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In Memoriam

Animal Genetic resources in Slovak Republic

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Abstract

The animal genetic resources represent very important part of agriculture. In order to be able to undertake actions for conservation of endangered breeds, monitoring of the actual state of populations has to be in place. This paper provides a short look on the actual state of animal genetic resources in Slovak Republic and its change over the time. The data for regular updating of EFABIS information system were used in the study. Results showed different trends in different species. In cattle there was decrease in dairy breeds, while increasing trend was observed in Slovak Spotted cattle and beef breeds. In the sheep rapid decrease in population size of traditional breeds was observed. In goat population sizes of traditional breeds increased. The numbers of animals of pig, horse, poultry and rabbit breeds were more-less stabilized. In many cases the preference of farmer for the single breed and his passion is the only reason for keeping the animals. In this regard the raising awareness is a very important part of animal genetic resources conservation.

Keywords: biodiversity, animal genetic resources, conservation, local breeds

Introduction

Biodiversity for food and agriculture is indispensable to food security, sustainable development and the supply of many vital ecosystem services (FAO, 2019). The animal genetic resources are generally defined as subset of biodiversity, which are used or may be used, for the production of food and agriculture. Within the animal genetic resources, the biggest attention is given to local breeds. The importance of the local breeds lies in their adaptability to local environmental conditions. In many cases this is especially applicable to harsh environment of remote regions. It is also expected to use their adaptability in changing climate conditions. From production point of view, these breeds present possibilities to produce animal products with reasonable inputs (feeding, veterinary, welfare). Moreover, cultural and environmental roles of these breeds have to be taken into account as well. Despite these facts, the decreasing trends of local population sizes and numbers of these local breeds are present for several decades. GANDINI and OLDENBROEK (1999) reported that the main reason of rapid erosion of farm animal diversity is lack of economic profitability of local breeds. In many cases this is connected to other threats identified by FAO (2015) including indiscriminate cross-breeding and introduction and use of imported breeds. The development of number of livestock breeds and population sizes in Slovak Republic will be presented in this paper.

Material and methods

The data on population sizes and purebred animals registered in herdbooks in years 2011 and 2018 were used. In case of poultry and rabbits, data on most important breeds (breeding animals from registered farmers) were used. These data are part of the animal genetic resources monitoring in Slovak Republic, which is performed in cooperation with the Breeding Services of the Slovak Republic, s.e. and authorized breeders` organizations of respective breeds. These data are used for regular updating of EFABIS/DAD-IS information system (TOMKA et al., 2013).

Results

The monitoring of animal genetic resources in Slovak Republic covers not only the endangered breeds, but also other main breeds of livestock. The number of breeds published in EFABIS, and number of breeds monitored in year 2011 and 2018 are presented in Table 1. Numbers of breeds published in the EFABIS and monitored breeds differ slightly. This difference is due to publishing of only well-established breeds, e.i. breeds with at least 3 farmers registered and number of animals is higher than 100. The trend of importing new breeds can be seen especially in sheep and goat. On the other hand number of poultry breeds is decreasing. Trends in these species can be explained not only by economic driven decisions of farmers. Many cases show that the decision is based on emotions and "fashion trends". This fact is especially applicable to breeds with very small population sizes and limited breeding animals. As a result of these decisions we witness fluctuating trend in number of breeds but also in size of populations.

Table 1. Number of breeds published in the EFABIS

Species	EFABIS	Monitored (2011)	Monitored (2018)
Cattle	16	15	15
Sheep	14	16	26
Goat	5	4	9
Pig	10	6	6
Horse	11	11	11
Goose	4	4	3
Duck	3	2	2
Rabbit	43	64	46
Chicken	20	28	18

The increase of number of beef cattle breeds was observed in last several years. Stabile and increasing trend was observed in Slovak Pinzgau and Slovak Spotted cattle (Table 2). Also the increase of population size in well-established beef breeds like Charolais and Limousine was observed. These findings are supported by ORAVCOVA et al. (2010), who stated that farmers are using crossing with beef breeds to transform their herds to beef production. Changes in effective population size of traditional dual-purpose breeds were presented in TOMKA et al. (2016).

Table 2. Cattle

	2011		2018	
	Population size	Purebred females	Population size	Purebred females
Slovak Pinzgau	12,409	1,763	12,164*	1,479*
Slovak Spotted (Simmental)	152,463	15,515	172,000*	16,189*
Holstein (black pied)	153,874	47,398	145,591	31,281
Holstein (red pied)	74,398	n.a.	51,608	5,997
Aberdeen-Angus	2,138	37	7,960	272
Beef Simmental	3,363	158	5,146	262
Blonde d'Aquitaine	1,466	35	1,693	130
Charolais	30,060	1,400	53,755	2,102
Hereford	857	65	1,472	13
Limousine	20,955	880	47,503	1,759
Piemontese	1,610	5	1,043	11
Galloway	n.a.	n.a.	1,241	39
Highland	n.a.	n.a.	1,404	153

*data for the year 2017, n.a. – not available

In the sheep rapid decrease of population size and purebred females registered in herdbooks in traditional breeds including Improved Valachian and Tsigai can be observed (Table 3). On the other hand increase in population size can be observed in specialized breeds like Lacaune and Suffolk. The increase in Askanian Merino and Valachian sheep should be accounted to farmers` enthusiasm (TOMKA et al., 2014). Not published results from survey among sheep breeders showed that one of the main reasons to keep Valachian sheep is patriotism.

Table 3. Sheep

	2011		2018	
	Population size	Purebred females	Population size	Purebred females
Askanian	714	24	418	52
Tsigai	144,777	10,602	113,135	5,433
Merino	25,477	507	11,424	68
Valachian	2,640	102	2,834	820
Improved Valachian	147,602	13,578	121,807	6,971
Berrichon Du Cher	1,113	163	1,635	130
Charollais	2,033	220	1,961	165
Ile-De-France	5,436	352	7,461	409
Lacaune	25,507	1,151	54,318	2,282
Oxford Down	1,915	244	573	128
Romanov	1,291	7	760	n.a.
Romney	699	56	2,721	166
Suffolk	4,964	332	7,675	373
East Friesian	2,429	133	3,001	3,001

n.a. – not available

In goat population size of White Shorthaired and Brown Shorthaired breed increased (Table 4). However the number of purebred females in herdbooks decreased in White Shorthaired goat. Based on effective population size both breeds were reported as endangered (TOMKA et al., 2016). Numbers of two other breeds increased over the time.

Table 4. Goat

	2011		2018	
	Population size	Purebred females	Population size	Purebred females
White Shorthaired Goat	6,321	934	8,166	752
Brown Shorthair Goat	703	50	1,912	152
Anglo-Nubian	58	16	1,232	95
Boer	186	40	574	130

The situation in pigs in Slovakia is more-less stabilized (Table 5). The decrease in some breeds leads to lack of breeding animals (TOMKA et al., 2014).

Table 5. Pig

	2011		2016*	
	Population size	Purebred females	Population size	Purebred females
Large White	5,800 – 10,400	2,161	6,500 – 11,600	2,000
Duroc	50 – 100	10	70 – 120	30
Hampshire	70 – 120	34	50 – 80	20
Landrace	1,700 – 3,000	584	800 – 1,400	300
Pietrain	70 – 120	42	50 – 90	35
Yorkshire	160 – 280	65	80 – 140	48

*data for the year 2018 were not available

The situation in horses is different due to different reasons of keeping animals and smaller population sizes. Numbers are changing over the time (Table 6), but detailed analyses showed oscillating trends (TOMKA et al., 2016). Despite support measures, decrease in population size and number of registered purebred females can be observed in Slovak Warmblood.

The situation in poultry (Table 7) and rabbits (Table 8) was reported to be alarming (TOMKA et al., 2014) and this not changed over the years. This can be explained by the previously mentioned fact that small farmers and households keep animals not just for the production. Moreover the trend of people moving to cities and changes in rural areas leads to decrease of small farmers and household numbers. The raising public awareness has to be in place in these situations. The efforts in this regard are undertaken by NPPC-RIAP Nitra (TOMKA et al., 2017). Similarly as in sheep, not published results from survey among goose breeders showed that one of the main reasons to keep Slovak breeds is patriotism.

Table 6. Horses

	2011*		2018	
	Population size	Purebred females	Population size	Purebred females
Furioso-Northstar	500 – 600	156	400 – 550	158
Shagya Arab	600 – 700	171	600 – 800	151
Hutsul	460 – 550	129	400 – 600	141
Lipitsa	630 – 750	152	800 – 1 000	181
Nonius	100 – 110	22	80 – 130	26
Noric of Murany	480 – 550	100	400 – 550	119
Slovak Warmblood	3 900 – 4 300	1650	2 000 – 3 000	836
Slovak Sport Pony	300 – 400	92	200 – 300	98
English Thoroughbred	800 – 900	105	1 900 – 2 000	80
Arab	300 – 400	74	400 – 600	98
Haflinger	320 – 400	88	250 – 350	128

* estimation for the end of year 2010

Table 7. Poultry

	2011		2018	
	Females	Males	Females	Males
Oravka (chicken)	295	42	350	42
New Hampshire (chicken)	484	53	401	46
Amrock (chicken)	166	22	186	21
Plymouth Rock (chicken)	497	47	314	35
Sussex White (chicken)	152	15	150	18
Welsummer (chicken)	218	34	40	5
Wyandotte (chicken)	378	64	97	13
Rouen (duck)	13	4	23	7
Slovak White (goose)	29	13	25	12
Suchovy (goose)	25	10	21	12
Pomorian (goose)	10	7	9	5

Conclusions

The comparison of actual state of monitored livestock populations with the state in 2011 confirms global trends of decrease of local populations, due to increasing number of specialized breeds. However in some breeds it can be observed, that number of animals stabilize at some point. This represents farmers and keepers, who raise animals not just for the production purposes. This fact suggests that more attention should be paid to raising awareness. The local breeds are not competitive to specialized ones and their preservation is strongly dependent on the preference of farmers for the single breeds.

Table 8. Rabbit

	2011		2018	
	Females	Males	Females	Males
Blue of Holitz	122	45	80	35
Liptovský lysko	n.a.	n.a.	50	25
Nitra	143	32	240	40
Slovak Pastel Rex	n.a.	n.a.	35	15
Slovak Greyblue Rex	125	92	95	30
Zemplin	135	28	60	20
Zobor	n.a.	n.a.	35	10
Belgian Giant	1 260	315	400	90
Burgundy	84	32	500	70
New Zeeland Red	62	25	270	55
New Zeeland White	490	235	300	100

n.a. – not available

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Exterior features and trends in stallion's lines of the Croatian Posavina horse

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Abstract

Croatian Posavina horse is one of the three autochthonous horse breeds in Croatia. During the past twenty-five years, his program of protection has been implemented. After inventarisation and stopping the decline of the population, the breeding organisation formed sire lines to efficiently improve the breeding program. The Croatian Posavina horse has defined 30 sire lines. It was necessary to determine the conformation of the sire lines, therefore 92 active studs were included in research and through it collaboration with the breeding association was achieved. The determined values of the withers height, chest circumference and the cannon bone circumference were 141.98 ± 4.34 cm, 194.17 ± 8.47 cm, and 22.18 ± 0.95 cm, respectively. According to the previous study, stallions in this research have smaller values for withers height (-1.55 cm; $p < 0.05$) and chest circumference (-5.82 cm) while the cannon bone circumference was wider (+0.28 cm). Comparing the dispersion parameters revealed that the phenotypic variation of the examined traits has decreased, which can serve as a good indicator of the consolidation of exterior traits. Monitoring of the breed through phenotypic level and planned mating, should be continued in order to provide its viability. In addition, stronger efforts should be made in promotion and economic affirmation of the breed, especially if long-term sustainability is expected. Good breeding practice encompass regular monitoring of the population trends by analysing phenotype variability within Croatian Posavina Horse populations. Furthermore, balance between sire and dam lines will help in preserving genetic diversity as one of the important component of conservation program.

Key words: Croatian Posavina horse, stallions, exterior, body measurements

Introduction

The Croatian Posavina horse is an autochthonous breed bred at the area of the Sava River in a controlled introduction of warmblooded and later coldblooded horse breeds. The base population of the Croatian Posavina horse was Busak. Until the middle of the 20th century, the Croatian Posavina horse was crossbred with Arabian horses to improve some features (interior, speed, strength and endurance) and therefore it appeared in a type of warmblood (ROMIĆ, 1965). The Croatian Posavina horse was suitable for agriculture and forestry work, drawing heavy loads and pulling wheeled vehicles, and pulling of small boats (cro. *teglenice*) at the coast of the rivers Sava and Kupa. In the formation of the Croatian Posavina horse was

mainly used Arabic, Ardenic, and in some areas Lipizzaner and Nonius studs. Arabic, Nonius and Lipizzaner breeds heavily influenced the temperament of the Croatian Posavina horse.
The introduction of mechanization in agriculture in the second half of the 20th century has reduced the need for the Croatian Posavina horse as a working animal, which led to the decrease of its population size. Nowadays, this breed is mostly used in agro-tourism and extensive systems for production of the horse meat. The systematic protection of the Croatian Posavina horse began in the middle of the nineties' of the 20th, which resulted into increasing numbers of the population. Actual total number of Croatian Posavina horses is 4.900 heads out of which are 277 stallions and 2639 mares.

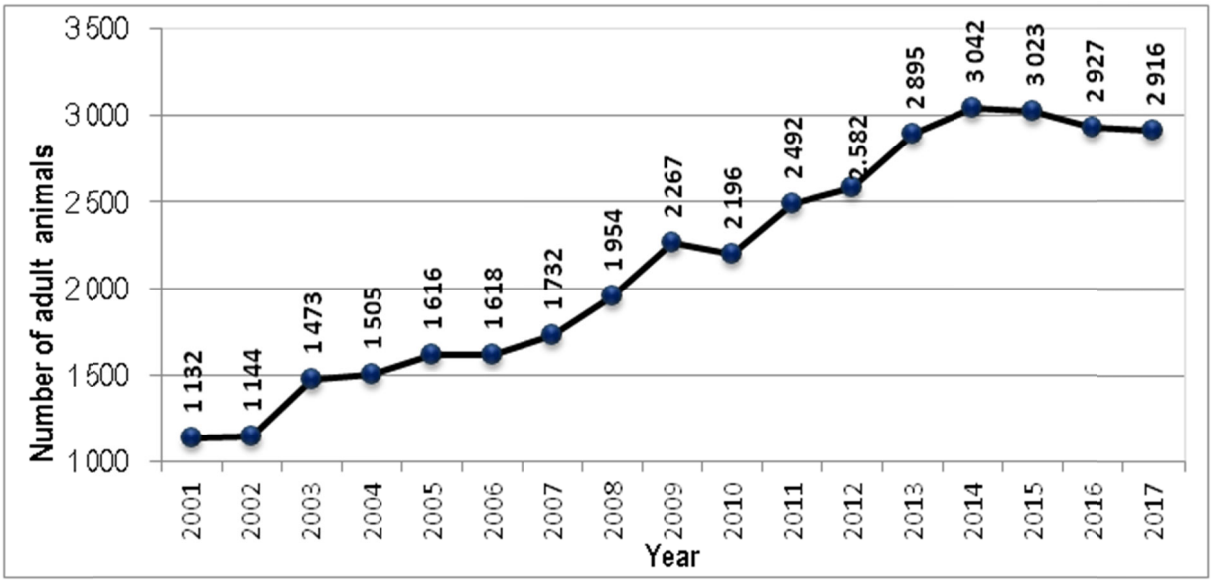

Figure 1. Number of Croatian Posavina horses per years (CAA, 2002 – 2018)

Croatian Posavina horse is a middle-weight horse of a firm constitution. Head is dry and small, forehead is wide, profile is straight, ears are small, eyes and nostrils are large and expressed. Neck is moderately long, well covered with muscles, firmly connected to the body. Chest is wide and deep. Shoulders are moderately long, diagonally positioned, well covered with muscles and firmly connected to the body. The back is medium long, strong and wide, while the connection over the loin is short and wide. Croup is wide, moderately struck down, split, and well covered with muscles. Belly is rounded and body is compact. Legs are dry and strong, joints expressed, and cannon bone is short. Feathers are short. The postures of the legs are correct and hoofs are wide and adequately built. Mane and tail are wavy and medium long. The most frequent coat colour is bay and black, while sorrel/chestnut, dun, grey or Isabella coat colour rarely appear. Sexual dimorphism is clearly expressed. Croatian Posavina horse is appreciated for its resistance, strength, modesty and adaptability.
Although the population of Croatian Posavina horse has 28 sire lines, only eight lines have four or more licenced studs in breeding. One of the breeding goal incorporated in the breeding program is to preserve wider genetic variability therefore in breeding are left sire lines with three and less active stallions. Genetic characterization and verification of the pedigree was carried out. In order to have a better insight into the structure of the population, we also wanted to research the variability of the basic exterior measurement of the Croatian Posavina stallions. Therefore, the aim of this research was to investigate the phenotype variation of sire lines in the Croatian Posavina horse population.

Material and methods

Dates were collected during scoring of the Croatian Posavina stallions after horses reached maturity. From reproductively active stallions (92 animals), body measures were taken (withers height, chest and cannon bone circumference). Information about exterior measures were analysed by GLM procedure of SAS package (SAS Institute, 1999) and Past 3.23 (HAMMER et al., 2001).

Results and discussion

Observed body measurements of the Croatian Posavina studs included in research (withers height, chest circumference, and cannon bone circumference) are present in Table 1. Withers height in this research (141.98 ± 4.34 cm) was smaller for -1.55 cm in comparison to the withers height observed in the research of IVANKOVIĆ and CAPUT (2004; 143.53 ± 3.10 cm), although the variability of the trait increased. Comparing the height of the withers of stallions from the middle of the last century (138.04 cm; ROMIC, 1965), there is a significant increase ($p < 0.05$) in withers height (+3.98 cm) of nowadays stallions. It is interesting to note that the stallions of the Croatian Posavina horse in withers are lower by 0.82 cm than the phylogenetically related Posavina horse in Slovenia (-0.82 cm)

Table 1. Body measurement of stallions of Croatian Posavina horse (cm)

Trait	LSMean ± SD	Min.	Max.
Withers height (cm)	141.98 ± 4.34	132.0	153.0
Chest circumference (cm)	194.17 ± 8.47	178.0	216.0
Cannon bone circumference (cm)	22.18 ± 0.95	19.5	25.0

The decrease of chest circumference (194.17 ± 8.47 cm) in this study was -5.82 cm in relation to the chest circumference observed in IVANKOVIĆ and CAPUT (2004; 199.99 ± 9.24 cm) however, they kept significant levels of variability. Compared to the circumference from the middle of the past century (153.66 cm; ROMIĆ, 1965), a significant increase was noted in the size of chest of nowadays stallions (+40.51 cm). This increase of chest girth can be explained by the genetic influence (introducing of coldblooded breeds into the population) and improved keeping and feeding management. It is worth noting that Croatian Posavina horse has a wider chest girth for + 5.98 cm than the Posavina horse in Slovenia (188.2 ± 1.57, SIMČIĆ et al., 2012)

The cannon bone circumference in this research (22.18 ± 0.95 cm) has a slight increase of +0.28 cm in relation to the cannon bone circumference mentioned in the research of IVANKOVIĆ and CAPUT (2004; 21.90 ± 1.27 cm) however, there was a high difference of +3.66 cm from research of ROMIC (1965; 18.52 cm). Possible explanation can be due to introduction of coldblooded breeds in the Croatian Posavina population in the second half of the 20th century. SIMČIĆ et al. (2012) reported the higher cannon bone girth in Slovenian studs (23.0 ± 0.16) than Croatian ones.

Table 2. Body measurement of eight most numerous sire lines of Croatian Posavina horse (cm)

Sire line	n	Withers height			Chest circumference			Cannon bone circumference		
		X ± SD	Min.	Max.	X ± SD	Min.	Max.	X ± SD	Min.	Max.
10	15	141.3± 5.45	132.0	153.0	196.3±7.49	183	211	22.33±0.90	21.0	24.0
11	12	141.6± 3.99	135.0	148.0	199.9±6.87	190	216	22.13±1.05	20.0	24.0
12	13	140.2± 2.95	135.5	146.0	191.7±8.33	178	207	21.69±1.09	19.5	23.0
15	7	142.2± 2.80	138.5	146.0	193.9±13.13	180	214	22.43±0.73	21.5	23.5
13	6	142.3± 5.13	136.0	151.0	191.5±4.32	186	197	22.42±0.86	21.5	24.0
14	6	142.2± 5.56	135.0	150.0	188.5±6.74	182	200	22.00±1.58	20.5	25.0
17	6	143.1± 4.25	137.5	149.0	194.8±8.13	186	210	22.33±0.41	22.0	23.0
16	5	144.9± 5.66	138.5	151.0	189.2±13.85	179	213	21.90±0.96	21.0	23.5

Out of the 30 sire lines present in the breeding of the Croatian Posavina horse, 24 of them were analysed. Eight lines have higher number of offspring and are more often used in breeding than the rest 16 investigated lines that have one to two offspring. Table 3 shows average values of the body measures of eight dominant sire lines of Croatian Posavina horse. Although some discrepancies among measures are observed, statistically they are not significant.

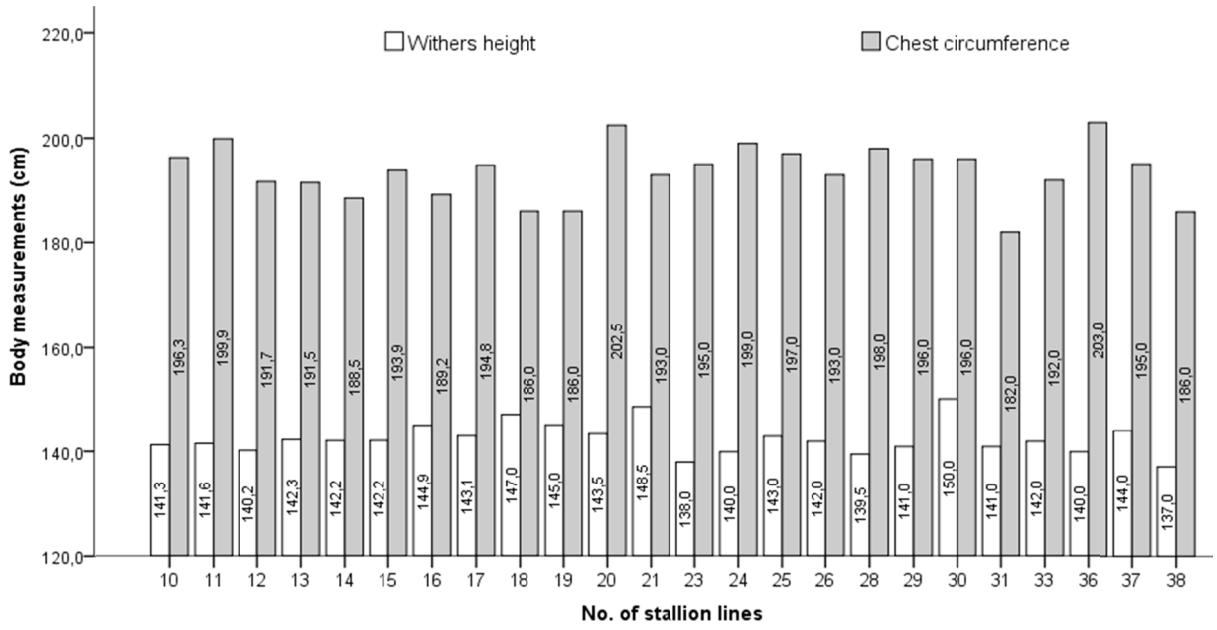

Figure 2. Average height of the withers and circumferences of the chest in sire lines of the Croatian Posavina horse

The Principal Component analysis of body measures of the Croatian Posavina horse describe that most of the variability can be attributed to the withers height (82.45%), then to the chest circumference (16.87%), and the smallest part of the variance to the cannon bone circumference (0.68; Table 3., Figure 1).

