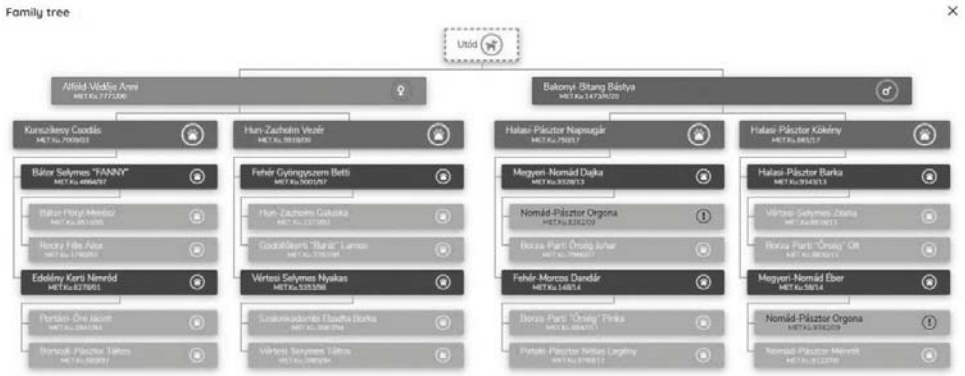

Figure 1. Family tree of the planned litter

Molecular genetic testing
The total number of alleles observed ranges from 4 (REN247M23, AHTk253) to 11 (AHT121) with an average of 6.76 (SD = 0.4). The lowest number of effective alleles (1.47) was found at the REN247M23 locus, and the highest (5.28) at the AHT121 locus. The mean number of effective alleles was 3.14 (SD = 0.21). Also, the REN247M23 locus had the lowest expected heterozygosity (0.32), and the AHT121 had the highest (0.81).
The mean expected heterozygosity (H_e) of the population was 0.65 (SD = 0.03). The mean observed heterozygosity (H_o) of the population was 0.65 (SD = 0.03). The lowest value of H_O was also associated with the REN247M23 locus (H_O = 0.33), the highest was associated with AHT121 (H_O = 0.82).
The lowest fixation index was observed at locus AHTk211 (F_{is} = -0.079). Seven STRs showed positive values, of which F_{is} = 0.00 occurred at the REN5P11 locus, and the highest value (Fis = 0.07) at the INU030 locus.
The average value of F_{is} for all loci 0.07 (SD = 0.08).
The two coefficients of inbreeding (pedigree 5.36%, n = 7713 and 6.76%, n = 768) showed no statistically proven difference (two-sided differential test, p = 0,104).
The 21 STRs showed no significant deviation from the Hardy-Weinbeg equilibrium in 11 cases. Weaker significance ($p < 0.01$) for 4 loci (CXX279, REN169O18, INRA21, AHT137), while

stronger significance ($p < 0.001$) for 6 loci (REN162C04, FH2054, AHT121, AHTh260, AHTh130, REN5P11) was observed (Table). The Table also presents the values of the Shannon information index. The genetic diversity of each microsatellite is more directly reflected in its relative indicator, the relative entropy, which can be called the effective genetic diversity. It can be seen that the microsatellite AHTh130, despite having 10 allelic variants, is characterized by the slightest entropy. In such microsatellites the risk of losing the very rare (low frequency) allelic variants in a short time is increased.

Table. Molecular test results of the loci. N: number of samples, N_a: number of alleles per locus, N_e: number of effective alleles, H_o: observed heterozygosity, H_e: expected heterozygosity, F_{is}: fixation index, I=Shannon's information index, I_{rel}=relative entropy, Signif: statistical analysis of the HWE