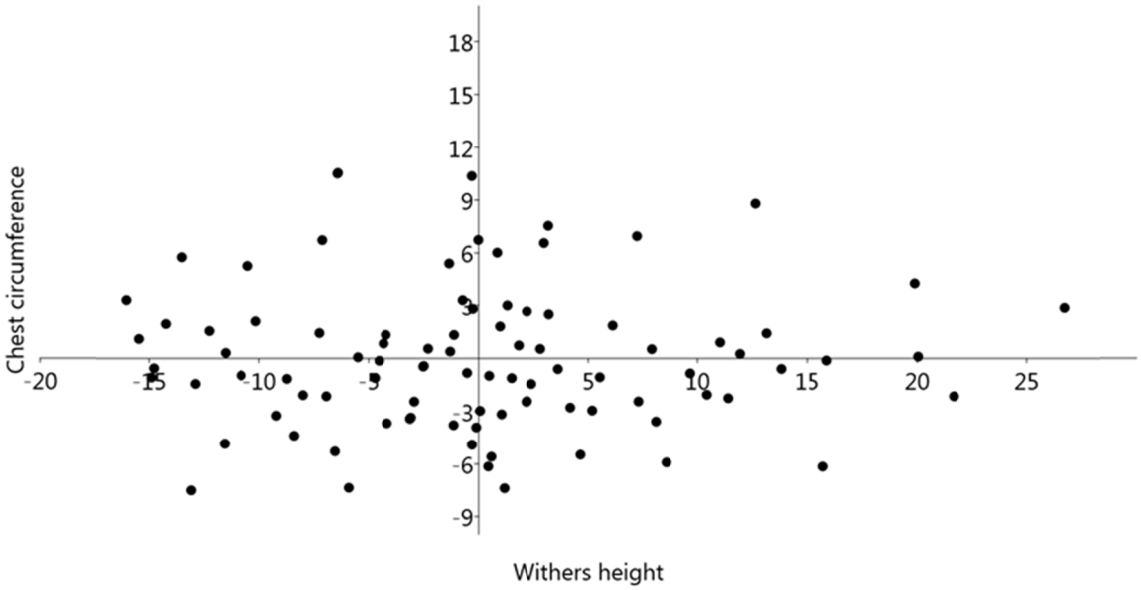

Figure 3. Principal Components Analysis plot of individuals according to withers height and chest circumference in Croatian Posavina horse

Table 3. Percentage of variance in body measurements of Croatian Posavina horse

Trait	Eigenvalue	% variance
Withers height	80.45	82.45
Chest circumference	16.46	16.87
Cannon bone circumference	0.66	0.68

Conclusion and recommendation

In all examined traits, the comparison revealed a decrease in phenotypic variability, which may indicate consolidation of conformation traits. Taking into account the results of both phenotypic and genotypic analysis it can be concluded that phenotypic consolidation of the breed may not have caused major loss of initial genetic variability. Monitoring of the breed should include the phenotypic and genetic level in order to protect existence of the breed.

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Pedigree analysis of the Hungarian Coldblood Horse breeding population

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Abstract

An effective gene conservation programme requires the knowledge of genetic diversity of the population. The genetic structure of Hungarian Coldblood Horse breed was studied from pedigree records. Herdbook data of the active breeding population in 2016 of registered Hungarian Coldblood horses were analysed. The generation interval varied between 8.71 and 10.22 for the analysed population. The proportion of known parents for the reference population was above 50% in the 6th generation. There were 3,434 and 427 horses covering the total genetic variability of the whole and reference populations. Most important ancestors were Belgian import stallions for the whole population, whereas it was a French import stallion for the reference stock. Average inbreeding coefficient of the reference stock is 1.8%. There are 27 horses having higher than 10% inbreeding coefficient in the reference population.

Key words: pedigree analysis, Hungarian Coldblood Horse

Introduction

Number of draught horses has been decreased across the world in the last decades, so conservation of these breeds is an increasing demand as they were part of the traditional livestock farming. Evaluation of within population genetic variability might give new information about the history and status of a breed before implementing a new breeding program for the management of the present breeding stock. Breeders want to be familiar with the genetic variability of various livestock species (WOOLLIAMS et al., 2002). The population genetic structure and variability can be described using pedigree data; the analysis produces information about the ancestors and relatives of the animals (MAIGNEL et al., 1996).

The Hungarian Coldblood Horse, as being heavy draught horse, was mainly bred in the western part in the Transdanubian region of Hungary. The breed is originated mainly from the Austrian Noriker and Pinzgauer horses. After the 2nd World War, Belgian and French Ardennes type cold-blooded stallions were imported to develop the weight of the breed and decrease the conformation heterogeneity of the population.

Genetic background of horses based on the pedigree information is well documented for various breeds. Population structure of English (MOUREAUX et al., 1996; BOKOR et al., 2013) as well as Arabian Thoroughbreds (GLAZEWSKA and JEZIERSKI, 2004;

CERVANTES et al., 2008) were estimated. DRUML et al. (2009) found low inbreeding for the Austrian Noriker population. DRUML et al. (2007) studied 11 draught horse populations based on microsatellite markers.
The aim of the research study was to analyse the pedigree information of the registered horses within the Hungarian Coldblood Horse studbook.

Material and methods

The basis of the current study was the Hungarian active breeding population of Hungarian Coldblood in 2016. The active population (1251 horses) was chosen as reference when needed. The base pedigree information was given by the Hungarian Coldblood Horse Breeding Association. There were the pedigree data of 18223 animals in the developed database. Generation interval shows the average age of parents at the time of their offspring's birth (JAMES, 1977). The value was calculated along four different pathways (sire–daughter, sire–son, dam–daughter and dam–son) on the basis of the recoded individuals' and their parents' birth dates. Pedigree completeness was characterized by the values of the number of full generations traced. The homozygosity of the population was characterized using the inbreeding coefficient (WRIGHT, 1922) and additive genetic relationship (BOICHARD, 2002). As BOICHARD et al. (2007) described, its precision greatly depends on the length and the completeness of the pedigree. All the above described parameters were computed using ENDOG (GUTIÉRREZ and GOYACHE, 2005) and POPREP (GROENEVELD et al., 2009) software.

Results and discussion

The longest generation interval was computed for the sire-to-son pathway (Table 1). The dam-to-daughter pathway was found to be the shortest generation interval among the different paths. The four pathways were compared pairwise using independent samples t-test. There were significant differences between stallions and mares ($P<0.05$) as stallions were used in breeding significantly longer than broodmares. Our values were slightly higher than those of found by VOSTRY et al. (2011).

Table 1. The generation intervals (years) of the Hungarian Coldblood population for the different pathways

Parent-offspring lineages	Number of pathways	Generation interval
Sire-to-son	1824	10.22^{a}
Sire-to-daughter	4603	9.28^{b}
Dam-to-son	1506	8.93^{c}
Dam-to-daughter	3688	8.71^{c}
Average	11621	9.20

different superscript letters show significant difference ($P<0.05$)

Figure 1 shows the pedigree completeness of the population based on birth years. Pedigree of more than 50% of horses born after 1970's was known at least six generations back. The average pedigree completeness was 4.24 for the reference population what is much smaller than those of estimated for Lipizzaner (15.2; ZECHNER et al., 2002) and English Thoroughbred (15.64; BOKOR et al., 2013) horses.

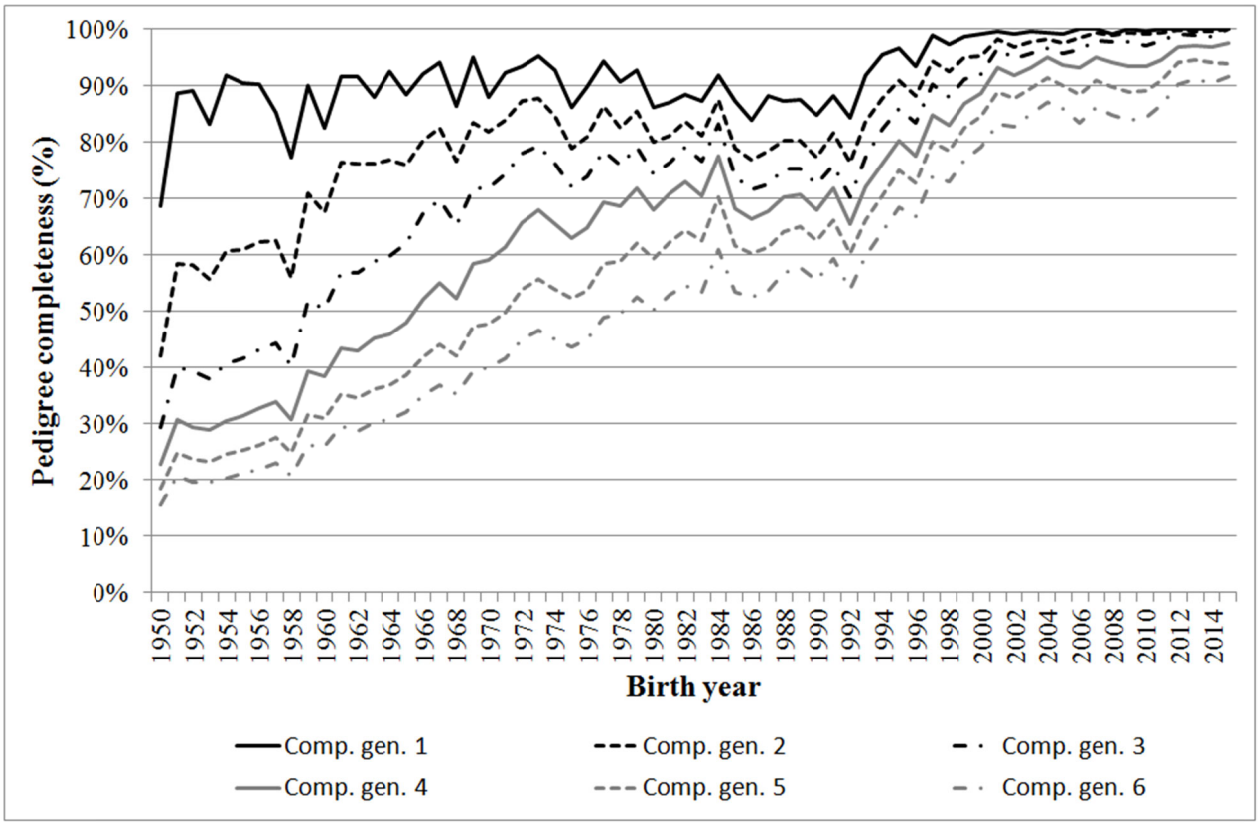

Figure 1. Evaluation of pedigree completeness

Table 2 gives information the genetic variability of the recent stock. Only 76 and 17 horses cover the 50% of the genetic variability for the whole and reference population, respectively. It can be seen easily, that the reference population could be covered with much less ancestors which show reasonable gene loss during the history of the breed.

Table 2. Concentration of genetic variability

Percentage of genetic variability	Whole population	Reference population
50%	76	17
60%	152	28
70%	338	43
80%	789	73
90%	1613	136
100%	3434	427

Ancestors having greatest impact on the total population are detailed described in Table 3. There were only stallions among the ten most important ancestors. The highest impact on the total population had two Belgian import stallions, 2281 Belga-23 and 2264 Belga-6. They were half-siblings, so the genetic variability of the stock was decreased in this way.

Table 3. Most important ancestors of the total population

Name of the horse	Gender	Birth year	Covering genetic variability (%)
2281 Belga-23	1	1946	4.18
2264 Belga-6	1	1947	3.70
2734 Perseron-201 Bizsu	1	1989	3.14
4380 Bikal-3	1	1955	2.14
1560 Nágocs-110	1	1980	1.79
2351 Tormás-61	1	1987	1.53
5899 Görösgal-17 (Tizes I.tm.)	1	1966	1.51
1899 Gölle-9	1	1977	1.47
5790 Andráshida-12	1	1965	1.22
4337 Szentdénes-15 (Huszonkettes)	1	1955	1.14

Ancestors responsible for the genetic variability of the reference population are shown in Table 4. The stallion imported from France, 2734 Perseron-201 Bizsu, born in 1989, covers 6.66% of the total genetic variability of the present stock. This high proportion variability is unreasonably high in the case of an indigenous breed and his importance higher than those of the two Belgian import stallions. The high overall influence of Percheron stallions should be taken into account in the future during the utilization of young stallions.

Table 4. Most important ancestors of the reference population

Name of the horse	Gender	Birth year	Covering genetic variability (%)
2734 Perseron-201 Bizsu	1	1989	6.66
1560 Nágocs-110	1	1980	5.76
2281 Belga-23	1	1946	4.66
2264 Belga-6	1	1947	4.38
2351 Tormás-61 (descendant of Perseron-1)	1	1987	3.85
1899 Gölle-9 (descendant of Perseron-4)	1	1977	3.02
4380 Bikal-3	1	1955	2.60
986 Gölle-2	1	1936	2.51
456 Szentgáloskér-2	1	1970	2.41
5790 Andráshida-12	1	1965	2.37

Because of the opened pedigree of the breed, the inbreeding of the population is quite low (Figure 2.), it is 1.8% for the reference animals. This low inbreeding is in agreement with DRUML et al. (2009) and VOSTRY et al. (2011) results for the Austrian (1.2) and Czech (1.51) Noriker population, respectively. VOSTRY et al. (2011) estimated higher inbreeding for Silesian Noriker (3.23) and Czech-Moravian Belgian (3.53) horses. The values of additive genetic relationship and inbreeding coefficient by year are very close to each other, so mating of related animals were not avoided. There are 41 horses having more than 25% inbreeding coefficient in the whole population whereas 27 horses having higher than 10% inbreeding coefficient in the reference population.

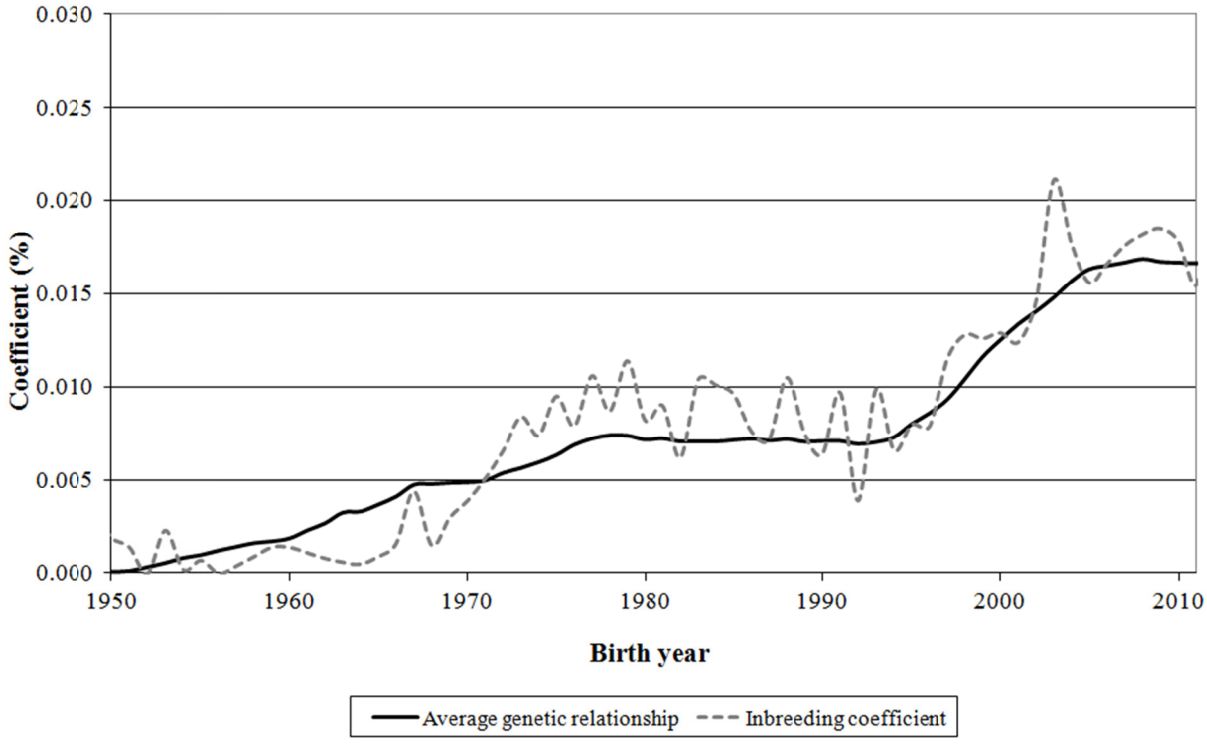

Figure 2. Changing of inbreeding of the population

Conclusions and recommendations

The generation interval varied between 8.71 and 10.22 for the analysed population. The shortest was estimated for mare-to-daughter pathway, whereas the longest was sire-to-son pathway. There were 3,434 and 427 horses covering the total genetic variability of the whole and reference populations, respectively. Most important ancestor was 2281 Belga-23 (4.18%) for the whole population, whereas it was 2734 Perseron-201 Bizsu (6.66%) for the reference population. There are 41 horses having more than 25% inbreeding coefficient in the whole population and 27 horses having more than 10% inbreeding coefficient in the reference population, respectively.

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Genetic connectedness of breeding populations of Hucul breeder countries

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Abstract

The Hucul horse is originated from the former Austrian-Hungarian Monarchy, from the wooden Carpathian region. The structure of the total population was described from available pedigree information of the breeder countries using Nei's minimum distance and F statistics. Appearance of stallion lines and mare families were also evaluated. Only three stallion lines have appeared in all examined countries. Among the 42 mare families only one, 4 Kitca, was found in each breeder countries. Genetic diversity might be decreased due to 11 mare families were appeared only in one country. Smallest pairwise genetic distance was found between sub-populations of the Czech Republic and Slovakia, whereas longest was found between those of Romania and Poland based on both measurement variables. Lowest within population differences were found for the small German population.

Key words: genetic distance, Hucul Horse breed

Introduction

The Hucul horse is originated from the former Austrian-Hungarian Monarchy, from the wooden Carpathian region. After the 1st World War, the original population was divided among the countries being from the ruins of the Monarchy and breeding has started in several countries. Genetic analyses of the recent stocks of the breeder countries have been started. Information about the Polish population was reported in several studies (MACKOWSKI et al., 2015; Kwiecińska and Purzyc, 2009), PJONTEK et al. (2012) the Slovakian whereas SOMOGYVÁRI et al. (2018) reported about the Hungarian breeding stock.
The handling of the within-population genetic variability and minimizing gene flow is necessary to establish appropriate management of the genetic stock. Simple demographic parameters are largely dependent on the management and mating policy and have a large impact on the genetic variability. The study of the population structure can highlight important circumstances affecting the genetic history. Overall, the main aim of the gene preservation is maintaining the largest genetic variability within the population.
The aim of the present research work was to discover the pairwise genetic distances among breeding populations of various Hucul breeder countries.

Material and methods

The structure of the total population was described from pedigree information of the breeder countries based on pedigree information summarized from national stud-book data. The appearance of stallion lines and mare families within countries should be taken into account during the analysis of gene flow, so it was collected and evaluated. The examined time interval was calculated based on the generation interval. In this way those animals were taken into account, which appeared between 2000 and 2016 as breeding animals within the analysed populations. The pairwise genetic distance among the sub-populations of the Hucul breeder countries were characterized using Nei's minimum genetic distance (NEI, 1987) and F statistics (WRIGHT, 1978). Both parameters were computed using ENDOG (GUTIÉRREZ and GOYACHE, 2005) software.

Results and discussion

Distribution of stallion lines across countries was evaluated. The seven stallion lines (Hroby, Goral, Prislop, Pietrosu, Ousor, Polan, Gurgul) were checked for all countries (Table 1.). The Goral, Ousor and Prislop lines appeared with breeding stallions within all countries. The Polish originated stallion line, Polan, has representative stallions only in three countries; Austria and Hungary have stallions from this line along Poland. Unfortunately, the lines were not equally distributed, only part of them is responsible for the genetic diversity within the analysed countries.

Table 1. Distribution of stallion lines across breeder countries

Stallion line	Austria	Czech Republic	Germany	Hungary	Poland	Romania	Slovakia
Goral	X	X	X	X	X	X	X
Gurgul	X	X	X	X	X		X
Hroby	X	X		X	X	X	X
Ousor	X	X	X	X	X	X	X
Pietrosu	X	X		X	X	X	
Polan	X			X	X		
Prislop	X	X	X	X	X	X	X

There were 42 mare families appearing in the total breeding population within the analysed time period (2000-2016). Only a single mare family, 4 Kitca, originated from Lucina, Romania appeared through mare descendants in all countries. Four other mare families (1 Panca, 17 Aglaia, 825 Agla and Bukovina) were found within six sub-populations. It should be noted that 11 mare families appeared only in one country. Further interesting thing that is most cases they are surviving outside the founder country. Furthermore, 2 Ritka and 3 Tatarca mare families have only three broodmares existing in the total population. Genetic diversity might be increased with the more equal distribution of these families. From gene conversation point of view, more equally distribution across mare family size would be favourable, as usually one (maximum two/three) families dominates within country populations.