Locus	N	N_a	N_e	H_o	H_e	F_{is}	I	I_{rel}	Signif
AHTk211	752	5	2.251	0.60	0.56	-0.079	0.925	39.8	ns
CXX279	768	6	2.836	0.66	0.65	-0.026	1.242	48.1	**
REN169O18	768	6	4.487	0.76	0.78	0.023	1.571	60.8	**
INU055	767	6	4.026	0.76	0.75	-0.006	1.472	56.9	ns
REN5P11	767	8	3.817	0.74	0.74	0.000	1.440	48.0	***
AHTh130	764	10	1.850	0.47	0.46	-0.029	0.871	26.2	***
INRA21	767	6	3.509	0.76	0.72	-0.067	1.408	54.5	**
AHT137	768	7	3.373	0.70	0.70	-0.001	1.331	47.4	**
REN169D01	767	7	2.799	0.62	0.64	0.030	1.175	41.9	ns
REN169D01	764	7	3.930	0.72	0.75	0.031	1.395	49.7	***
AHTk253	765	4	2.639	0.63	0.62	-0.006	1.040	52.0	ns
INU005	765	7	3.294	0.68	0.70	0.031	1.389	49.5	ns
INU030	767	8	2.861	0.60	0.65	0.070	1.099	36.6	ns
FH2848	761	6	3.969	0.73	0.75	0.020	1.489	57.6	ns
REN105L03	759	5	2.770	0.66	0.64	-0.037	1.204	51.9	ns
AHT121	767	11	5.278	0.82	0.81	-0.015	1.803	52.1	***
FH2054	764	5	2.022	0.51	0.50	-0.015	1.027	44.2	***
REN162C04	767	7	2.316	0.559	0.57	0.016	0.939	33.4	***
AHTh171	767	10	3.924	0.757	0.75	-0.017	1.511	45.5	ns
REN247M23	767	4	1.474	0.330	0.32	-0.026	0.597	29.8	ns
REN64E19	762	7	2.554	0.636	0.61	-0.046	1.093	38.9	ns

With FEDO.HU, the pedigree data have been placed in a public, freely accessible and organised electronic database. From the parentage and breeding data, the COI calculator provides a professionally verified, accurate method to calculate the inbreeding values of the planned litters. The current COI of 5.36% for the Kuvasz population, based on literature data, is considered as a low inbreeding coefficient (GOLEMAN et al., 2021; DZITSIUK et al., 2017; GOLEMAN et al., 2018). The importance of the calculator is confirmed by the fact that we found individuals with approximately 20% COI value in the current population. By checking the planned mating, the website can efficiently help to change breeders' attitudes, prevent close inbreeding and maintain the genetic diversity and health of the breed.

Based on the 142 alleles observed in STR studies, the Kuvasz is comparable to other local breeds with low inbreeding (RADKO et al., 2017; GOLEMAN et al., 2021; DZITSIUK et al., 2017; BIGI et al., 2018; RADKO et al., 2021). Genetic diversity of the breed is considered to

be conserved, with a mean F_{is} of -0.07 +/- 0.08 indicating population stability, although the variance may push this value into the positive range minimally. Only 6 of the 21 loci studied deviate significantly from the Hardy-Weinberg equilibrium. In a previous study including several Hungarian dog breed populations, 90% of the loci in deviated from the Hardy-Weinberg equilibrium. The authors used 10 STR loci in their work and drew attention to the fact, that the use of more markers than this is justified for pedigree verification, especially for highly inbred breeds (ZENKE et al., 2007). Our own study uses 21 genetic markers so the results can be considered reliable.

Expected and observed heterozygosity values are in agreement and STR tests did not show significant inbreeding. The coincident values suggest that results based on pedigree data as well as molecular genetic calculations are equally reliable when determining COI.

Relative entropy exceeds 50% only in a minority of microsatellites. This suggests that in a larger proportion of microsatellites genetic diversity is reduced, occasionally critically threatened. This result draws attention to the need for careful breed maintenance.

The two inbreeding coefficients (5.36% and 6.76%) are statistically identical, which means that both methods revealed the same degree of inbreeding and homozygosity. The molecular inbreeding coefficient is usually higher than the other one. Results obtained from pedigree data analyzation indicate more diverse population, what is due to the fact that the relatedness between the founding ancestors is ignored.

Conclusion and recommendation

The breeding practice where breeders aim to have dogs that are "healthy, behaving and looking like their breed members, and provide sustainable performance" is now called Dog Breeding 2.0. (BROECKX, 2020).

With the FEDO.HU system, the Hungarian dog breeding has got a transparent, professionally supervised, freely accessible tool, which can provide a basis for a change in approach that can lead to the development of what we call Dog Breeding 2.0.

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