Table 2. Distribution of mare families across breeder countries

Mare family	Austria	Czech Republik	Germany	Hungary	Poland	Romania	Slovakia
1 Panca (Ro)	4	1		25	13	6	5
2 Lucina (Ro)	4	4		12		3	
2 Ritka (Ro)		1			2		
3 Tatarca (Ro)				3			
4 Kitca (Ro)	11	3	3	123	16	30	3
5 Plosca (Ro)	1			12		3	
11 Rotunda (Ro)	1	3		27		8	23
11 Zuza (Sk)		5					
12 Sarata (Ro)	13	1	24	122		9	
17 Aglaia (Ro)	2	6		10	98	7	29
18 Barna (Sk)		10					
19 Kavka (Sk)		2					16
23 Klapta (Sk)		14					
39 Franca (Sk)		1			8		4
48 Mulica (Pl)		2					13
70 Sekacka (Sk)		15		3			2
71 Róza (Sk)					12		
84 Hurka (Sk)		4					
84 Polonia (Pl)	23	9	1		523		86
86 Deremoxa (Ro)	8		4	53		6	15
90 Machocha (Sk)	1				13		77
108 Morza (Sk)		6					
111 Rumina (Sk)		15					
825 Agla (Sk)	1	33	2		4	2	104
862 Dagmar (Sk)		37	2				9
882 Gelnica (Sk)	3	8		70			51
Agatka (Pl)			2		219		
Árvácska (Hu)				228			2
Aspiráns (Hu)	2			168	1		2
Bajkálka (Pl)	5				146		1
Bukovina (Sk)	1	23	3	1	1		113
Czeremcha (Pl)	3				96		
Jagoda (Pl)	1		1		132		
Laliszka (Pl)	13				188		
Nakoneczna (Pl)	9	2			130		3
Reda (Pl)					61		
Szroczka (Pl)	2	6			200		26
Valuta (Sk)		6	1				
Wolga (Pl) (b.Hu)	1				313		
Wrona (Pl) (b.Hu)	4			1	144		
Wydra (Pl) (b.Hu)	1			3	154		
Zyrka (Pl)					52		

The pairwise genetic distance among countries can be seen in Figure 1. As the colour is getting darker, the distance between subpopulations getting larger. There were the smallest distance between the Czech and the Slovak populations. The reason might be that the two countries were joined to one near 70 years and this made the breeding rules quite uniform. The separation of the countries couldn't made large population changes yet, furthermore, the international breeding organization of the breed (Huzul International Federation, HIF) makes cooperation among the breeder countries. The distance was also low between Austrian and the Polish sub-populations. The reason might be that Austria re-started breeding of Hucul horses in 1992 based on mainly imported animals from Poland. The Hungarian and Romanian populations are also quite close to each other. It was supposed based on experimental way, but in this way we could strengthen it scientifically. The continuous imports from Lucina increased the number of mare families and stallion lines in Hungary and the genetic diversity was also improved in this way.

The highest pairwise genetic distance was estimated between the Polish and Romanian populations. Genetic distance for the Polish stock is the highest for populations except the Austrian. The reason might be, that compared to the high number of horses in Poland, only a few animals were imported from other countries, especially from Lucina. Nowadays, it is demand in all HIF member countries to increase number of mare families within the sub-populations, so migration of breeding animals is increasing. It would be interesting to repeat this work after few generations.
There is a small population in Germany, mainly for utilization and not for breeding. Animals were imported from Poland, Austria, Slovakia and Hungary, their sub-population is in similar distance from all other countries.

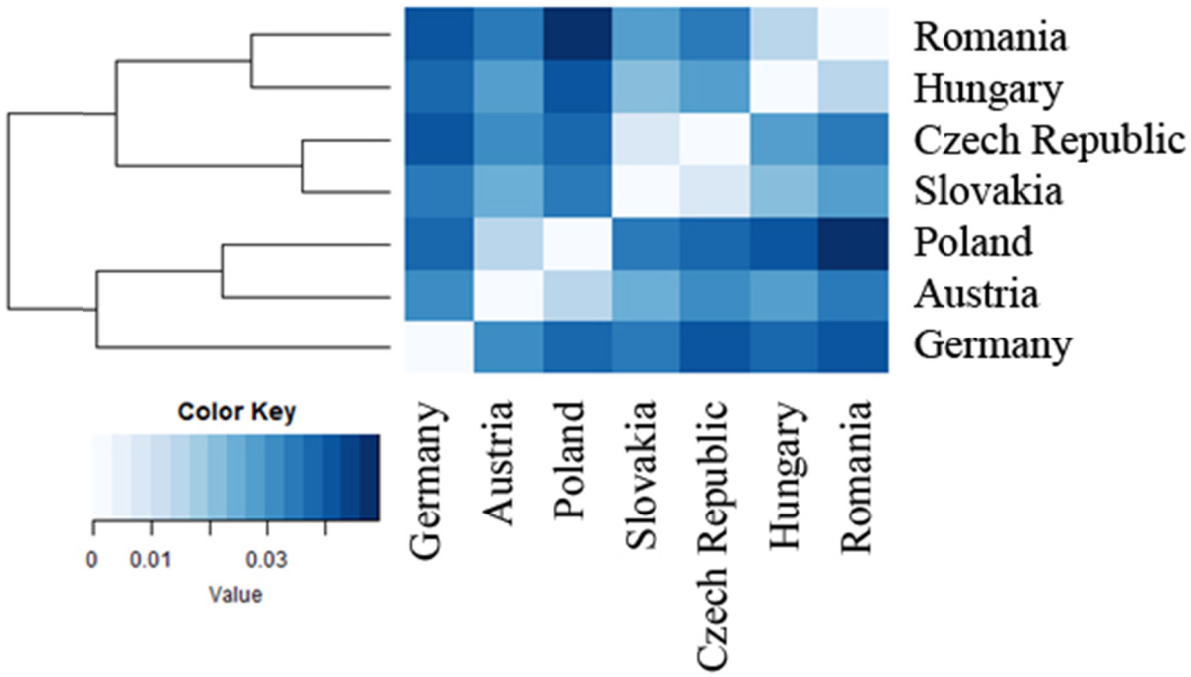

Figure 1. Nei-based genetic distance among populations of Hucul breeder countries

Genetic distances were also calculated also using F statistics. The overall F_{ST} value (0.02476) was below 0.05, so we estimated low differences among the sub-populations. The variability of the total population (F_{IT}) was 0.01393, so there is moderate heterogeneity and low fragmentation within the total breeding stock. The pairwise differences between the Hucul breeder countries were graphically illustrated (Figure 2.) based on the F_{ST} values. Highest differences were found between Romanian and the Czech and also between Romanian and Polish populations. Moderate differences were found between the Czech and Hungarian, between Romanian and Slovakian as well as between German and Austrian populations. Small difference was found between the Slovakian and the Czech breeding stocks (reason might be the common historical background) and also between Austrian and Slovakian stocks. The smallest within population differences were estimated for the German population. The reason might be the small population and also the large amount of imported and lack of own bred animals.

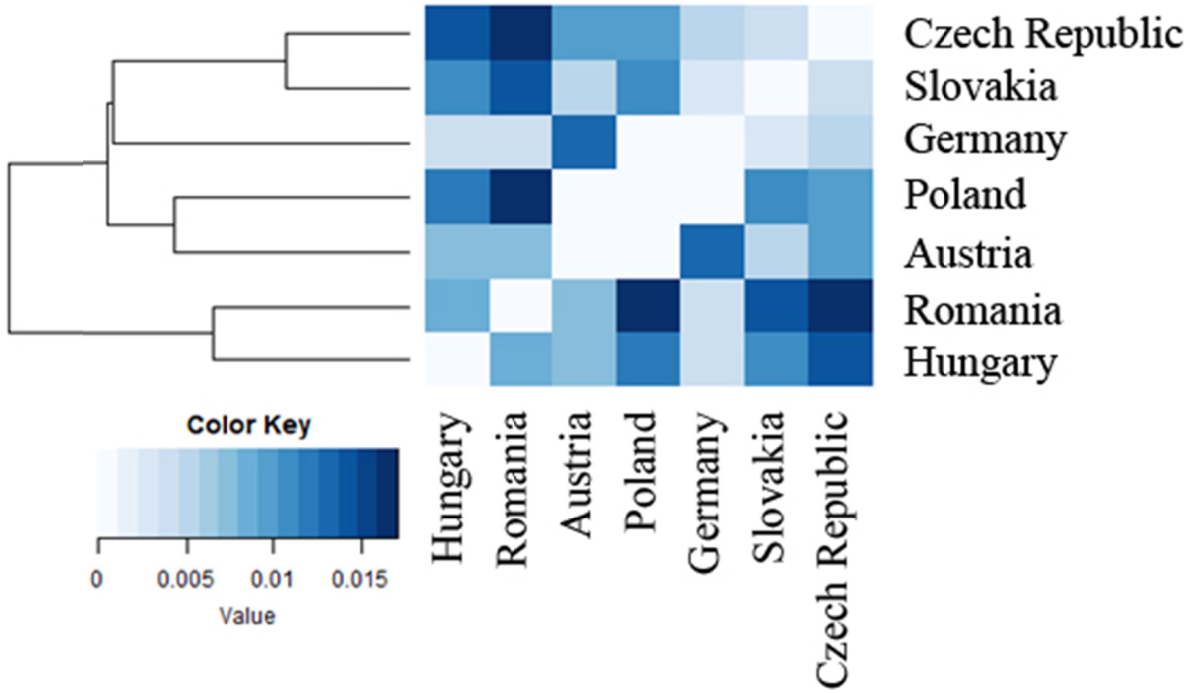

Figure 2. Genetic distance among populations of Hucul breeder countries based on F statistics (F_{ST})

Conclusions and recommendations

Only three stallion lines have appeared in all examined countries. Among the 42 mare families only one, 4 Kitca, was found in each breeder countries. Genetic diversity might be decreased due to 11 mare families were appeared only in one country. Smallest pairwise genetic distance was found between sub-populations of the Czech Republic and Slovakia, whereas longest was found between those of Romania and Poland based on both measurement variables.

Acknowledgements

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Genetic diversity of the Hungarian Furioso-North Star Horse population

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Abstract

The most common goal of animal conservation programmes is to maintain genetic diversity. The genetic structure of Furioso-North Star horse breed was studied from pedigree records. Herdbook data of the active breeding population in 2016 of registered Furioso-North Star horses were analysed. The generation interval varied between 10.05 and 12.40 for the analysed population. There were 3,328 and 534 horses covering the total genetic variability of the whole and reference populations, respectively. Most important ancestor was the English Thoroughbred North Star III (2.67%) for the whole population, whereas it was Furioso III-4 (VI.tm) (6.65%) for the reference population, respectively. There were 23 horses having higher than 25% inbreeding coefficient in the reference population.

Key words: pedigree analysis, Furioso-North Star horse

Introduction

The most common goal of animal conservation programmes is to maintain genetic diversity. Complete pedigree information is considered very important in horse breeding. The Furioso-North Star breed is originated from Mezőhegyes Stud (founded in 1785) based on two stallions founder stallions, Furioso Senior and North Star Senior (EDWARDS and GEDDES, 1991). This breed is an important gene resource.
The demand to get to know the genetic variability of livestock animals has been continuously increasing (WOOLLIAMS et al., 2002). In pedigree analysis, information about the animal's ancestors and collateral relatives is used to carry out analyses to estimate parameters, which characterize the genetic structure and diversity of the populations (MAIGNEL et al., 1996). This analysis will suggest appropriate strategies to monitor matings and manage genetic variability to enlarge the selection basis useful for a selection program (VALERA et al, 2005). Many publications in recent years have described the genetic structure of different breeds of horses based on analysis of levels of inbreeding and founder contributions. The complete or partial results of genetic diversity and population parameters were reported in the literature for various horse breeds, including Andalusian (VALERA et al., 2005), Brazilian Sport Horse (MEDEIROS et al., 2014), Dutch harness horse (SCHURINK et al. 2012), Hanoverian (HAMANN and DISTL, 2008), Holstein (ROOS et al., 2015), English Thoroughbred (BOKOR et al., 2013), Lusitano (DA SILVA FARIA et al., 2018b), Old Kladruber

(VOSTRÁ-VYDROVÁ et al., 2016), Spanish Arab Horse (CERVANTES et al., 2008) and Quarter Horse (DA SILVA FARIA et al., 2018a) breeds.
Therefore, the aim of the research study was to analyse the pedigree information of the registered Hungarian Furioso-North Star population.

Material and methods

The basis of the current study was the Hungarian active breeding population of Furioso-North Star Horse breed in 2016. The active population (3208 horses) was chosen as reference when needed. The base pedigree information was given by the Furioso-North Star Horse Breeding Association. There were the pedigree data of 16746 animals in the developed database.
Pedigree completeness was characterized by the values of the number of full generations traced. Pedigree completeness expresses how much generation equivalent information we have on average in any individual's pedigree.
The homozygosity of the population was characterized using the inbreeding coefficient (WRIGHT, 1922). The inbreeding coefficient was defined as the probability of an individual having two genes identical by descent. Its precision depends on the length and the completeness of the pedigree (BOICHARD et al., 1997). Generation interval shows the average age of parents at the time of their offspring's birth (JAMES, 1977). The value was calculated along four different pathways (sire–daughter, sire–son, dam–daughter and dam–son) on the basis of the recoded individuals' and their parents' birth dates. All the above described parameters were computed using ENDOG (GUTIÉRREZ and GOYACHE, 2005) and POPREP (GROENEVELD et al., 2009) software.

Results and discussion

The longest generation interval was computed for the sire-to-son pathway (Table 1). The dam-to-daughter pathway was found to be the shortest generation interval among the different paths. The four pathways were compared pairwise using paired samples t-test. There were significant differences between every pathways ($P<0.05$) and stallions pathways were two years longer than those of broodmares.

Table 1. The generation intervals (years) of the Furioso-North Star population for the different pathways

Parent-offspring lineages	Number of pathways	Generation interval	Standard error
Sire-to-son	2904	12.40[a]	0.096
Sire-to-daughter	4716	12.08[b]	0.074
Dam-to-son	2245	10.39[c]	0.093
Dam-to-daughter	3813	10.05[d]	0.072
Average	13678	11.31	0.042

different superscript letters show significant difference ($P<0.05$)

Our recent findings were longer than found by MOUREAUX et al. (1996) estimations for Anglo-Arabians (11.5). Their results for Selle Francais and French Trotters (11.7 and 11.8) were also greater than our results. BOKOR et al. (2013) found similar values for the Hungarian English Thoroughbred population (11.41) to our estimations for the Furioso-North

Star horses. Our values were higher than those of found by ROOS et al. (2015) in the Holstein breed (10.31).
The pedigree completeness of the population is shown in Figure 1. More than 60% of horses born after 1980's had known pedigree data at least six generations back. The average pedigree completeness was 4.62 for the reference population. Because of the historical storms (wars, etc.) of the breed as well as some pedigree filling problems, these values were smaller compared to Lipizzaner (15.2; ZECHNER et al., 2002) and English Thoroughbred (15.64; BOKOR et al., 2013) horses.
There were ancestors up to the 20th generation back for some horses in the reference population, six of them had it to the 29th generation back. The evaluation of the pedigree showed that those ancestors were English Thoroughbreds due to the used breeding method.

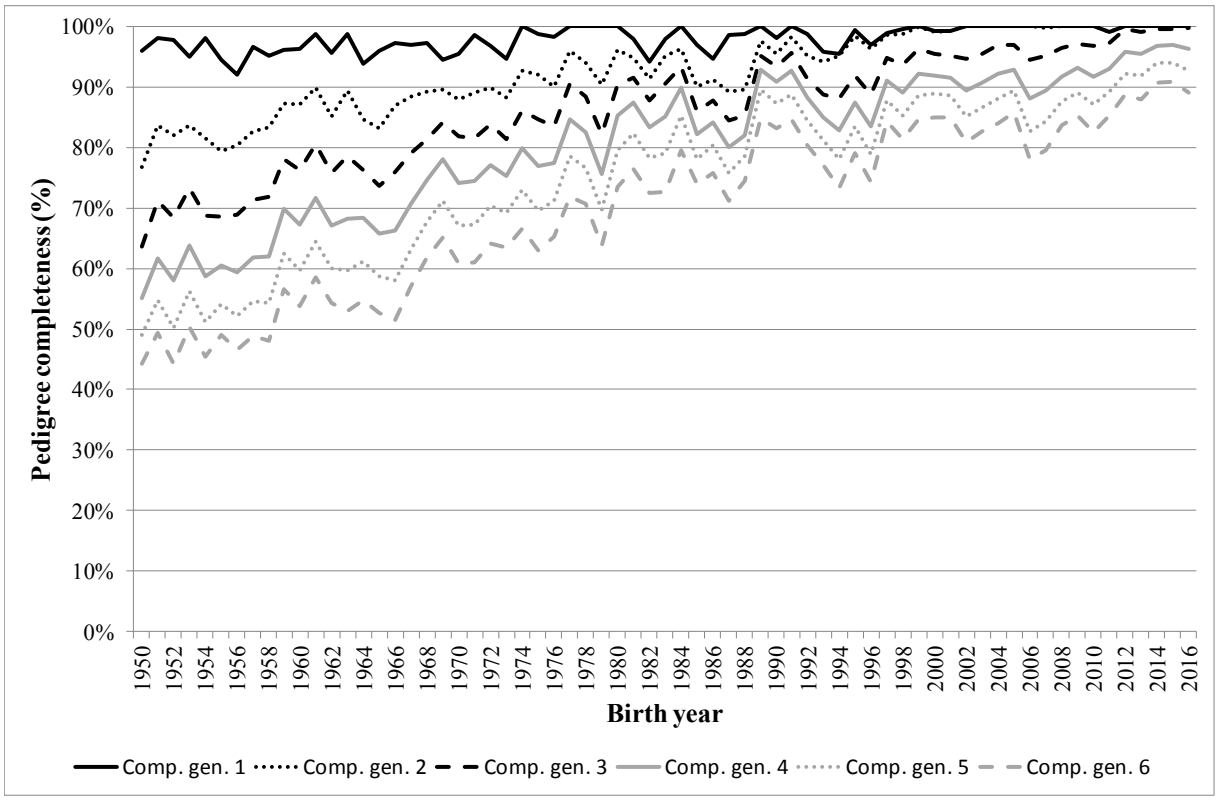

Figure 1. Evaluation of pedigree completeness

Ancestors having greatest impact on the total population are described in Table 2. Every horses responsibly for at least 1% of genetic variability were stallions. There were two English Thoroughbreds among the seven most important ancestors. Most important ancestor was North Star III (2.67%).

Table 2. Most important ancestors of the total population

Name of the horse	Gender	Birth year	Covering genetic variability (%)
North Star III	stallion	1872.	2.67
Furioso III-4 (VI.tm)	stallion	1969.	1.98
Furioso XXIII	stallion	1889.	1.82
Furioso XXVIII	stallion	1902.	1.74
Buccaneer	stallion	1857.	1.59
Galopin	stallion	1872.	1.56
Furioso XXI	stallion	1881.	1.07

Ancestors responsible for the genetic variability of the reference population are shown in Table 3. Furioso III-4 (VI.tm), born in 1969, covers 6.65% of the total genetic variability of the present stock. There was only one broodmare (North Star IV) among the ten most important ancestors.

Table 3. Most important ancestors of the reference population

Name of the horse	Gender	Birth year	Covering genetic variability (%)
Furioso III-4 (VI.tm)	stallion	1969.	6.65
Furioso XXVIII	stallion	1902.	4.30
Furioso XXIII	stallion	1889.	3.99
North Star VIII-13 (III.tm)	stallion	1985.	2.91
Furioso II-4 (X.tm)	stallion	1969.	2.84
Orosháza Hadfi-365	stallion	1978.	2.65
North Star XXII-4 (XXV.tm)	stallion	1929.	2.51
Masetta	stallion	1974.	1.92
North Star IV	mare	1886.	1.89
Furioso XII-35 (XX.tm.)	stallion	1987.	1.73

Table 4. Animals with highest inbreeding coefficient (IC) within the reference population

Name of the horse	Gender	Sire	Dam	IC
The Bart Furioso III-84 Boglár	mare	Furioso The Bart-17 Tévedés	Furioso-106 Bóbita	0.27
Catalin XII-31 Dóra	mare	Catalin VII-9 (XII.tm)	Catalin-79 Dáma	0.27
Catalin XII-22 Dia	mare	Catalin VII-9 (XII.tm)	Catalin-79 Dáma	0.27
Hadfi Furioso-37 Lenke	mare	Szentes Hadfi-5 Mandarin	Furioso-63 Levendula	0.27
Furioso Hadfi-16 Dórika	mare	Szentes Hadfi-5 Mandarin	Furioso-63 Levendula	0.27
Hadfi Furioso-59 Leonardo	stallion	Szentes Hadfi-5 Mandarin	Furioso-63 Levendula	0.27

Horses having highest inbreeding coefficient within the reference population are shown in Table 4. Every horse was born from a father-daughter mating. The most inbred horse is The Bart Furioso III-84 Boglár in the present breeding stock.
There are 35 horses in the whole population and 23 horses having more than 25% inbreeding coefficient in the reference population, respectively.

Table 5 gives information the genetic variability of the recent stock. Only 146 and 24 horses cover the 50% of the genetic variability for the whole and reference population, respectively. It can be seen easily, that the reference population could be coved with much less ancestors which show reasonable gene loss during the history of the breed.

Table 5. Concentration of genetic variability

Percentage of genetic variability	Whole population	Reference population
50%	146	24
60%	289	35
70%	567	52
80%	1074	81
90%	1815	143
100%	3328	534

Conclusions and recommendations

The generation interval varied between 10.05 and 12.40 for the analysed population. The shortest was estimated for Dam-to-daughter pathway, whereas the longest was sire-to-son pathway. There were 3328 and 534 horses covering the total genetic variability of the whole and reference populations, respectively. Most important ancestor was the English Thoroughbred North Star III (2.67%) for the whole population, whereas it was Furioso III-4 (VI.tm) (6.65%) for the reference population.

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Common origin of local cattle breeds in western region of Carpathians

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Abstract

The aim of this study was to analyse the genetic relationships and the state of diversity within and across three local breeds originating from western Carpathians by using microsatellite markers. In total of 214 biological samples representing the gene pool of Slovak Pinzgau, Slovak Spotted and Brown Carpathian cattle (UA) were genotyped using a set of 11 microsatellites that are primarily recommended by the ISAG for paternity testing. All of applied biostatistics approaches indicated that most of the genetic variation was conserved within individuals on the metapopulation level (92%), while the subdivision of cattle populations explained only 6% of variation. Similarly, the Wright F_{ST} index (F_{ST}=0.036±0.004) and Nei's genetic distances (D_A=0.211±0.031) pointed out to relatively high level of genetic similarity among breeds under consideration. The highest genetic identity revealed Slovak Spotted and Brown Carpathian cattle. On the other hand, the analysis of genetic variability conserved within each breed showed only negligible loss of genetic diversity.

Key words: Brown Carpathian cattle, genetic diversity, microsatellites, Pinzgau cattle, Slovak Spotted cattle

Introduction

The great changes in livestock management in recent years due to the increased specialization and mechanization in combination with a strong focus on high-yielding breeds and breeds that mainly offer provisioning ecosystem services have led to a substantial loss of local breeds' genetic diversity (MARSONER et al., 2018). The situation is critical especially in Europe, where up to 40% of the breeds can be classified as endangered by the significant loss of genetic diversity (FAO, 2011). However, such indigenous native breeds are valuable genetic resources as they are well adapted to local environments, represent cultural heritage and in some cases, the certified products obtained from local breeds provide an additional value that distinguishes them from non-native breeds (GINJA et al., 2013; DI TRANA et al., 2015; OVASKA and SOINI, 2016; UPADHYAY et al., 2019).

During the last decades a large number of genetic diversity studies in cattle based on microsatellite and SNPs (single nucleotide polymorphisms) loci was carried out to assess population structure and genetic variability in order to provide insight into origin, history and adaptation of local breeds (GLOWATZKI-MULLIS et al., 1995; DALVIT et al., 2008; GINJA et al., 2010; GAMARRA et al., 2017; KUKUČKOVÁ et al., 2017). When comparing STR (short tandem repeat or microsatellite) and SNP markers, STR markers proved enough statistical power in comparison to SNPs and need of twice as many SNP markers to provide the same effectiveness as STRs for genetic identification (FERNÁNDEZ et al., 2013).
The main objective of present study was to assess the genetic relationships among and within three local breeds bred in western part of Carpathians; namely Slovak Spotted, Slovak Pinzgau and Brown Carpathian cattle, based on microsatellites genotyping. Subsequently, the analysis of genetic variability was conducted to estimate the degree of their endangerment status in respect to the loss of intra-population diversity.

Material and methods

A total of 214 animals representing the gene pool of Slovak Pinzgau (N=79), Slovak Spotted (N=90) and Brown Carpathian (UA) (N=45) cattle were included in the study. All of animals under consideration were genotyped by using s set of eleven microsatellite markers (*BM1818*, *BM1824*, *BM2113*, *ETH10*, *ETH225*, *ETH3*, *INRA23*, *SPS115*, *TGLA122*, *TGLA126*, and *TGLA227*) recommended by the International Society for Animal genetics (ISAG) for paternity testing.
The genetic relationships among and within analysed breeds were evaluated based on three different approaches: principal component analysis (PCA), Nei's standard genetic distance, and Wright's F statistics. The PCA analysis was performed using R package adegenet (JOMBART and AHMED, 2011). The matrices of Nei's D_A genetic distances and Wright's F_{ST} index was computed using R package StAMPP (PEMBLETON et al., 2013). Subsequently, the amount of inbreeding-like effect within (F_{ST} or Θ) and among subpopulations (F_{IS} or f) was measured according to method described by WEIR and COCKERHAM (1984) using GenAlEx 6.5 software (PEAKALL and SMOUSE, 2012). An analysis of molecular variance (AMOVA) estimating the genetic structure indices using information on the allelic content of haplotypes, as well as their frequencies stored entered as a matrix of Euclidean squared distances was performed by 10,000 permutations with Arlequin v3.5 software (EXCOFFIER et al., 2005).
The level of intra-population genetic variation characterized by the allele frequencies, mean number of alleles (MNA), observed heterozygosity (H_o), gene diversity (H_e) often referred to as expected heterozygosity, and Shanon's information index (I) was calculated using GenAlEx 6.5 software (PEAKALL and SMOUSE, 2012). The same statistical environment was then used to estimate the probability of random mating in population by Chi-square goodness-of-fit test and by the likelihood ratio test that analyse departure from the Hardy-Weinberg equilibrium (HWE) at each marker.

Results and discussion

The analysis of molecular variance showed that the subdivision of cattle populations based on their origin explained only 6% of variation conserved in analysed dataset. Within individuals on the metapopulation level was partitioned 92% of variations and the rest (2%) was explained by the differences among individuals within populations (Table 1).

Table 1. Summary of ANOVA analysis

Source	DF	SS	MS	Est. Var.
Among populations	2	80.916	40.458	0.265
Among individuals	211	861.334	4.082	0.088
Within individuals	214	836.000	3.907	3.907
Total	427	1778.250		4.259

DF – degree of freedom, SS – sum of squares, MS – mean squares, Est. var. – estimate of variance

To describe the degree of genetic differentiation across analysed breeds was used first two principal components that correspond to 8.31 % (PC1) and 5.40 % (PC2) of variability conserved within all of animals in analysis. As can be seen in Figure 1 the level of genetic differentiation within and among breeds determined by the PCA analysis was only low. This was confirmed by the Nei's genetic distances (D_A=0.211±0.031) and F_{ST} index (F_{ST}=0.036±0.004) as well. Both of this parameters pointed out to high genetic similarity between Slovak Spotted and Brown Carpathian cattle (D_A=0.176; F_{ST}=0.031), while the higher genetic differentiation was found between Brown Carpathian and Slovak Pinzgau cattle (D_A=0.238; F_{ST}=0.038).

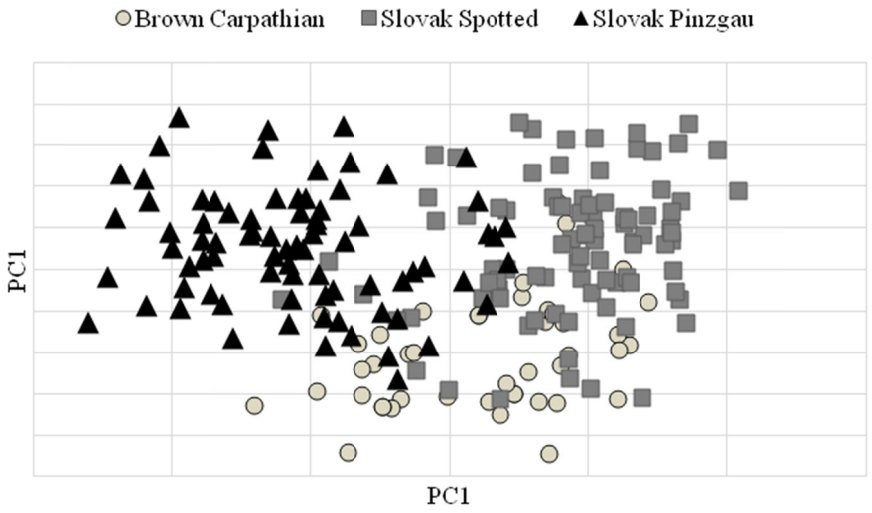

Figure 1. Genetic relationships among analysed breeds based on principal component analysis

Each of applied approaches in this study revealed high genetic similarity among analysed breeds that is in accordance to previous studies. For example GLAZKO et al. (1996a) found that the genetic structure of the highland group of Pinzgau cattle was similar to that of Brown Carpathian breed with respect to biochemical genetic systems, mainly transferrin and amylase-I loci. It is thought that the similarity found may be accounted for by close ecological and geographical breeding conditions of the groups of cattle studied. Vice-versa BERDICHEVSKIĬ et al. (1992) presented that the Brown Carpathian is genetically most similar to Swiss cattle. Moreover, this authors showed that the Brown Carpathian had unique traits of the genetic structures mostly in case of the erythrocyte antigens B-system. Comparative analysis of genetic structure of Brown Carpathian cattle and other local breeds bred in Carpathians regions performed by GLAZKO et al. (1996b) demonstrated that Brown

Carpathian breed formed a single cluster and were readily distinguishable from thoroughbred Swiss cattle. Originally, the Swiss breed participated in forming the gene pool of Brown Carpathian cattle. MASHUROV et al. (1993) suggested that the selective advantage of some gene associations including the loci of the biochemical genetic systems could be a specific characteristic of the Brown Carpathian breed compared to other genetically related breeds (MASHUROV et al., 1993).

With respect to the genetic diversity the analysis didn't showed significant loss of variability within evaluated breeds. The mean number of alleles (7.273±0.354) was comparable with previous studies in dairy as well as dual-purpose breeds (HANSEN et al., 2002; MACHADO et al., 2003; ŠIDLOVÁ et al., 2014). The effective number of alleles ranged from 3.583±0.273 (Slovak Spotted) to 3.917±0.302 (Slovak Pinzgau). Relatively higher level of polymorphic information content of loci in population of Slovak Pinzgau cattle compared to others revealed also the Shannon's information index (from 1.436±0.067 to 1.548±0.072). The significant decrease of heterozygosity due to higher intensity of relatives mating wasn't found. Expected heterozygosity and gene diversity clearly indicated the higher proportion of heterozygotes (H_o = 0.712, H_e = 0.718±0.012) and sufficient level of genetic diversity within each breed. The low impact of inbreeding confirmed the Wright's F_{IS} index (F_{IS} = 0.015±0.008) as well. The highest proportion of alleles unique to a single population showed Slovak Pinzgau cattle (Figure 2).

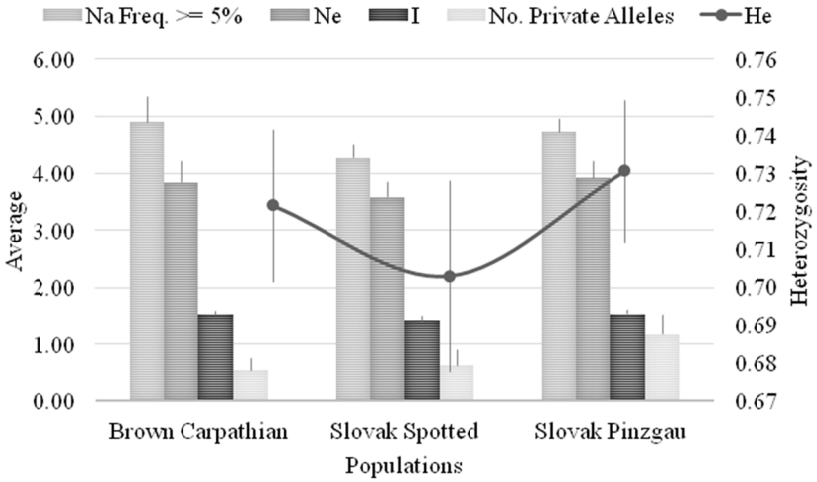

Figure 2. Allelic patterns within analysed breeds

(Na Freq. >= 5% – number of different alleles with a frequency higher than 5 %; Ne – effective number of alleles; I - Shannon's Information Index; No. Private Alleles – number of alleles unique to a single population; He – gene diversity)

Conclusion and recommendation

The results of this study confirmed high genetic similarity among local breeds originating from western part of Carpathians mainly as a consequence of close ecological and geographical breeding conditions as well as common breeding history due to the implementation of similarly breeds in order to improve their production performance. But the analysis also indicated that state of genetic variability within and across analysed local breeds is sufficient to maintain genetic diversity in the future. Obtained results also indicated that the microsatellites still provide valuable basis for the development of conservation strategies especially in situation where genealogical information are not available. However, the future

application of high-density SNP genotyping array would be beneficial to examine the proportion of genetic admixture and gene flow among analysed local breeds at more detailed level.

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Analyses of factors affecting the longevity of Hungarian Simmental beef cows

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Abstract

The Hungarian Simmental cow is the dual purposed breed, because it is good at milk and meat production. Beside the good milk and meat production and quality, the Simmental cow has some important traits, for example longer productive life. The longevity is the time period between first calving and culling. It was only 2.7 for Hungarian Simmental Cattle in 2012 (BEDŐ, 2014). The aim of this study was to analyse the longevity of Hungarian Simmental breed, to evaluate the effects of sex of first calf, size of herd, age at first calving, year, month and season of first calving. Based on Cox-regression, the season of the first calving and the size of herd had significant effects on longevity. The sex of the first calf was not significant for the analysed animals. For cows, having first calving in winter, were estimated lower risk ratio. The biggest risk ratio was estimated for cows calving in summer. The highest risk ratio was estimated for the medium size herd (20-50) and the lower risk ratio was for the small size of herd (<20).

Key words: Simmental cow, longevity, culling, Cox-regression

Introduction

In the world and also in Hungary, over the last decades the breed and type composition of the cattle population has changed significantly. Consumer's demands has changed, the demand on milk and meat has increased and this resulted in the appearance of new specialized breeds. The Hungarian Simmental cattle is a dual purposed breed, because it is good in milk and meat production. Beside the good milk and meat production and quality, the Simmental cattle has some important traits, for example good conformation, high and long term fertility, excellent mothering ability and longevity.

Longevity or productive life is the most important functional trait in the selection of cattle. The longevity is the time period between first calving and culling. It was only 2.7 in Hungarian Simmental Cattle in 2012 (BEDŐ, 2014). Longer productive life increases profits and decreases the costs of replacement. Decreasing of longevity was resulted increasing in culling. DUCROCQ et al. (1998) explained the importance of distinguish between disposal mostly beyond the control of dairy managers (such as the sale of a profitable but sterile cow, this is the involuntary culling) and the voluntary disposal (when the cow is healthy but not profitable). If the rate of involuntary culling is decreased, a higher voluntary culling can be applied, resulting in a larger profit (VAN ARENDONK, 1986).

There are numerous previous studies in this field, for example LASSEUR-LANDAIS (1992) reported, that number of calving affects longevity. In BOICHARD (2010) reported that primary is to solve the increase in longevity to reduce replacement and the number of non-productive cows.

The aim of this study was to analyse the longevity of Hungarian Simmental breed, to evaluate the effects of sex of first calf, size of herd, age at first calving, year, month and season of first calving.

Material and methods

The results were collected by the Hungarian Simmental Breeder's Society. The filtered database contained the results of 4,385 cows. Cows, which calved after 2016 were censored, all in all 2,037 cows. The evaluation model contained groups and covariates variable.

First of all, we would like to introduce the fixed effects:

- year of first calving
- season of first calving
- month of first calving
- weaning weight of calves
- sex of first calf
- size of herd
  - number of annual calving is under 20
  - number of annual calving is between 20-50
  - number of annual calving is over 50

Covariates:

- age at first calving
- weaning weight of first calf

The mathematical evaluation was carried out using Kaplan-Meier procedure (KAPLAN AND MEIER, 1958) and Cox Regression (COX, 1972) in SPSS 13.0. The Kaplan-Meier procedure is a method of estimating time-to-event models in the presence of censored cases. The Kaplan-Meier model is based on estimating conditional probabilities at each time point when an event occurs and taking the product limit of those probabilities to estimate the survival rate at each point in time. Cox Regression allows including predictor variables (covariates) in a model and it will provide estimated coefficients for each of the covariates, allowing assessing the impact of multiple covariates in the same model. Cox Regression can be used to examine the effect of continuous covariates.

The data were prepared for analysis with the help of the Microsoft Office Excel and Microsoft Office Access and statistical analysis using SPSS for Windows.

Results and discussion

Number of calving within the examined population is presented in Figure 1. It can be seen that with the increasing of the number of calving, the number of cattle decreases. More than 1,000 cattle (23.3 %) had only one calving. In this population, average of longevity was 3.37 years. In this topic there are some researches that were published in literature. NAGY and TŐZSÉR (1988) reported that the longevity of Simmental x Hereford (F_1) was 5.6 years. DÁKAY et al. (2006) evaluated five breeds (Hungarian Grey, Hereford, Aberdeen Angus, Limousin, Charolais) and two crossbred genotypes (Simmental x Hereford F_1, Simmental x Limousin F_1). The longest life span was reached by Hereford crossbred and Hungarian Grey and the shortest by Limousin crossbred cows.

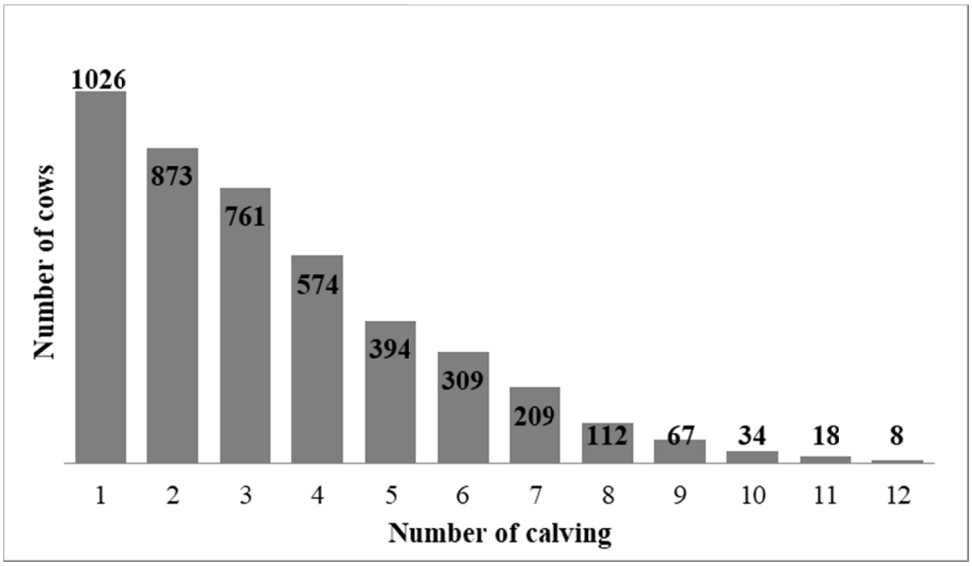

Figure 1. Distribution of number of calving (N=4,385)

Application of the Kaplan-Meier Method for longevity analysis

Significant effects of some factors (size of herd, sex of first calf, season and month of first calving) are shown in Table 1. It can be seen, that the size of the herd, the season of the first calving and the month of the first calving were significant effects on longevity and culling (Table 1).

Table 1. Significant effects of some factors influencing longevity

Factors	Chi^2		
	Log Rank (Mantel Cox)	Breslow (Generalized Wilcoxon)	Tarone-Ware
Size of the herd	85.460 *	60.116 *	71.964 *
Sex of first calf	0.440	2.531	1.860
Season of first calving	58.797 *	39.245 *	45.563 *
Month of first calving	80.613 *	66.597 *	73.415 *

*: $P<0.05$

Application of the Cox Proportional Hazards Model for longevity analysis

The model included the weaning weight of the first calf and the age of the cow at first calving as covariates. Both of these effects were significant. The later age at first calving (1.25) and the bigger calf weight (0.99) resulted bigger risks ratio for the mother, than the normal age at the first calving and normal weaning weight of calf.

Figure 2. shows the risk ratios of the effect of the size of the herd on longevity. The highest risk ratio was estimated for the medium size (20-50) and the lowest risk ratio as well as lowest number of cows was received for the small sized herds (<20). The risk ratio of cows in category 2 and category 3 was 80% and 40% higher compared to category 1, respectively. Category 3 (>50) contained the highest number of cows.

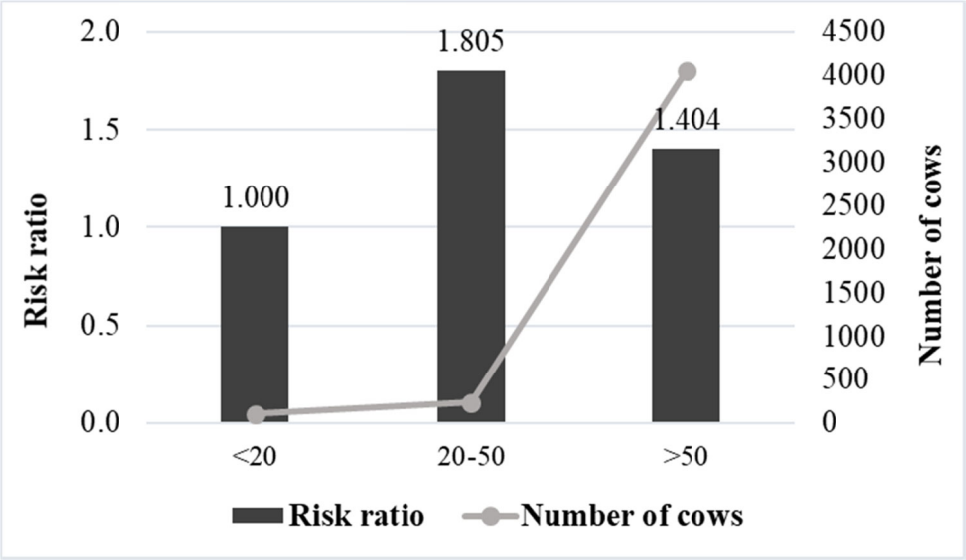

Figure 2. Effect of herd size on the relative culling risk

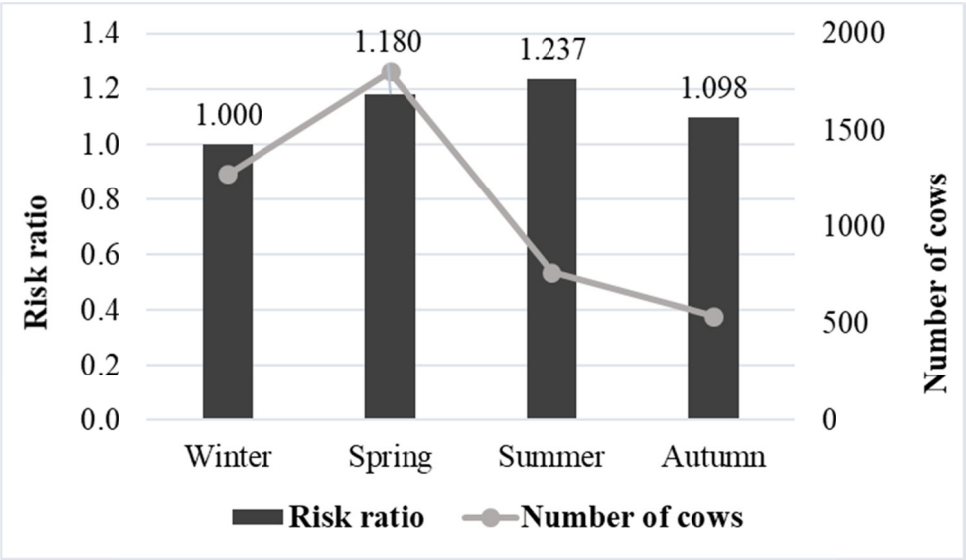

Figure 3. Effect of season on the relative culling risk

The relationship between the calving season and culling risk is shown in Figure 3. It can be seen that winter was chosen as the reference group and cows having first calving in winter had lower risk ratio. The biggest risk ratio was estimated for cows first calved in summer. Most calving was received in spring during the analysed time period.

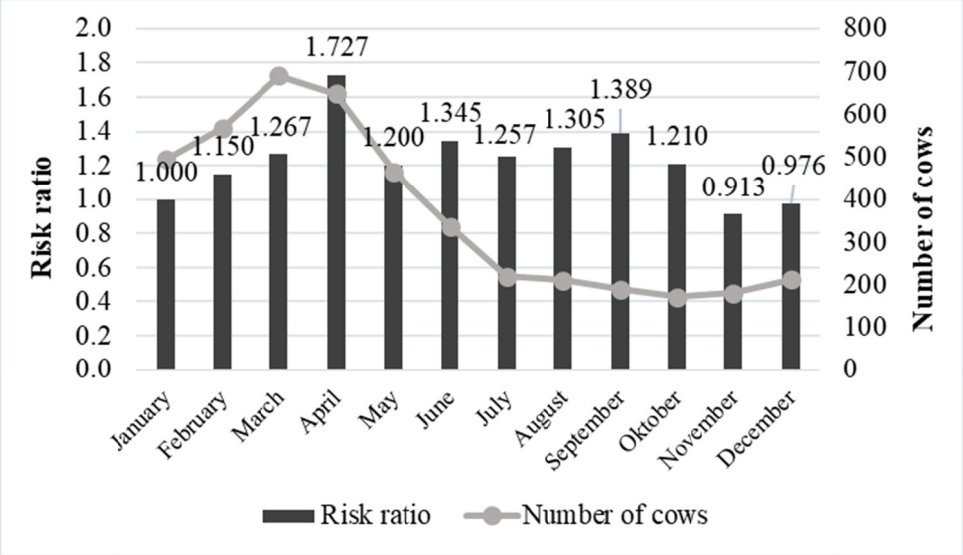

Figure 4. Effect of month on the relative culling risk

Distribution of first calving is in agreement with DÁKAY et al. (2006) results estimated for five beef cattle populations as they found most calving was in March and April (Figure 4). During the evaluation of effect of month of first calving, January was chosen as a reference group. Cows having first calving in April had 72 % higher risk of culling than those who calved first in January. In contradiction with the risk ratio, most of the cows had the first calving in April. The lowest risk ratio was in November.

Conclusion and recommendation

Based on Cox-regression results of this study, the season of the first calving and the size of herd were significant effects for longevity. The sex of the first calf was not significant in the database.

Cows, which first calved in winter, had lower risk ratio. The highest risk ratio was estimated for cows calving in summer. The highest risk ratio was estimated for the medium size herd (20-50) and the lower risk ratio was for the small size of herd (<20). The risk ratio of cows with results in category 2 was 80 % and category 2 was 40 % bigger than cows in category 1. The category 3 (>50) contains the highest number of cows.

Acknowledgements

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Cryopreservation of in vitro matured bovine oocytes

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Abstract

Cryopreservation of matured oocytes generally shows variable results worldwide because of problems encountered during fertilization and embryo development. The aim of present study was to examine the approach of freezing of the bovine cumulus-oocyte complexes (COC) without surrounding ovarian tissue. Prior to freezing the oocytes were matured *in vitro* and afterwards frozen by an ultra-rapid cooling technique in minimum volume using 300 mesh electron microscopy nickel grids as a carrier. After vitrification/warming of the oocytes their developmental competence was verified by in vitro fertilization (IVF) procedure and subsequent embryo culture until blastocyst stage. Using this design we obtained more than 56% cleavage rate and about 9.4% reached the blastocyst stage, which proves that the cumulus-oocyte complexes after vitrification/warming can retain their developmental ability. In conclusion, our preliminary experiments show that cryopreservation of matured cumulus-oocyte complexes is, in our conditions, more promising than the vitrification of ovarian tissue fragments. However it requires further optimization of an oocyte cryopreservation regimen.

Key words: cattle, ovary, oocytes, cryopreservation, in vitro fertilization

Introduction

Important role in solving the issue of the preservation of animal genetic resources belongs to a cryopreservation and subsequent long-term storage of genetic material from valuable breeds of livestock or genetically valuable individuals. Cryopreservation of the entire ovary, their surface tissues or ovarian follicles represents a possible source of female gametes in future. This biological material can be collected and frozen for uncertain period, and after thawing, it may be transplanted to the health ovary as a graft, or cultured *in vitro* as a source of oocytes for *in vitro* fertilization (IVF) and *in vitro* embryo production (IVP).
Cryopreservation of ovarian tissue offers many advantages over mature oocytes or embryos to preserve female germline of endangered animals. First, the ovary contains a large pool of oocytes enclosed in primordial follicles. Ovarian tissue can be collected from animals of almost all developmental stage (adult, pre-pubertal and foetus) and status (alive or dead) (CLEARY et al., 2001). Finally, primordial follicles are more resistant to cryodamage, because their oocytes have a relatively inactive metabolism, lack of metaphase spindle, *zona pellucida* and cortical granules and low amount of lipids (HOVATTA, 2005). According to FAHEEM et al. (2011), isolated ovarian fragments may have good post-thaw survivability, providing a source of oocytes for IVM. Nevertheless, cryopreservation of ovarian tissue is still problematic and, therefore, should be optimized. Cryopreservation of matured (metaphase

II) oocytes gives poor results because of problems encountered during fertilization and embryo development.
The aim of our study was to verify an alternative approach of freezing of the cumulus-oocyte complexes (COC) without surrounding ovarian tissue. This approach included: (1) the isolation of COC from the ovaries by ovarian follicle aspiration; (2) in vitro maturation (IVM) of COC by their incubation in IVM medium and (3) vitrification/warming of matured oocytes and checking of their developmental competence by in vitro fertilization (IVF) and subsequent embryo culture.

Material and Methods

The ovaries were isolated from undefined cows at a local abattoir and transported to the laboratory. The oocytes were recovered from antral follicles (2-8 mm) by the aspiration of follicular fluid using sterile syringe with a needle (Figure 1.). Cumulus-oocyte complexes (COC) were collected into a Petri dish with a holding medium (M199-HEPES) and only COC with several layers of cumulus cells and homogeneous ooplasm were selected for in vitro maturation (Figure 2.). COC were matured during 24 h incubation in a maturation medium containing TCM 199 (Gibco), sodium pyruvate (0.25 $mmol.l^{-1}$), gentamycin (50 $\mu g.ml^{-1}$), foetal bovine serum (FBS) 10% and FSH/LH (1/1 I.U., Pluset) at 38.5 °C and 5 % CO_2.

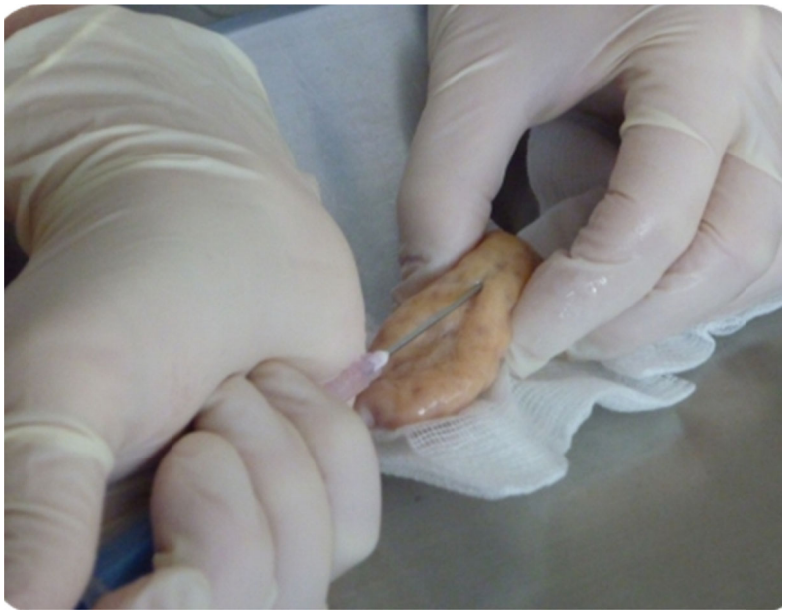

Figure 1. Isolation of oocytes from the ovary by follicle aspiration

Cryopreservation of oocytes
For cryopreservation of in vitro matured oocytes, ultra-rapid cooling technique in minimum volume was used. Selected matured oocytes with cumulus cell layers were placed into equilibration solution (ES: 3% ethylene glycol in M199-HEPES, supplemented 10% foetal calf serum and 50 µg/ml gentamycin) for 12 min. Following equilibration the oocytes were transferred to vitrification solution (30% EG + 1M sucrose in M199-HEPES with 10% foetal calf serum) at room temperature for 25 sec. The oocytes (10-15) in a small drop were placed with a glass micropipette onto 300 mesh nickel grids (electron microscopy grade, Figure 3.), an excessive medium was removed by a filtration paper and then the oocytes were

immediately plunged into liquid nitrogen for storage (several weeks). For warming, nickel grids were directly transferred into thawing solution (0.5M sucrose in M199-Hepes, at 37˚C) for 1 min. The warmed oocytes were transferred to the diluent solutions (0.25M, 0.125M and 0.0625M sucrose in M199-HEPES) for 3 min, and then washed twice in M199-HEPES with FCS for 5 min. Oocyte survival was evaluated on the basis of the integrity of the oocyte membrane and the *zona pellucida* after 20 h culture post-thawing.

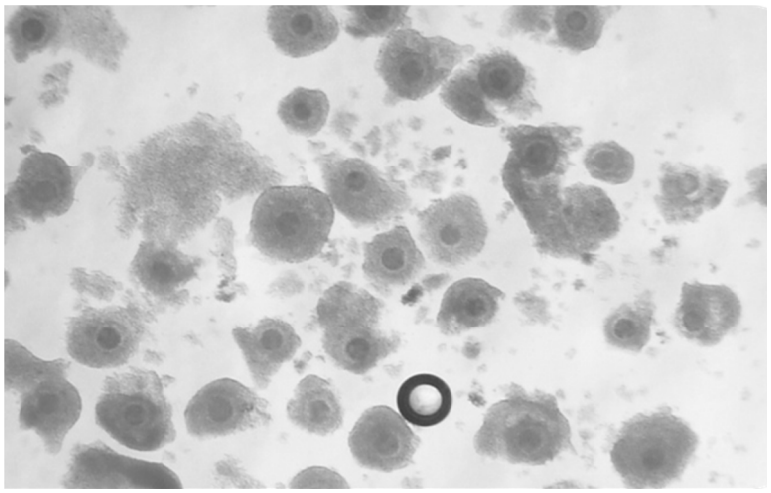

Figure 2. Cumulus-oocyte complexes (COC) selected for in vitro maturation

Figure 3. Electron microscopy nickel grid (300 mesh) with aluminium foil handle for vitrification of oocytes

In vitro fertilization (IVF) of vitrified-warmed oocytes and embryo culture

Warmed oocytes were washed in IVF-TALP medium (TALP solution, 10 µg/mL heparin, 50 µg/ml gentamycin) and put into 100-µl droplets of IVF medium under a mineral oil, where the sperm (at 2×10^6 per ml) and PHE solution (20µM penicylamine, 10 µM hypotaurine, 1 µM epinephrine) was previously added, and incubated for 18 h at 39°C in 5% CO_2. Following insemination, presumptive zygotes were vortexed in centrifuge tubes containing 0.5 ml

holding medium for 45 s to remove residual cumulus cells. Denuded zygotes were transferred to the dish with the granulosa cell (about 10%) layer for 24 h. Afterwards, the embryos were transferred to a new dish with the granulosa cells (about 30- 40% of confluence) in B2 medium with 10 % FBS. On the Day 2 since insemination, the cleavage, and on Day 7, 8 and 9 - the number of blastocysts were counted.
Total cell number of resulted blastocyst was counted under a Leica fluorescent microscope after staining of randomly chosen day 7 (D7) blastocysts with a DAPI fluorescent dye by 15 min incubation in a Vectashield medium with DAPI (Vector Laboratories, Burlingame, USA).

Results and Discussion

In our initial study we investigated the status of oocytes frozen in the fragments with antral follicles. Our previous experiments did not confirm that the oocytes, frozen in small antral follicles from ovarian tissue fragments using either solid-surface vitrification or liquid vitrification are able to mature *in vitro.* The reason for this failure was an extensive cellular damages revealed on a histological and ultrastructural levels (MAKAREVICH et al., 2018).
In the present study we aimed to to test the approach of freezing of the cumulus-oocyte complexes (COC) without surrounding ovarian tissue. Prior to freezing the oocytes were matured by the incubation in maturation medium and afterwards immediately frozen by an ultra-rapid cooling technique in minimum volume, previously described by MARTINO et al. (1996) and ISHII et al. (2018). The results of oocyte vitrification and *in vitro* fertilization are presented in Table 1.
From a totally 300 vitrified oocytes, 273 were warmed and afterwards only 203 oocytes were selected for IVF procedure. As a control group, 410 freshly isolated oocytes were after maturation *in vitro* subjected to the IVF procedure. Cleavage rate of vitrified oocytes was significantly lower (56.7%; $p < 0.05$) than that of a fresh control oocytes (74.9%). In the vitrified group only 19 blastocysts were developed (9.4%) in comparison with 22% in the fresh control group oocytes ($p < 0.05$).

Table 1. Development of fresh or vitrified-warmed oocytes after IVF

Groups	Oocytes totally	Oocytes vitrified	Oocytes warmed	Oocytes in IVF	Embryo cleavage	Blastocyst rate
Vitrified	330	300	273	203	115 (56.65%)[a]	19 (9.36%)[a]
Control	462	-	-	410	307 (74.88%)[b]	90 (21.95%)[b]

[a] versus [b] – difference is significant at $p < 0.05$ (Chi-square test)

Total cell number of blastocysts at Day 7 in vitrified/warmed group was slightly but not significantly lower when compared to the fresh control blastocysts (Table 2). This little difference was probably due to a less rate of blastomere division after vitrification/warming compared to intact control blastocysts. Insignificant difference however, suggests that frozen oocytes can give a rise to blastocysts of comparable quality to fresh oocytes.

Table 2. Total cell number of blastocysts

Groups	No. blastocysts	Cell number (x ± s.e.m)
Vitrified/warmed	6	84 ± 15.4
Control (fresh)	11	91 ± 6.8

Difference between groups is not significant (t-test)

In the group of vitrified/warmed oocytes, on the second day after fertilization, we observed occurrence of 2-cell embryos with unevenly sized blastomeres, as well as 3-cell embryos, what indicated asynchronous cell division in this group. Similarly, in this group the incidence of unevenly sized blastomeres in the Day 9 blastocysts was observed (Figure 4). Oppositely, in the control group mostly regularly divided 2- and 4- cell embryos were observed on the 2nd day after fertilization.

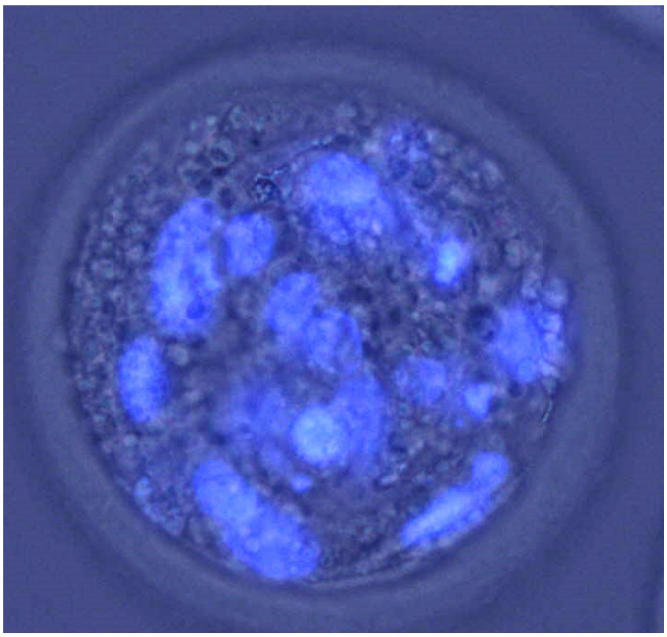

Figure 4. Day 9 blastocyst with unevenly sized blastomeres.

Using this design we obtained more than 56% cleavage rate and about 9.4% reached the blastocyst stage, which proves that the cumulus-oocyte complexes after vitrification can retain their developmental ability. Similar results were published earlier also by CHIAN et al. (2004, 7.4 %) and ZHOU et al. (2010, 5.4 %). However, VAJTA et al. (1998), PAPIS et al. (2001) and ISHI et al. (2018) obtained more than 10 % of blastocyst after bovine oocytes cryopreservation.

Conclusion and recommendation

Our experimental data suggest that obtaining the blastocyst rate exceeding 10% is really possible, but it perhaps requires further optimization of our oocyte cryopreservation regimen. In conclusion, our preliminary experiments show that cryopreservation of matured cumulus-oocyte complexes is more promising than the vitrification of ovarian tissue fragments.

Acknowledgements

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Analyses of meat producing performance in Tsigai rams by real-time ultrasound

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Abstract

Sheep (Ovis aries) is one of the first domesticated animals and sheep husbandry is a valuable trade for its meat, wool, milk and hide. Out of these product, the sheep meat, or mutton, is the most requested and it accounts for 2.82% of the world's meat production. For export as well as sale in the domestic markets of lamb, the appropriate meat conformation with the best grade is a desirable breeding characteristic. Since the breeding stock cannot be slaughtered, we have to estimate the traits of sheep meat indirectly by observing the animals using ultrasonography to obtain objective measurements. Ultrasonic devices are efficiently used to assess meat grades in the swine sector, to a lesser extent in cattle, but relatively few uses in the domestic sheep sector. Our study adopted this ultrasonography examination using the registered Tsigai rams to assess the meat/body grades. This is done by measuring the eye muscle depth (EMD) and the back fat thickness (BFT) of the sheep. It is known that rib eye has high heritability and those animals having good and desirable meat traits are well inherited. In Hungary, the annual meat consumption is 2 kg/person/year out of which only 0.3kg/person/year come from mutton, which is very low. From the point of view of Hungary's sheep breeding, the real-time ultrasound technology offers the possibility of a potential method for selection of breeding stocks for sheep meat.

Key words: Tsigai, ultrasound technology, eye muscle depth, back fat thickness

Introduction

The Tsigai sheep probably originated from Asia Minor, then migrated to the eastern territories of the Balkan-Peninsula and later to Bulgaria, Yugoslavia, Hungary and Czechoslovakia. It is one of the national breeds of Romanians. From there, it spread to Moldavia and Bessarabia (VERESS et al., 1982). The black headed and footed, white haired variety of Hungarian Tsigai sheep was introduced in the Great Plain of Hungary in the 18th century, a few years ahead of Merino (JÁVOR et al., 2006). Due to its good adaptability, it has spread quickly, mainly in the southern parts of the country. In the past 200 years, it occupies a considerable proportion of Hungarian shepherds (1-10%) but has never been able to play a leading role (JÁVOR et al., 2006).
Tsigai sheep has a very good milking and meat production ability and possesses a good mothering behaviour. In Hungary, today there are two types of Tsigai, one of which is native and the other selected for milk production, namely, the Dairy Tsigai (SÁFÁR and GÁSPÁRDY, 2014). Also, the Tsigai meat is reported more delicious than that of the Merino

and is considered one of the best and finest (VERESS et al., 1982). The breed has improved in several respects as a result of selections and cross breeding. Over time, the reproductive capacity, meat quality, muscularity and body weight have improved significantly. In Hungary, the current purpose of Tsigai breeding is to preserve the genetic capability of the breed and to maintain its genetic diversity (BÖŐ, 2003).
In the sheep sector, the biggest income for the sheep farmer is the sale of lambs and meat (85%) and wool (15%) (STEWART, 2011).
The use of real-time ultrasound technology (UST) can aid in the objective estimation of carcass traits in live animals. Ultrasound technology has been used quite effectively in the swine industry and to a lesser extent in the beef cattle industry for measurement of carcass traits in live animals. Real-time ultrasound technology offers the possibility of estimating carcass characteristics in live animals and represents a potential method for selection of breeding stocks (BEDHIAF et al., 2006). Still, there is relatively very little adoption of ultrasound technology in the sheep industry (HIEMKE et al., 2004).

Material and methods

Our investigations were carried out at the University of Debrecen Institute For Agricultural Research and Educational Farm. On 4th December 2017, Tsigai rams (n=12) were randomly selected for the study of using ultrasonography in measuring carcass traits. The main goal was to select individuals with the deepest eye muscle which could be the basis for later selection. Briefly, the selected rams were examined with the Pie Medical Falco Vet100 ultrasound machine with a 6-8 mHz linear probe set over the loin between the 12th and 13th ribs by spreading the wool sideways such that the lubricated probe was in touch with the skin directly. This measurement was done only in standing position. The ear tag number, EMD, BFT and body weight were recorded. The herd-book classification of the ewes, the weight at weaning and end of own performance test on farm (ÜSTV), birth type (singles, twins and triplets) were also considered during the evaluation. In addition to rams, the production results of the Tsigai ewes (n=47) in 2017 where weaning weight and yearling body weight were recorded. The results are processed in Microsoft Excel and SPSS 13.1 v. program.

Results and discussion

The Table 1. shows the production results of the Tsigai ewes. As a first step, we compared the lamb weight gain of rams and ewes. As per literatures available, rams are comparatively heavier than the ewes (Table 1. and 2.). The average annual body weight was 54.7 kg.

Table 1. Production results of Tsigai ewes

Ewes (n)	Weaning weight (g/day)	Yearling body weight (kg)
47	357	54.7

Table 2. Tsigai stock data

Ear tag number	Weaning weight (g/day)	ÜSTV weight gain (g/day)	Birth type	Herd-book classification
7584	430	244	Single	A
7555	382	103	Single	K
7547	368	308	Single	K
7514	370	282	Twins	K
7491	270	295	Twins	A
7558	368	256	Single	K
7475	333	205	Twins	K
7509	397	218	Single	A
7508	438	333	Single	A
7534	300	231	Twins	K
7553	324	282	Twins	K
7515	397	282	Twins	K

The average weaning weight was 364.7 g/day (Table 3.). This can be due to the good maternal qualities of the ewes, as the lambs were fed solely with milk until weaning. The standard deviation of the weaning weight was found to be 50.3. The ÜSTV data provide information about the individual's performance. We found that during the fattening period, the weight gain of the Tsigai was 253.2 g/day. The standard deviation in this case is 60.5.

Herd-book classification and birth type were needed to determine whether these factors affect the weight gain and the size of the EMD and BFT. Half of the lambing was as single, the other as twins and no triplets were recorded. The weaning weight of single-born individuals was significantly higher than the twins ($p<0.05$). In terms of herd-book classification, 4 individuals of the rams population were registered in the 'A' herd-book, 8 individuals were registered in the 'K' herd-book. The herd-book classification did not significantly affect the weight gains, the EMD and the BFT ($p>0.05$).

The main purpose of our research was to investigate the development of the EMD and BFT and its influencing factors. In addition to BFT, EMD and weight data (at the time of ultrasonography), the average weight gain data are also discussed for the sake of transparency (Table 3).
The Table 3. shows that the BFT is 0.27 cm and the EMD is 2.3 cm.

Table 3. The EMD, BFT and the weight data of Tsigai rams

Data	Mean	Standard deviation
Gain (g/day)	364.7	50.3
ÜSTV (kg)	253.2	605
Weight (at the time of ultrasonography, kg)	35.21	4.51
BFT (cm)	0.27	0.06
EMD (cm)	2.3	0.2

Figure 1. shows the ratio of BFT to EMD. The smallest measured value was 1.88 cm, the highest was 2.71 cm. The standard variation in BFT was also very small, but it can be clearly deduced that the smaller rib eye was associated with a smaller proportion of fat thickness.

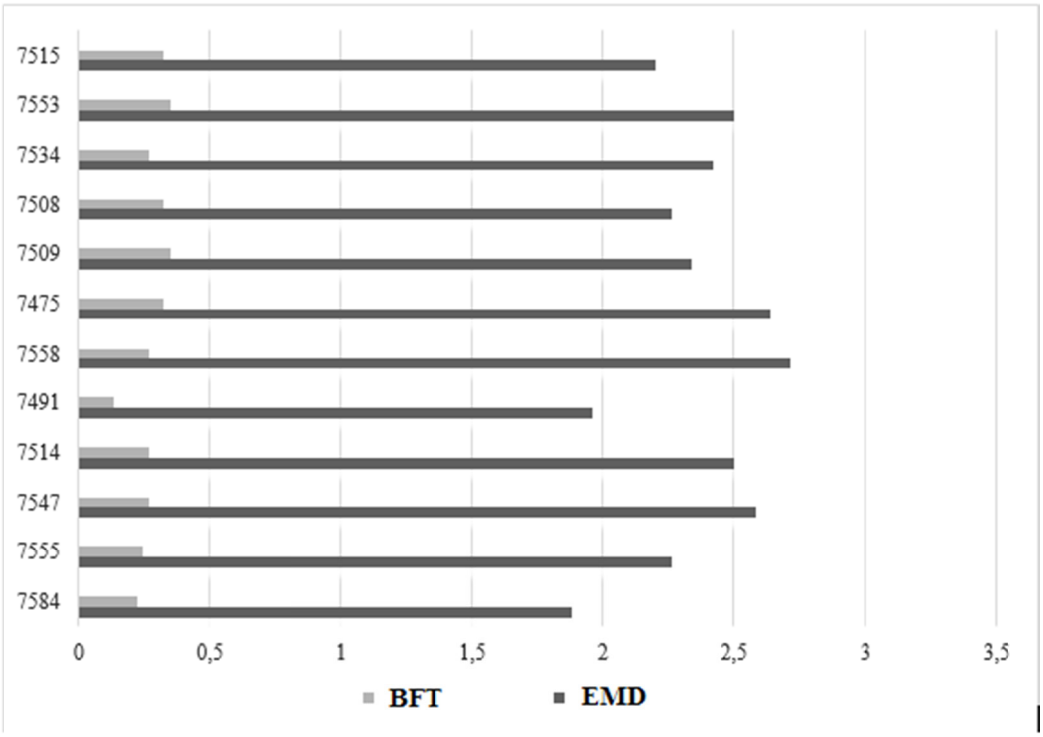

Figure 1. Ratio of the EMD and BFT of the Tsigai stock (cm)

It should be noted that measuring the weight of the examined animal at the same time as the ultrasonic measurements was important so as to ascertain if there was any correlation of weight gain with EMD and BFT. By analysing this data, we concluded that the increase in body weight is related to the thickness of the fat and the size of the rib eye.

The average EMD of the Tsigai was 2.3 cm (Figure 2). The stock was relatively homogeneous, with no significant differences in individuals. The largest EMD were 2.71 and 2.64 cm.
From Figure 2. and 3., it can be inferred that there is less dispersion in the EMD data than that of the BFT. The smallest fat thickness measured was 0.13 cm and that of the highest was 0.35 cm. In this case, the body weight was only 0.5 kg higher than the average body weight.

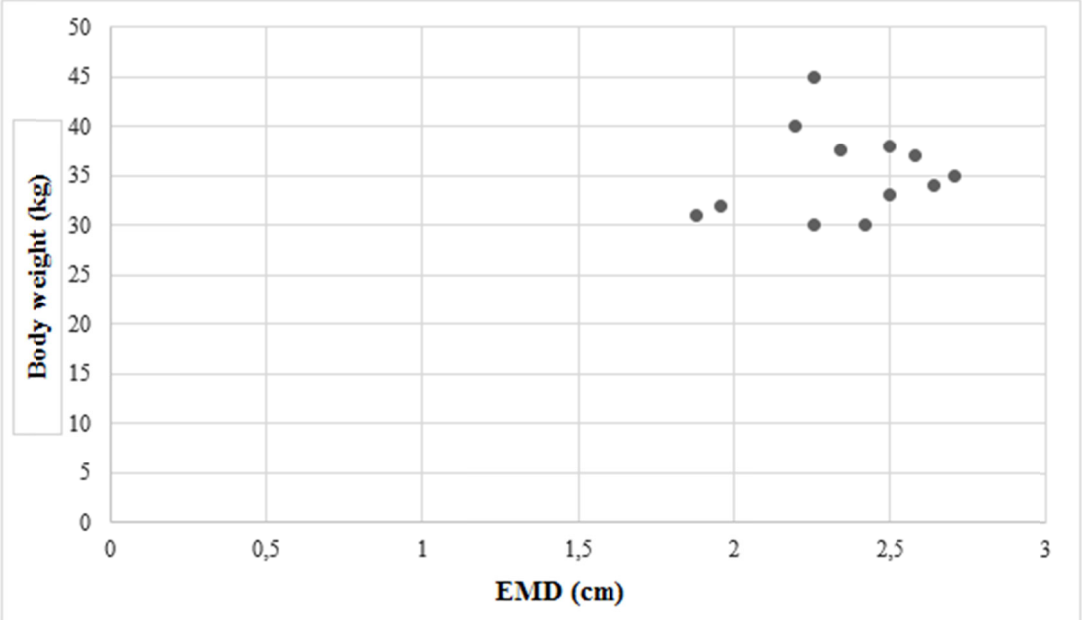

Figure 2. The EMD parameters of the Tsigai stock

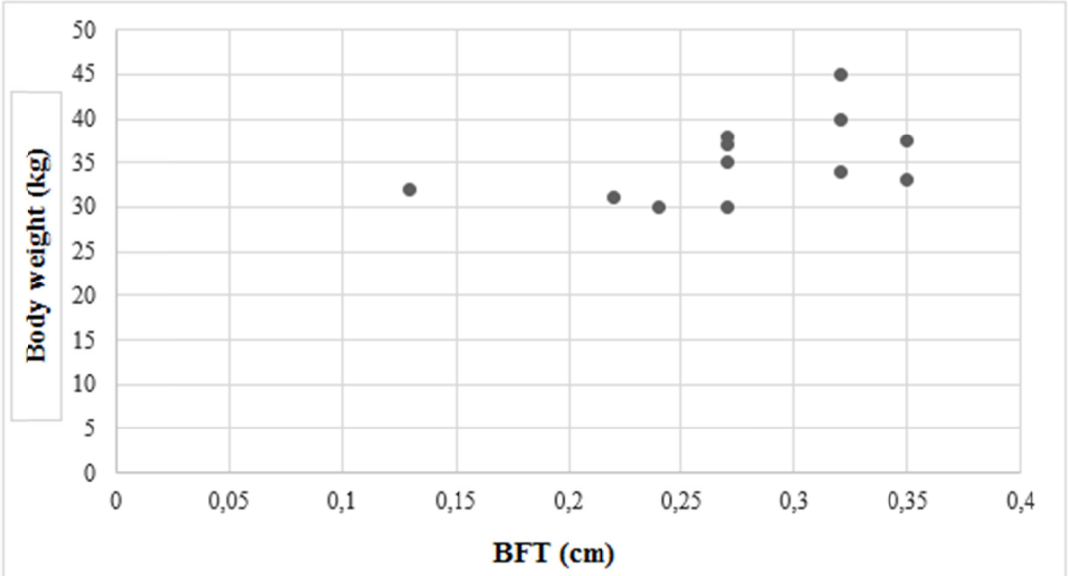

Figure 3. The BFT of the Tsigai flock

Conclusion and recommendation

In the study, 12 Tsigai were examined for their EMD and BFT with ultrasound system. We examined the weaning weight, the ÜSTV, the herd-book classification and the birth type. Our goal was to select the best individuals that possessed the largest EMD and the smallest BFT. We chose those individuals that are best suited for further breeding, based on the results as shown in Table 4.

Table 4. Selected Tsigai rams

Ear tag number	EMD (cm)	BFT (cm)
7475	2,64	0,32
7547	2,58	0,27
7514	2,5	0,27
7534	2,42	0,27
7508	2,26	0,24

Acknowledgements

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Sensory characteristics of lamb meat of three Zackel sheep types in Serbia

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Abstract

Meat quality characteristics (*m. longissimus dorsi*) were evaluated in lambs of three types of autochthonous Zackel sheep: Sjenica sheep, Lipe sheep and Vlashko Vitoroga sheep, reared in traditional habitats in a sustainable management system. For the evaluation of sensory characteristics of lamb meat, quantitative descriptive analysis was performed, based on structural intensity scale of seven points (ISO 6564:1985). All Zackel meat samples had an overall acceptability, the most favorable being in Sjenica sheep, with detected differences between Sjenica and Vlashko Vitoroga sheep ($p<0.05$). Sustainable consumers appreciate sensory characteristics of lamb meat from agroecological farming system and prefer lamb meat as local food.

Keywords: local food, autochtonous Zackel sheep, ecosystem service, preserving biodiversity

Introduction

The established criteria for strategic environmental, economic and social sustainability enhanced the importance of traditional nature conservation (ALLIEVI et al., 2011, EUROSTAT, 2011.). Nowadays, ecosystem services have an active role in good practice management of natural resources. Five principles for integrating livestock systems within the agroecology include: adopting management practices that aim to improve animal health, decreasing the inputs needed for production, reducing emissions, enhancing diversity within animal production systems to strengthen their resilience and preserving biodiversity by adapting management practices (DUMONT et al., 2013).

Many studies indicate that it is a human society faces with hunter-gatherers, agrarian societies and industrial societies prepared for transition towards sustainable society, fundamental re-orientation of society and the economy. Promotion of animal genetic recourses conservation get a new chance transforming materialist consumerism based on following attitudes like health-promoting lifestyle, recycling, organic food, water conservation, maintaining of civil society, favouring the eco-labelled products and using renewable energy resources (ARTO et al., 2011). Considering the importance of biodiversity for agriculture, global food and health security, the Republic Project was focused on supporting the biodiversity and the importance of autochthonous Zackel sheep and value-added animal products on the sustainable development of region (SAVIC et al., 2018). The active sustainable society approach promote the conservation and rational use of autochthonous and endangered Sjenica sheep, Lipe sheep

and Vlashko Vitoroga sheep, types of Zackel sheep reared based on grazing of native pastures in traditional habitat. The aim of this paper is to examine lamb meat sensory characteristics of three Zackel sheep types in Serbia as local food. Local food is one of indicators of environmental sustainability.

Material and Methods

The study was carried out on 24 lamb carcasses of both sexes, eight lambs per each of three types of autochthonous Zackel sheep. The lambs were slaughtered at 90-100 days of age, mean weight of 26.74 ± 1.85 kg, 25.24 ± 2.12 kg and 23.42 ± 2.35 kg for Sjenica sheep, Lipe sheep and Vlashko Vitoroga sheep, respectively. All Zackel type lambs were produced in an extensive, sustainable management system. Sjenica sheep is reared on the Sjenica-Pester plateau (900-1200 m altitude) in South Western Serbia, the Lipa sheep in grassland fields in lower Morava valley in Northern Serbia and the Vlashko Vitoroga type in the lowland pastures in South Eastern Banat region. Feeding systems were based on grazing of native pastures.
The selection and training of the evaluators were conducted in accordance with ISO 8586-2:2012 (Sensor Features - General guidance for the selection, training and monitoring of assessors; Part 2: Sensory assessors (experts). Quantitative descriptive analysis (evaluation of the acceptability-odour) was performed according to ISO 6564:1985, the structural intensity scale / eligibility of seven points, with the score of 7 being the maximum intensity / eligibility, and score of less than 3.5 marked the product as unacceptable. The odour intensity, flavour intensity, flavour quality and overall acceptability were scored. Colour and odour of the meat samples were analysed before thermal treatment. Before cooking the fat was removed from the *m. longissimus dorsi*. The samples of *m. longissimus dorsi* were grilled (70 ºC internal temperature) and cut into thin slices. Sensory analysis was done by an 8 member trained taste panel professional commission.
Statistical analysis of the data was done by GraphPad Prism statistics 7.05 for Windows, GraphPad Software, San Diego California, USA. The tested parameters were presented by descriptive statistical method (mean value, standard deviation). One-way ANOVA with Tukey's multiple comparison test was performed to test the average value differences of sensory characteristics in lamb meat between three types of Zackel sheep.

Results and discussion

Meat quality characteristics (*m. longissimus dorsi*) were evaluated in lambs of three types of autochthonous Zackel sheep: Sjenica sheep, Lipe sheep and Vlashko Vitoroga sheep, reared in traditional habitats. The sensory characteristics of lamb meat of Sjenica sheep, Lipe sheep and Vlashko Vitoroga sheep are presented in Table 1.

Table 1. Sensory characteristics of lamb meat of three Zackel sheep types

Sensory characteristic	Sjenica sheep M±SD CV%	Lipe sheep M±SD CV%	Vlashko Vitoroga sheep M±SD CV%
Colour	6.19±0.35 5.82%	5.50±0.26 4.86%	5.19±0.25 4.99%
Odour	5.93±0.32 5.40%	5.25±0.27 5.09%	6.18±0.37 6.01%
Juiciness	6.06±0.32 6.05%	5.81±0.37 5.80%	5.56±0.32 5.55%
Softness	6.18±0.26 4.18%	6.12±0.23 3.78%	5.31±0.25 4.78%
Odour and flavour (aroma)	6.12±0.23 3.87%	5.87±0.35 6.02%	5.81±0.37 6.40%
Overall appraisal	6.12±0.25^{A} 3.87%	6.00±0.25^{a} 4.45%	5.62±0.37 4.11%

A - indicates significant difference ($p<0.01$) compared to Vlashko Vitoroga sheep; a - indicates significant difference ($p<0.05$) compared to Vlashko Vitoroga sheep

The tested meat samples had a fine structure on cross section. Using quantitative descriptive analysis for sensory evaluation, it was determined that Sjenica lamb meat had the highest tenderness, juiciness, meat aroma and overall acceptability. All Zackel meat samples had preferable overall acceptability, the most favourable was in Sjenica sheep, with detected differences between Sjenica and Vlashko Vitoroga sheep (6.12 ± 0.25 and 5.62 ± 0.37 respectively, $p<0.01$), as well as between Lipe sheep and Vlashko Vitoroga sheep (6.12 ± 0.25 and 6.00 ± 0.25 respectively, $p<0.05$, but no statistical difference between Sjenica and Lipe sheep was detected (Table 1). The established results are in accordance with a number of studies. They have confirmed that the interaction between breed and nutritional regimens with specific grass composition of pastures in the traditional habitat, has a big impact on odour and flavour of lamb meat. Local sustainable consumers appreciate flavour of pasture fed lamb meat. "Towards sustainability" strategy supports consumers to favour local food, eco-labelled products, ecosystem conservation, vibrant small business and health-promoting lifestyle. This strategy is a good base for the promotion of Zackel lamb meat as a local food.

Conclusion and recommendation

Recognizing that locally adapted animal breeds gained genetic resistance and adaptability through the evolutionary process, breeding strategies in sustainable farming practices today are far more attuned to the preservation and utilization of autochthonous breeds. Investigations of specific characteristics and added value of autochthonous sheep breeds, especially advanced characterization of the productive potentials and specific product characteristics are important topics for a sustainable conservation strategy and producing a highly appreciated local food.

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STR-Polymorphismen in einer historischen Rasse, im Ciktaschaf

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Zusammenfassung

Die Bestandsstruktur der gefährdeten Cikta Schafrasse wurde anhand von neun Mikrosatelliten-Polymorphismen bewertet. Aus 72 Tieren von drei Herden wurden Blutproben genommen, um die genetischen Indizes in der ungarischen Population zu bestimmen. Insgesamt waren die beobachteten und wirksamen Allel-Zahlen im Durchschnitt 5,63 bzw. 3,76. Hohe Werte für die erwartete Heterozygosität (0,65–0,87) zeigten den Auszucht Status an. Die Diskriminanzanalyse basierend auf den Genotyp-Frequenzen zeigte eine moderate genetische Diversität unter den Cikta-Herden, da nur drei Loci (OarCP49, CSSM47 und OarHH41) signifikant Unterscheidung (P <0,05) von den Subpopulationen hatten. Die geringeren Entfernungen von Mahalanobis zu Gruppen-Zentroiden bestätigten ebenfalls, dass die Rasse durch die drei Herden fast gleich stark vertreten ist. Das moderate Niveau der Diversität zwischen den Herden wurde auf die langfristigen Auswirkungen eines Bestandsengpasses aus den 70er Jahren zurückgeführt. Für die Erhaltung seltener Allele und Diversität bei Cikta-Schafen sind fortlaufende Informationen von Mikrosatelliten erforderlich.

Schlüsselwörter: ungarischer Ciktaschaf, Mikrosatelliten-Polymorphismen, relativer Shannonsche Index, Mahalanobis-Distanz

Abstract
STR polymorphisms of a historical sheep breed, the Cikta

The population structure of the endangered Cikta sheep breed was evaluated by means of nine microsatellite polymorphisms. Seventy-two individuals from three flocks were sampled to determine genetic indices in the Hungarian population. Overall, average observed and effective allele numbers were 5.63 and 3.76, respectively. High values of expected heterozygosity (0.65-0.87) indicated outbred status. Discriminant analysis based on genotype frequencies revealed moderate genetic diversity among Cikta flocks, since only three loci (OarCP49, CSSM47 and OarHH41) contributed significantly (P<0.05) to differences between subpopulations. Low squared Mahalanobis distances from group centroids also confirmed that the breed is almost equally represented by the three flocks. Moderate level of diversity between flocks was attributed to the long-term effects of a population bottleneck dating back

to the 1970s. Continuous microsatellite information is required for the preservation of rare alleles and diversity in Cikta sheep.

Key words: Cikta sheep, short tandem repeats, relative Shannon index, Mahalanobis distances

Einleitung

Traditionelle landwirtschaftliche Nutztierrassen stellen wertvolle genetische Ressourcen dar, die zur Bewältigung zukünftiger Herausforderungen der Tierzucht wie Klimawandel und starker Verlust der Biodiversität erforderlich sind. Daher sollten alle Anstrengungen unternommen werden, um bestehende Populationen lokaler autochthoner Rassen sorgfältig zu erhalten und zu beschreiben. Die Cikta ist eine von mehreren in Ungarn registrierten einheimischen Schafrassen. Nach der Entwicklungsgeschichte haben Schwaben (eine ethnische deutsche Bevölkerung) die Ciktaschafe in Ungarn eingeführt und propagiert, als sie sich im 17. Jahrhundert in der Region ansiedelten. Cikta wurde ursprünglich als eine Dreinutzungsrasse (für Fleisch, Milch und Wolle) verwendet und hat sich sehr gut an die unterschiedlichen geographischen und klimatischen Bedingungen Ungarns in den letzten Jahrhunderten angepasst (KORTH, 1825; BOHM, 1878).
Die Bestandgröße von Cikta schwankte, war jedoch ein fester Bestandteil des ungarischen Schafbestandes. Jedoch Cikta wurde nie zur führenden oder bestandsreichsten Rasse Ungarns. Nach dem Zweiten Weltkrieg wurde dieses wertvolle Schaf fast vollständig von der Zucht ausgeschlossen (KOPPÁNY, 2000; JÁNOSI u. Mitarb., 2017). Im Jahr 1974 begann das Nationale Tierzuchtinspektorat mit der Erhaltung von Cikta zu beschäftigen, indem es 40 Mutterschafe und 3 Widder im Land sammelte. Der Tierbestand wuchs im folgenden Jahrzehnt auf 200 Tiere an. Dieser Pool war die Wurzel der heutigen lebenden Nukleuszucht von etwa 600 Tiere. Daher gilt der nationale Cikta-Bestand als Nachkomme dieses einzigen homogenen Schafbestandes. Die Cikta als geschützte indigene Rasse existiert heutzutage in einigen kleinen Herden mit staatlicher Unterstützung.

Ziel ist es, die mehrzweckige Nutzung der Rasse zu erhalten. Cikta gehört historisch zu den Gruppen der Zaupel-Schafen, zusammen mit den lebenden Vertretern der sogenannten Steinschaf-Gruppe, wie den Alpines Steinschaf, dem Waldschaf, dem Tiroler Steinschaf und dem Krainer Steinschaf (BAUMUNG u. Mitarb., 2006). Die Untersuchung von NEUBAUER und Mitarbeitern (2015) haben bestätigte, dass der Cikta genetisch gut von den Rassen des Zackel-Typs (z. B. ungarisches Racka und Siebenbürgisches Racka) und anderen zahlreicheren Rassen in Ungarn getrennt ist. Basierend auf paarweisen Neischen genetischen Distanze PICHLER und Mitarbeitern. (2017) haben den Zaupel-Typ (westeuropäisch; aus dem Krainer Steinschaf analysierte Proben) zweifellos vom Zackel-Typ (osteuropäischer Herkunft) unterschieden.

Ziel der vorliegenden Studie ist es, die populationsgenetische Analyse der Cikta-Rasse anhand der von der FAO empfohlenen Mikrosatelliten-Polymorphismen zu erweitern. Darüber hinaus wurde auch die Diskriminierungskraft der Mikrosatelliten getestet, um das Niveau der genetischen Identität zwischen den Subpopulationen der Rasse zu bestimmen.

Material und Methoden

Die zweiundsiebzig Tiere, die für die vorliegende Prüfung ausgewählt wurden, waren Vertreter der ältesten Familien. Die ausgewählten Tiere mit 6-5-4 Vorfahren-Generationen gehörten 36 Familien (mütterliche Linien) an. Zwei lebende Vertreter aus jeder alten Familie wurden ausgelassen.
Im Oktober 2015 wurden bei drei Farmen Blutproben entnommen. Eine der ausgewählten Farmen war der staatliche Nationalpark Duna-Dráva (Station Nagydorog mit 20 Familien und 40 Exemplaren), während die anderen beiden privaten Farmen im Besitz von Herrn T. Nagy (Pénzesgyőr, mit 11 Familien und 22 Exemplaren) und Herr J. Jánosi (Szécsénke, mit 5 Familien und 10 Exemplaren). Auffanggefäße, die EDTA als Antikoagulans enthielten, wurden bis zur Verarbeitung bei -20 ° C gelagert. Nach dem Auftauen wurde die DNS aus den Blutproben mit dem Wizard Genomic DNA Purification Kit (Promega, USA) extrahiert.

Die Amplifikation der DNS wurde mittels einer programmierbaren PCR-Maschine (DNA Thermal Cycler, Perkin Elmer, USA) durchgeführt. Neun von FAO empfohlene Mikrosatelliten-Loki bei Schafen (FAO, 2011) wurden in zwei Sätzen von Multiplex-Reaktionen analysiert, wobei der erste Satz Primer für die Loci BM0757, BM8125, OarCP49, BM0827 und OarHH47 enthielt, während der zweite Satz Primer für Loci CSSM47, MAF214, OarHH41 und OarVH72 enthielt. Die 5'-Enden der Forward-Primer wurden mit Fluoreszenzfarbstoffen markiert.
Touchdown-PCR wurde für die Amplifikation von Mikrosatelliten-Loki angewendet.
Der PCR-Produktnachweis, die Bestimmung des Mikrosatelliten-Allels und die Analyse wurden unter Verwendung eines ABI PRISM 3130XL Genetic Analyzer durchgeführt, der von ABI PRISM 310 Data Collection Software (Applied Biosystems, USA) gesteuert wurde. Zur Identifizierung von Mikrosatelliten-Allelen wurde die 310 GeneScan Analysis Software 3.1 verwendet, die interne Standards zur Längenbestimmung verwendet.
Genotyper Software (Applied Biosystems, USA) wurde zur Dateninterpretation und zur Überprüfung der Länge von Mikrosatelliten verwendet.
Grundlegende populationsgenetische Parameter - wie die Anzahl der verschiedenen Allele (N_a), die Anzahl der effektiven Allele (N_e), die Anzahl der beobachteten (H_o), erwarteten (H_e) und fehlerfreien erwarteten Heterozygotie (uH_e) - wurden für Cikta-Populationen mit Hilfe des Microsatellite Analyzer (DIERINGER und SCHRÖTTERER, 2003) berechnet.

Der Vergleich der Herden erfolgte durch Diskriminanzanalyse. Die Kategorisierung erfolgte mit der Bayes-Klassifikation, wo der Vergleich der Gruppen war basiert auf Gruppen-Zentroiden. Die Abweichung einer Beobachtung vom Zentroid wurde anhand des Mahalanobis-Abstandes gemessen (DELL INC., 2015).

Ergebnisse und Auswertung

Die Anzahl der Allele betrug durchschnittlich 5,63. An allen Orten war die Anzahl der Allele bei Mikrosatelliten über vier (mit Ausnahme von CSSM47), was die Empfehlung der FAO (1998) für genetische Diversitäts-Studien erfüllt. Eine Übersicht über die Parameter der genetischen Diversität ist in Tabelle 1 dargestellt.
Die Werte des relativen Shannonschen-Information-Indexes liegen in einem größeren Bereich (von ca. 40 bis 60%). Der I_{rel} ist der Anteil des tatsächlichen Shannonschen-Wertes und des theoretisch höchsten Shannonschen-Wertes (unter gleichen Allelfrequenz-Bedingungen). Diese Zahl ist vorteilhaft, wenn Mikrosatellitenorte mit verschiedenen Allelzahlen (Haplotypzahlen) verglichen werden sollen (GÁSPÁRDY u. Mitarb., 2018).

Tabelle 1. Ergebnisse der Statistik für Mikrosatelliten in Cikta Schafbestand (Mittel ± SD)

Mikrosatelliten	N_a	N_e	I	I_{rel}, %	H_o	H_e
BM0757	5,33 ± 0,58	3,43 ± 0,49	1,41 ± 0,05	54,5	0,72	0,79
BM8125	4,67 ± 0,58	3,35 ± 0,84	1,31 ± 0,26	56,4	0,80	0,73
OarCP49	5,67 ± 0,58	4,12 ± 0,18	1,53 ± 0,05	59,2	0,88	0,78
BM0827	6,00 ± 1,00	4,59 ± 0,74	1,63 ± 0,13	58,1	0,92	0,81
OarHH47	4,00 ± 0,00	2,66 ± 0,62	1,07 ± 0,17	46,1	0,52	0,60
CSSM47	3,33 ± 1,53	2,54 ± 0,55	0,98 ± 0,28	42,2	0,77	0,65
MAF214	6,00 ± 0,00	3,60 ± 0,26	1,46 ± 0,09	52,0	0,84	0,74
OarHH41	9,33 ± 1,53	6,15 ± 0,58	1,97 ± 0,10	56,9	0,82	0,87
OarVH72	6,33 ± 1,53	3,44 ± 0,31	1,42 ± 0,05	47,3	0,81	0,71
Hauptdurchschnitt	5,63 ± 1,71	3,76 ± 1,10	1,42 ± 0,29	52,5 ± 6,01	0,78 ± 0,11	0,74 ± 0,08

N_a = Anzahl der Allele; N_e = Anzahl der effektiven Allele; I = Shannonscher-Information-Index; I_{rel} = relative Shannon-Information-Index; H_o = beobachtete Heterozygotie; H_e = erwartete Heterozygotie

Der Durchschnitt der beobachteten und erwarteten Heterozygotie lag immer über 0,50 und lag in einem größeren Bereich von 0,52-0,92 bzw. 0,65-0,87. Die Werte der fehlerfreien erwarteten Heterozygotie (uH_e, Zahlen werden hier nicht dargestellt) entsprechen vollständig den Werten der erwarteten Heterozygotie.

Tabelle 2 gibt einen Überblick über die Länge der Mikrosatelliten für jede Herde. Unterschiede wurden nur in drei Fällen (OarCP49, CSSM47 und OarHH41) festgestellt, was bedeutet, dass die Herden einen hohen Identitätsgrad aufweisen und die Familien der Cikta-Rasse genetisch nahe sind. Die Untersuchung von SHARMA u. Mitarb. (2016) wurde in den gefährdeten Tibetischen Schafen von Indien durchgeführt, die alle unsere Mikrosatelliten enthielten. In allen Fällen wurde eine unerwartete Identität im Allelbereich (Größe) festgestellt.

Tabelle 2. Größe der Mikrosatelliten bei den Cikta-Beständen gezeigt in Basispaaren (Mittel ± SD)

Mikrosatelliten	Nagydorog n = 80	Pénzesgyőr n = 44	Szécsénke n = 20	Alle n = 144	P-Wert
BM0757	182,4 ± 4,6	183,2 ± 10,0	183,8 ± 5,3	182,8 ± 6,5	0,645
BM8125	114,4 ± 2,3	114,8 ± 2,8	114,2 ± 2,1	114,5 ± 2,4	0,596
OarCP49	$91,0^{b}$ ± 8,4	$88,5^{ab}$ ± 7,9	$86,3^{a}$ ± 6,3	89,5 ± 8,1	0,020
BM0827	220,6 ± 3,5	220,0 ± 2,9	219,9 ± 2,7	220,3 ± 3,2	0,513
OarHH47	133,8 ± 3,0	134,1 ± 3,4	134,7 ± 2,5	134,0 ± 3,0	0,283
CSSM47	$129,5^{a}$ ± 1,3	$130,0^{b}$ ± 1,5	$131,0^{b}$ ± 1,0	129,9 ± 1,4	0,005
MAF214	206,6 ± 21,3	202,4 ± 19,0	202,4 ± 22,0	204,8 ± 20,8	0,334
OarHH41	$129,8^{b}$ ± 8,0	$125,9^{a}$ ± 6,1	$131,8^{b}$ ± 7,5	129,2 ± 7,7	0,015
OarVH72	131,1 ± 5,7	132,8 ± 6,4	129,3 ± 5,2	131,2 ± 5,9	0,073

$^{a, b}$ - die unterschiedliche Buchstaben drücken die statistisch bewiesene Abweichung ($P < 0,05$) aus

Das gemeinsame Wilksche Lambda der Diskriminanz-Funktion betrug 0,606. Dieser höhere Wert zeigt die signifikante ($F = 18,2$ und $P < 0,001$) Unterscheidungsfähigkeit der Funktionen an. Nicht alle unabhängigen Variablen waren signifikant ($P > 0,05$), folglich spielt nicht jeder Mikrosatellit eine Rolle bei der Isolierung der Herden und Familien pro Herde. Es gab nur drei Mikrosatelliten (OarCP49, CSSM47 und OarHH41), die den Unterschied zwischen den

Herden im Vergleich zu den anderen deutlich erhöhten, was sich aus ihren P-Werten und den relativen Effekten aus Tabelle 3 ergibt.

Tabelle 3. Ergebnisse der Diskriminanzanalyse

Mikrosatelliten	Wilksche Lambda	F-Wert	P-Wert	R^2	Relative Wirkung (%)
BM0757	0,607	0,048	0,953	0,220	0,18
BM8125	0,607	0,036	0,965	0,303	0,14
OarCP49	0,665	4,752	0,011	0,232	18,23
BM0827	0,612	0,497	0,610	0,382	1,91
OarHH47	0,607	0,038	0,963	0,192	0,15
CSSM47	0,778	13,904	<0,001	0,351	53,33
MAF214	0,614	0,604	0,549	0,261	2,32
OarHH41	0,652	3,703	0,028	0,263	14,21
OarVH72	0,637	2,488	0,088	0,338	9,54

Als Klassifizierungsergebnis betrug die Gesamtgenauigkeit 64,2%. Die Genauigkeit der Herden war wie folgt: Nagydorog 85,2%, Pénzesgyőr 21,4% und Szécsénke 60,0%. Niedrige Präzisionswerte zeigen an, dass die Herden (und Individuen, die Familien repräsentieren) ähnlich sind, und die Rasse wird fast gleichermaßen von den drei Herden vertreten.
Die quadratischen Mahalanobis-Entfernungen von den Gruppenschwerpunkten waren wie folgt: 9,6 (P = 0,56), 9,7 (P = 0,257) und 11,1 (P = 0,184). Diese niedrigeren und nicht signifikanten Werte bestätigen, dass es keine paarweise Trennung der Schwerpunkte der verglichenen Herden gibt. Die Tiere der kleineren Populationen von Pénzesgyőr und Szécsénke gehören zu den Individuen der bestandsreichsten Nagydorog-Herde, auch wenn die Funktion (Root) 1 hauptsächlich darin besteht, zwischen Szécsénke und den anderen zu diskriminieren (Nagydorog und Pénzesgyőr; P für alle Wurzeln <0,001). In vertikaler Richtung (Wurzel 2) ist keine Tendenz erkennbar, dass Punkte unter oder über der Mittellinie (0) fallen und der Signifikanz-Test ergab für alle verbleibenden Wurzeln nach Entfernung der ersten Wurzel, P = 0,102. Bei der vom Aussterben bedrohten Tsigai-Rasse unterschieden sich die Herden (5 Herden, jeweils 48 Mutterschafe) bei allen acht Mikrosatelliten stark (Wilks λ = 0,059, p <0,001). Der durchschnittliche Anteil der korrekten Einstufung (83,7%) war äußerst zufriedenstellend (GÁSPÁRDY u. Mitarb., 2013).

Schlussfolgerungen und Empfehlungen

Unsere Ergebnisse, die auf Mikrosatelliten-Loci-Informationen basieren, haben die Tatsache bestätigt, dass der Cikta-Bestand des frühen 21. Jahrhunderts tatsächlich aus genetisch eng verwandten Tieren, Familien oder Herden besteht. Die Ähnlichkeit wird auf den schrumpfenden Population des kleinen Tierbestandes in den 70er Jahren und die Folge eines Engpass-Effekts zurückgeführt. Da die Herden sich in mütterlicher Abstammung mehr teilen als die in dieser Studie betrachteten, kann der Unterschied zwischen den Herden noch geringer sein. Trotz dieser Situation spiegelte sich in den meisten Mikrosatelliten die große Allel-Vielfalt wider. Der Grad der Heterozygotie, der als hoch angesehen wird (ca. 75-80%), kann auf die Wirkung von Widdern, die in einem größeren Anteil als gewöhnlich kamen vor, zurückgeführt werden. Ab 2011 trägt das neu eingeführte zentralisierte Aufzucht-Programm dazu bei, die Zuchtböcke unter den Herden gut zu organisieren. Ein interessanter Vorschlag zur Erhaltung der Vielfalt innerhalb der Rasse ist der regelmäßige Austausch von Weibchen

zwischen den Herden. Dies kann implementiert werden; der Transport von Tieren beinhaltet jedoch auch immer mehr umsichtige Tiergesundheits-Maßnahmen. Die Beharrlichkeit der genetischen Unveränderlichkeit und der Zweifel an dem akzeptablen Niveau der genetischen Veränderung nehmen einen zentralen Platz beim Schutz seltener Haustiere ein. Die Herden ändern sich jedoch ständig in ihrer genetischen Zusammensetzung, trotz des Willens, Beständigkeit und Unveränderlichkeit zu bewahren.

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The role of the Slovenian autochthonous Drežnica goat breed in the area of sustainability of the Alpine pasture and Alpine dairy farming

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Abstract

In Slovenia, there is only one conserved autochthonous breed of goat – the Drežnica goat. It is a critically endangered breed, consisting of approximately 750 goats. In terms of the purpose of rearing and its original location, two types of Drežnica goat were developed in the past: the dairy type in the Bovec area and the meat type in the Drežnica region. Still today, the Drežnica goat is in close connection with traditional farming practises such as seasonal Alpine dairy farming of the dairy Drežnica goat, whereas its meat counterpart is characterised by an even more specific way of farming practise that is unique to Slovenia. However, these traditional practices are in rapid decline. Therefore, it is necessary to precisely document and protect the variety of the Drežnica goat farming practises and to promote this valuable heritage for means of public awareness and relevant government institutions.

Key words: Drežnica goat, autochthonous breed, Alpine pasture, unique farming practises

Introduction

In the past, goat rearing had great significance in the Slovenian territory. This influence is evident also from the fact that among all species of farm animals most of the local names have been derived from the term goat, and many of them are still preserved: Kozjek, Kozarica, Kozarski potok (Slovenian term for goat is "koza"; NOVAK, 1970). However, goat rearing in the past was not a respected activity and for goats the derogative term "poor farmer's cow" was often used (ŽAN LOTRIČ, 2016). The neglect of goat rearing as a livestock husbandry resulted from a number of grazing bans and sometimes even from bans of goat rearing in the stable (ŠALEHAR et al., 2014).
However, over the last years, small ruminants rearing in Slovenia have become more important due to the extensive farming on grassland and pastures (ŽAN LOTRIČ et al., 2015).
Four goat breeds are mainly used in Slovenia: the foreign Boer goat, locally adapted Slovenian Alpine goat and locally adapted Slovenian Saanen goat. The fourth goat breed is the only Slovenian autochthonous goat breed called the Drežnica goat. This breed is highly appreciated for its excellent adaptability to poor conditions. The Drežnica goat has the lowest population size among autochthonous breeds in Slovenia. It comprises of 750 goats and is listed as a critical endangered breed (Register, 2018). The concentration of a major part (90%) of the total population is in a restricted geographical area, which means within a radius of less than 30 km (ŽAN LOTRIČ et al., 2013).

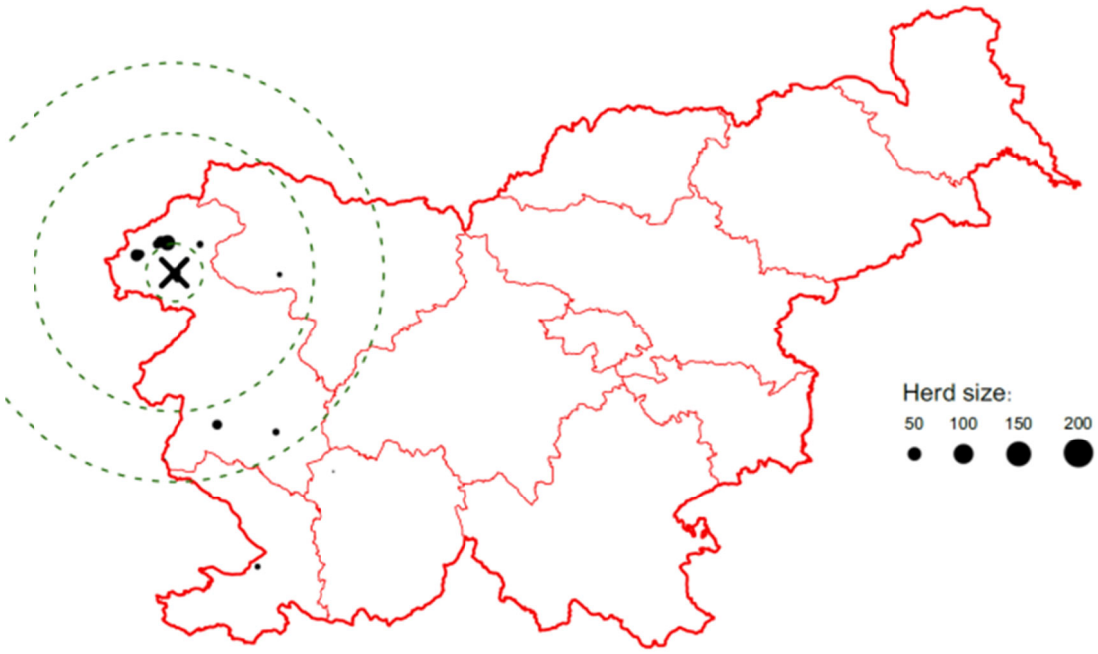

Figure 1. Location and flock size of the Drežnica goat with a calculated geographic centre of gravity (x – Drežnica and Bovec areas) and radius, presenting 50% (inner circle), 90% (middle circle) and 95% (outer circle) of the population (ŽAN LOTRIČ et al., 2013)

The population of the Drežnica goat is divided into two types according to the purpose of rearing on its original locations. The dairy type of the Drežnica goat was developed in the Bovec area where the cheesemaking tradition dates back to the 13th century, whereas the meat type of the Drežnica goat has developed in Drežnica region. Flocks of goats for meat production are prevailing. The Drežnica goat has a seasonal production, it is well adapted to the harsh Alpine conditions and is also kept for the landscape management in these regions. Meat type of Drežnica goat is intended primarily for kid production while the dairy type is reared for milk production and kids. With mountain pasturing through the vegetation period, the Drežnica goat is also intended to prevent overgrowth of the steep and remote agricultural areas.

The dairy Drežnica goat is strongly associated with pastoralism and Alpine dairy farming in the summer time. This form of farming system represents a long cultural tradition in Slovenia. The meat type of Drežnica goat represents the majority of the total breed population and its rearing is recognized by still more specific traditional farming practices than the dairy type and is unique in Slovenia. These elements of local culture originated a long time ago and still characterize Slovenian`s identity today. However, farming practices of both types of Drežnica goat is rapidly decreasing. The Drežnica goat can be conserved only by traditional farming practices which are unique ways of managing both dairy and meat types of goat.

The dairy type of Drežnica goat involved in traditional Alpine dairy farming

Summer grazing has evolved over centuries through the adaptation of human activities to harsh climatic and topographic conditions and has been, and still is, an integral element of Alpine agriculture (MACK *et al.*, 2013). The transhumance systems involve the seasonal movement of dairy ruminants from the permanent farms in the valleys to the mountain

pastures during summer. This system of grazing conserves the landscape from reforestation (MACK *et al.,* 2013). However, dairy farming has experienced a change towards concentration on fewer and larger farms (MARINI *et al.,* 2011). Instead of moving dairy breeds of ruminants to summer pastures, raising meat breeds of ruminants recently gained much greater importance (ŽAN LOTRIČ, 2018). These trends have a strong impact on the livelihoods of Alpine pastoralists and transhumance systems that have come under pressure and face an uncertain future (MACK *et al*., 2013). Research has shown that animals grazing in the high Alpine pastures produce milk which is healthier and richer in nutrients. It contains more protein and, above all, significantly higher levels of healthy unsaturated fatty acids, such as omega-3 and especially linoleic acid (COLLOMB *et al,* 2002; RENNA *et al*, 2012).

In Slovenia, there is a long tradition of moving livestock to the Alpine pasture. This farming practice by dairy breeds of small ruminants has always been concentrated in the upper Soča valley. These mountains are distinguished according to the type of species of farm animal. On the Tolmin and Kobarid mountains, mainly because of favourable grazing conditions, cattle, especially dairy cattle, were grazed. There were very few small ruminants on these mountains, none on most. On the contrary, sheep and also goat grazing prevailed on the steep and rocky mountain pastures of the Bovec Mountains. Hence the name "the sheep or goat mountains".
The dairy Drežnica goats are reared only by a few breeders and represent a very small part of the Drežnica goat population in the Bovec area. Of the total of 11 flocks of the dairy Drežnica goat in Slovenia, six reside in the Bovec region**,** where the main population of the dairy Drežnica goat is reared. The Encyclopaedia of Slovenia states: "*Bovec is the only province in Slovenia where the inhabitants produced forage mainly for sheep and goat farming and goat-herded them on the steep and highest mountains*."
During the vegetation period, only a few flocks of dairy Drežnica goats still graze on Bovec highland mountain pastures in the area of the upper Soča valley. According to the Slovenian official data on Alpine pasture in 2018, all dairy goats were on the highland mountain pasture in the Soča valley. Despite only having information about the location of goats grazing at our disposal, and no available data on the breed of goat, we can state with relative certainty that it concerned the Drežnica goat. According to our knowledge of the terrain in the Soča valley, no other goat breeds are on mountain pastures.

Table 1. Average milk yield and chemical composition of goat`s milk per breeds in 2018 (ZAJC et al., 2019)

Breed	Number of goats	Lactation period (days)	Milk yield (kg)	Fat (%)	Protein (%)	Lactose (%)	Dry matter (%)
Dairy type of Drežnica goat	144*	207	362	4.2	3.4	4,4	12.0
Slovenian Saanen goat	179*	222	503	3.3	2.9	4.3	10.5
Slovenian Alpine goat	507*	243	458	3.1	3.1	4.4	10.6

* Number of goats included into the breeding program

The dairy Drežnica goat is characterized by a relatively good milk yield, in average it produced 362 kg of milk per lactation with 4.2% fat and 3.4% proteins (ZAJC *et al.,* 2019). The breed is known as the dairy goat breed with the highest content of fat and protein in comparison to more intensive breeds in Slovenia such as the Slovenian Saanen goat and the Slovenian Alpine goat (Table 1).

By producing unique dairy products from goat and cow milk, the dairy Drežnica goat breeders conserve the tradition of Alpine dairy farming. The cheese-making tradition in this area dates back to the 13th century. The demand for dairy products with a unique flavour is mostly higher than the supply. Dairy goat products made from Drežnica goat milk in the Alps are mainly sold to permanent consumers and local restaurants. Goat cheese with its gentle aroma and pleasant taste, made from milk from the mountain pastures, is considered in the culinary art as an excellent addition to various dishes.

Figure 2. Dairy type of Drežnica goat on Bovec`s mountain Bošca (1,370 m.a.s.l; ; photo: Metka Žan)

The meat type of Drežnica goat and conservation of the autochthonous and unique farming practice

For a long time, cultivated Alpine summer pastures have been a valuable source of feed for ruminants in Slovenia, and using them has significantly shaped the cultural landscape. The meat type of Drežnica goat presents the majority of the breed's population and is concentrated in the Drežnica region. Animals are intended primarily for kid production. With mountain pasturing on the high altitude the meat type of Drežnica goat is also intended to prevent overgrowth of the steep and remote agricultural areas. According to the Slovenian official data on Alpine pasture in 2018 the majority of goats for meat production, regardless the breed, were on highland mountain pasture in the Soča valley. Although the data on the breed of goat used for meat production is not available, we could conclude on the information we have on the area in question that it concerns the meat type of Drežnica goat.

Specific farming practice of the meat type of Drežnica goat is unique in Slovenia. About three quarters of the year or even more, goats are free ranged on high altitude mountain pastures in the area above of the Drežnica village. These extensive areas where approximately 250-300 goats graze, easily facilitate more animals. Goats belong to different breeders that mostly live in the valleys of the Drežnica area.

Goats usually graze in the altitude in the range of 900 – 1,773 m.a.s.l. In the summer, when it is hot, goats go to the pastures that lie higher, where the grass is juicier and dew stays longer.

Goats are allowed to roam and scavenge for their own food. They rely entirely on morning dew for water supply.

The Drežnica goats are herded to graze in the spring, and at the end of the year they are caught by breeders in the same area, tied to a rope and brought back to the barn one by one. Catching goats to bring them back to the valley usually requires more than one visit in the mountains where the goats are scattered. It often occurs that due to their scattered grazing, not a single goat is caught. There are cribs in which feed or minerals act as bait to help catch the goats. Goats that are less accustomed to humans (especially the young ones) are more difficult to catch, so the cribs are a great tool to help animal breeders catch them. In late autumn and early winter, Drežnica goats descend onto lower pastures, where they find shelter easier and it snows less often, to eat bushes, allowing breeders in these lower areas to catch them.
All does and bucks are under the constant control of breeders who do regular visits on the mountain pastures where all the animals are examined regardless of the ownership. Breeders use a specific type of voice for calling goats to attract them to their vicinity. Goats are supplied with salt, minerals and dry bread. At the same time, breeders also perform regular health check-up. Breeders regularly monitor goats on mountain pastures by observing them with binoculars.

Figure 3. Meat type of Drežnica goat on the Krasji vrh mountain pasture (1,773 m.a.s.l.; photo: Metka Žan)

Meat type of Drežnica goats on mountain pastures are marked in a special way. Breeders use a knife and pliers for notching with wedge-shaped or rounded cutting so that the animals have a different mark on the ear, specific for an individual farm.
Due to their lively temperament and extraordinary sense of establishing hierarchy, goats are tied up in most barns. It is not possible to use a classic, free range in a communal-living barn with the Drežnica goat, because animals that are lower on the hierarchical scale in this case will not even get to the forage or others might even kill them. Breeders who have enough stable space house the animals into smaller pens and tie up only the most problematic animals. The way of rearing causes goats to become semi-feral; otherwise, this could affect

their survival on pasture during the long pasture season. Goats remain in the stables for 3-4 months to give birth and to wean their kids. At the end of March, and depending on weather conditions, the circle is repeated and the meat type of Drežnica goat return to the mountain pastures for the following months.

Conclusion and recommendation

The Slovenian autochthonous Drežnica goat is great at adapting to the modest and extensive management systems in the highland mountain pastures in the Bovec and Drežnica regions, which are the breed's autochthonous areas. There are two distinguishable types of Drežnica goat – the dairy and meat type, and each type belongs to a specific tradition of breeding practices which are original and unique in Slovenia and have developed over centuries.
Using traditional farming practices of both types of Drežnica goat on pastures at high altitudes fulfils many functions, inter alia control natural forest re-growth (trees and bushes) and consequently maintaining an open landscape that is attractive for tourism, protection from natural hazards, production of livestock products, sustaining traditions, biodiversity conservation, etc. The important role of the Drežnica goat in Slovenian agricultural space comes in the form of natural and cultural heritage. Its traditional farming practices present intangible cultural heritage of national significance and should be protected and included into the Register. Unfortunately, the younger generation do not realise the benefits of conserving such traditional practices of goat farming, and the latter is in decline. Therefore, we can only hope that the younger generations will continue this kind of traditional goat farming in these areas as their ancestors once did. The Drežnica goat is a national treasure marked with Slovenian identity and the specific way of farming in its original area must be conserved and protected appropriately. The principles of traditional farming practices of both types of Drežnica goat have been used for hundreds of years and helped to maintain the settlement of remote places in Slovenia and the cultivated landscape. To keep these areas demographically vital and settled in the future, special emphasis should be placed on the conservation of specific traditional farming practices designated for the Drežnica goat.

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Prolactin genotype is associated with egg production in Hungarian Yellow hens

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Abstract

Prolactin hormone has crucial roles in regulation of egg production, since broodiness is induced by elevated prolactin concentrations, which causes temporary suppression of the ovary, and interruption of laying. In the present study, 436 Hungarian Yellow hens were genotyped for the 24-base-pair indel in prolactin gene (*PRL*). Body weight measurements were taken biweekly from the day of hatching to 14 weeks of age, and egg production was monitored between 40 and 45 weeks of age. Average egg weight and egg production intensity was determined. Frequencies for *DD*, *ID*, *II* genotypes were 0.23, 0.48, and 0.28, respectively. *PRL* genotype associated ($P<0.05$) with egg production intensity, and had no effect ($P>0.05$) on body or egg weight in the monitoring period. The insertion (*I*) allele proved to be more beneficial for egg production, which is in agreement with the allele substitution pattern in several other breeds and crosses.

Key words: prolactin, Hungarian Yellow chicken, polymorphism

Introduction

The Hungarian Yellow chicken, developed in the early 20th century, originated from primitive native Hungarian and some foreign breeds (chiefly Rhode Island Red and New Hampshire). The impetus was to establish a stable dual-purpose population well suited to local needs, including backyard and free-range production. However, in the modern poultry industry, introgression from non-commercial chickens is rarely used, resulting in very narrow genetic and allelic diversity in commercial versus noncommercial breeds.
Since only a few breeds were used in formation of modern lines, local breeds have essential roles in maintenance of genetic diversity and to ensure future success and sustainability of the poultry sector (MUIR et al., 2008; BODZSÁR et al., 2009). The aims of this study were to evaluate effects of a prolactin gene (*PRL*) insertion-deletion (indel) on some important production traits, and to provide an overview on the genetic structure of the Hungarian Yellow population, based on the distribution of genotypes.

Prolactin (a hormone from the pituitary) has crucial roles in regulation of egg production, since broodiness is induced by elevated prolactin concentrations, which results in the interruption of the laying period (SHARP et al., 1984). Prolactin secretion can be stimulated by dopamine, a neurotransmitter that operates by activating hypothalamic dopamine D1 receptors (YOUNGREN et al., 2002). Prolactin and dopamine receptors are involved in a

wide spectrum of biological activities; however, only limited genotype-growth associations have been reported in chickens (BHATTACHARYA et al., 2011).

Material and methods

The animals analyzed in present study were hatched and raised at the genetic resource farm located in Mosonmagyaróvár, managed by the Széchenyi István University, Faculty of Agricultural and Food Sciences. The experimental population was kept under the same feeding and housing conditions with *ad libitum* access to food and water. The 436 individuals involved in this study were randomly selected from 1280 hens. This source population was constructed through crossing the elite breeding stock formed by 32 males and 320 females.
The maintenance of the breed is carried out in one central facility divided into 32 separated, identical floor-pens. In each year, after about 45-46 weeks of age, the population is selected upon several criteria (breed standard specifications, initial egg production, and body weight) to create the parental stock of the next generation. Since 1948, trap-nests and individual tagging have been applied to achieve pedigreed egg production data on the maternal side. On the paternal side, pedigree breeding is assured by the construction of separated breeding stocks, each with one rooster.

For genotype-trait associations, body weight (BW) measurements were taken biweekly from the day of hatching to 14 weeks of age, and egg production was monitored between 40 and 45 weeks of age. Body weight was also recorded at the beginning and the end of the egg collection period (BW40, BW45). Eggs laid during this period were measured and average egg weight (EW) was determined. Egg production intensity (EPI) was calculated as follows: EPI = (number of eggs laid/days of the egg collection period) × 100.
Blood samples were drawn from wing veins into collection tubes supplied with EDTA as anticoagulant, and then kept at -20°C until further processing. The DNA extraction from whole blood was carried out by means of the Wizard Genomic DNA Isolation kit (Promega, USA) following the attached instructions. DNA concentration and purity were assessed using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and both 260/280 and 260/230 ratios of samples analyzed were higher than 1.8.

Polymerase chain reaction (PCR) with agarose gel electrophoresis was applied to determine the *PRL* genotype of the animals. With slight modifications, the genotyping method for the 24 bp insertion in *PRL* was used as described by JIANG et al. (2005; Table 1).
The amplification reactions contained the following components: 2× PCR Mastermix 12.5 µl (Promega, USA; Thermo Fisher Scientific, USA), forward and reverse primers 1–1 µl (0.4 µM), DNA template 1 µl (100 ng), and nuclease free water up to 25 µl final volume. PCR program consisted of the next steps: 1 cycle initial denaturation at 95°C for 4 min, followed by 35 cycles at 95°C for 1 min, 1 min at 56°C and 1 min at 72°C, respectively, terminated by 1 cycle at 72°C for 5 min.
Alleless were separated, and visualised under UV illumination in 3% agarose gels stained with ethidium-bromide; the 24-bp length difference enabled the clear discrimination of the alleles.

Table 1. Primer sequence, annealing temperature (Ta) in °C, and the length of PCR products (bp)

Locus	Primers 5'-3'	Ta	Sequence ID	Length
Promoter of *PRL*	F: GGTGGGTGAAGAGACAAGGA R: TGCTGAGTATGGCTGGATGT	56	FJ663023 or FJ434669	177 or 201

HARDY–WEINBERG equilibrium (HWE) was analyzed by Chi-square (χ^2) tests of the observed and expected genotype frequencies in the Hungarian Yellow chicken population.
The effects of *PRL* genotype on production traits were analyzed with general linear model (GLM procedure) in SPSS for Windows v.16.0 (SPSS, USA): $Y = \mu + H + P + G_{PRL} + e$, where: Y = phenotypic records of the analyzed traits (body weight at different ages, egg weight, EPI); μ = population mean of the traits; H = effect of hatching; P = effect of the floor-pen; G_{PRL} = effect of the *PRL* genotype; e = residual random error.
Heterozygosity and polymorphism information content were calculated as described by NAGY et al. (2012).

Results and discussion

The analyzed *PRL* indel was polymorfic in the population, two alleles and three genotypes (*DD*, *ID*, *II*) were detected. The *I* allele was represented by a 201 bp PCR-product, whereas the *D* allele was 177 bp long. Allele and genotype frequencies are presented in Table 2.
From Chi-square test results, it can be inferred that the genotypes distribute according to HWE (Table 2). The high heterozygosity may be attributed to the breeding program that supports the mating of nonrelated individuals by mixing the males from one floor-pen with the females of the adjacent pen from year to year.

Table 2. *PRL* allele and genotype frequencies (%), results of the Chi-squre test for HWE, polymorphism information content (PIC) and heterozygosity (He) in the Hungarian Yellow population

Allele frequency	Genotype frequency[a]	χ^2	P-value[b]	PIC	He
I = 53 *D* = 47	*DD* = 23 (22) *ID* = 48 (50) *II* = 29 (28)	0.511	0.47	0.37	0.50

[a] HWE expected frequencies are presented in parentheses; [b] Degree of freedom (df) = 1

PRL genotype was associated (P<0.05) with egg production intensity, and had no significant (P>0.05) effect on body or egg weight in the monitoring period (Table 3).

The insertion allele proved to be more beneficial for egg production, which is in agreement with the allele substitution pattern in several other breeds and crosses (BAGHERI SARVESTANI et al., 2013; BEGLI et al., 2010; CUI et al., 2006; Table 4).

Table 3. *PRL* genotype effects on various traits (estimated marginal mean ± standard error)

Traits	*PRL* genotype (n)		
	DD (102)	*ID* (210)	*II* (124)
Body weight at week 8 (g)	541.8 ± 5.8	560.4 ± 3.7	568.2 ± 5.9
Body weight at week 10 (g)	789.4 ± 7.9	806.2 ± 6.3	793.3 ± 5.9
Body weight at week 12 (g)	886.7 ± 10.5	915.8 ± 6.8	913.4 ± 7.7
Body weight at week 14 (g)	969.6 ± 16.4	1003.4 ± 12.7	1010.8 ± 14.0
Body weight at week 40 (g)	1757.1 ± 18.4	1791.7 ± 17.3	1772.7 ± 16.6
Body weight at week 45 (g)	1941.2 ± 21.94	1961.7 ± 15.39	1911.3 ± 22.19
Egg weight (g)	54.85 ± 0.47	52.83 ± 0.49	53.78 ± 0.35
Egg production intensity (pcs)	49.52 ± 1.11[b]	55.76 ± 0.83[a]	55.08 ± 0.81[a]

[a, b] Values with different superscripts in the same row differ significantly (P<0.05)

Table 4. Insertion allele frequency in different breeds

Source	Breed (n)	*I* freq.	Annual egg number
CUI et al. (2006)	White Leghorn (30)	1.00	300
JIANG et al. (2005)	White Leghorn (72)	1.00	300
BHATTACHARYA et al. (2011)	White Leghorn (612)	0.70	300
	Hungarian Yellow (436)	0.53	150-210
CUI et al. (2006)	White Rock (30)	0.22	160
JIANG et al. (2005)	Silkie (222)	0.00	80
CUI et al. (2006)	Yangshan (30)	0.05	<70

No such effect of the *PRL* indel genotype was detected in Ningdu Sanhuang hens, whereas the *D* allele frequency (0.91) was vastly predominant in the experimental population (XU et al., 2011). The insertion frequency greatly differs among breeds, and is generally higher in egg-type chicken populations (JIANG et al., 2005). The 24 bp insertion allele provides a binding site for ecotropic viral integration site-1 encoded factor (Evi-1), that can down-regulate *PRL* expression, and leads to less frequent brooding and, to some extent, can improve egg production (CUI et al., 2006; JIANG et al., 2005).

Based on the highly polymorphic *PRL* promoter, six main (frequency over 0.01) haplotypes could be separated in White Leghorn lines, and significant (P<0.05) difference was uncovered between the body weight (at 16, 64 weeks of age and at sexual maturity) of some of the haplogroups (BHATTACHARYA et al., 2011). These observations highlight the importance of further promoter variations, and support a haplotype-based approach for *PRL* and growth association studies.

Conclusion and recommendation

PRL insertion allele was associated with improved egg production in Hungarian Yellow hens. The analyzed marker can be used for breed improvement; however, breed production characteristics should be maintained during preservation, results can be applied in designed crosses with modern egg or meat type breeds and hybrids in attempt to achieve moderately high-producing backyard varieties.

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Comparison of two staining techniques of Oravka cock semen using specific markers by flow cytometry

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Abstract

Cryopreservation of poultry genetic resources in the gene bank is necessary for the conservation of endangered species. The goal of this study was to compare two staining techniques (with and without DRAQ5 dye) for sperm viability and acrosome status. Heterospermic pooled semen from the Oravka cock line (n = 6) was diluted in a Kobidil^{+} extender and frozen in a cryoprotectant solution containing 8% glycerol (GL) in liquid nitrogen vapours before being plunged into the liquid nitrogen. Afterwards, quality of post-thaw spermatozoa using specific markers (DRAQ5, Yo-Pro-1, Sytox Green, PI and PNA) was evaluated. Our results showed significant differences ($P<0.05$) among the experimental groups (DRAQ5^{+}/PNA^{+} vs PNA^{+}, 9.76±3.35% vs 17.31±3.30%, DRAQ5^{+}/Yo-Pro-1^{+} vs Yo-Pro-1^{+}, 27.98±7.10% vs 8.87±0.82% and DRAQ5^{+}/SYTOX^{+} vs PI^{+}, 19.55±5.61% vs 29.67±3.99%). These differences may be due to various binding mechanisms of each marker tested. Therefore, the choice of a proper marker for a spermatozoa viability and acrosome status evaluation should be done carefully.

Key words: Oravka breed, Semen, Cryopreservation, DRAQ5 dye, Viability

Introduction

Conservation of poultry genetic resources in a gene bank is necessary for the preservation of endangered species. Cryopreserved biological material can be used for recovering of the lost variation within breeds and restoring of the breeds, which have become endangered as a result of destruction of their natural conditions (SAWICKA et al., 2011).
The local Oravka chicken breed has not been extensively investigated for an *ex situ* cryopreservation. This breed was created by the crossbreeding of local hens in the Orava region with Rhode Island, Wyandotte and New Hampshire breeds (HANUSOVÁ et al., 2014) and was recognized in 1990 (CHMELNIČNÁ, 2004). However, WEIS et al. (2010) categorized the Oravka breed as a critical endangered breed under the conditions of the Slovak Republic. Oravka is a dual-purpose breed kept for egg and meat production, respectively.

To date, semen cryopreservation is the only effective method of storing reproductive cells *ex situ* (IAFFALDANO et al., 2016a) and is extensively investigated as a possible effective method of maintaining male genetic material for the establishment of a cryobank. However, cryopreserved cock semen has limits, due to its presumably low spermatozoa motility with the primary role of the sperm to fertilize the ovum. In this regard, is necessary to improve and standardize germplasm cryopreservation technology for *ex situ in vitro* preservation programmes (HOU et al., 2008).

The analyses, essential for the study of cock semen quality, generally include the evaluation of spermatozoa motility and viability. Standard techniques of spermatozoa analysis are fluorescence microscopy or flow cytometry using fluorescent staining techniques. Flow cytometry is an automatic system able to provide precise assessment of spermatozoa quality (PETRUNKINA and HARRISON, 2007) because of its high sensitivity, repeatability (CHRISTENSEN et al., 2004, 2005) and determination of a large number (10.000) of spermatozoa in a short period of time (RIJSSELAERE et al., 2005). The increasing availability of fluorescent probes has stimulated interest in methods for evaluating many properties of spermatozoa quality (GRAHAM, 2001).
Several staining techniques are available for evaluating cell viability and can be used alone or in combination with other dyes for the assessment of spermatozoa quality. Spermatozoa viability can be detected by staining with propidium iodide, a non-permeant DNA-specific dye, alone or in combination with membrane-permeant stains, such as SYBR 14 (GARNER and JOHNSON, 1995), DAPI and Yo-Pro-1 dyes (KUŽELOVÁ et. al., 2015). Assessment of the acrosome integrity has also been used in association with fluorescently labeled plant lectins (NAGY et al., 2003).

The aim of the present study was to compare the effect of various cryoprotectants and two staining techniques on the post-thaw quality of Oravka cock semen quality evaluation using flow cytometry.

Material and methods

Experimental design
Semen was routinely collected from six cocks twice a week by dorso-abdominal massage into prepared sterile tubes. Only samples with a minimum total motility of 70% were used in the experiments. Samples containing urine and cell debris were removed. The samples were pooled to avoid the effects of individual differences among cocks. Semen sample was centrifuged (600 x g) at 4°C for 5 min, seminal plasma was removed and aliquots of semen (250 µl) were diluted (1:1; v/v) with Kobidil^{+} extender (Landata Cobiporc, France) at room temperature and cooled to 4°C. Kobidil^{+} contributes to maintenance of the osmotic balance of the diluted semen, limits the development of bacteria, meets the energy needs of the spermatozoa and guarantees the pH stability of the environment.

Next, freezing medium composed of Kobidil^{+} and glycerol (GL, Sigma-Aldrich, Germany) at the final concentration of 8% was added at the ratio of 1:1 (v/v) into the sample and kept at 4°C for 45 min. Cryoprotectant concentration used in our experiment was given according to the available literature (HANZAWA et al., 2010; SASAKI et al., 2010; SANTIAGO-MORENO et al., 2011; MPHAPHATHI et al., 2016) and our preliminary study. The semen was packaged into 0.25 ml plastic straws during the equilibration period. The straws were suspended horizontally in liquid nitrogen vapours at 5 cm above the liquid nitrogen level for 15 min (–125 to –130°C) before being plunged into the liquid phase (–196°C) for storage.

Following 2–3 days of storage in a liquid nitrogen, the straws were thawed at +4°C for 2 min and analysed by CASA (data not show) and flow cytometry.

Flow cytometry

The fresh or frozen/thawed samples were washed and centrifuged in $PBS^{(-)}$ (Sigma-Aldrich, Germany) at 600 x g for 5 min and subdivided into seven tubes for subsequent flow cytometric assessment of DRAQ 5-positive, DRAQ 5-positive/apoptotic, DRAQ 5-positive/necrotic, DRAQ 5-positive/acrosome damaged spermatozoa and apoptotic, necrotic and acrosome damaged without DRAQ 5 staining, as described below. The detection of apoptotic and necrotic spermatozoa was performed using a specific nuclear fluorochrome Yo-Pro-1 (Molecular Probes, Switzerland) and Sytox Green (Molecular Probes, Switzerland) in combination with the DRAQ5 nuclear dye (Biolegend, Germany) in order to detect only the spermatozoa from seminal plasma and propidium iodide (PI, 50 µg/ml, Molecular Probes, Lucerne, Switzerland) for necrotic spermatozoa evaluation without co-staining with DRAQ5. Further, the evaluation of acrosome integrity using a fluorescein-labelled lectin from peanut agglutinin (Arachis hypogea; PNA Alexa Fluor 488, Molecular Probes, Lucerne, Switzerland) in co-staining with and without DRAQ5 dye was performed. Briefly, the semen samples were washed and centrifuged in $PBS^{(-)}$ at 600 x g for 5 min. The supernatant was discarded, and the cell density was adjusted to $1x10^6$ cells/ml. One microlitre of the Yo-Pro-1 solution (100 µmol/l) and 0.1µl of DRAQ5 (5mM) were added to 500µl of the cell suspension to determine portion of apoptotic spermatozoa. 0.1 µl of DRAQ5 and 2 µl of PNA (0.5mg/ml) were added to 500µl of the cell suspension to evaluate acrosome integrity. Samples without DRAQ5 dye were stained at the same concentrations, as described above. Incubation was performed in the dark at room temperature for 15 min. After incubation samples were washed in $PBS^{(-)}$ and centrifuged at 600 x g for 5 min, the supernatant was discarded. To detect percentage of necrotic spermatozoa firstly 0.1 µl of DRAQ5 were added to the cell suspension and incubated for 15 min in the dark at room temperature. Afterwards, samples were washed in $PBS^{(-)}$ and centrifuged at 600 x g for 5 min, the supernatant was discarded and 2.5 µl of SYTOX Green (30µM) were added to 500µl of the cell suspension and incubated for 15 min in the dark at room temperature. After the second incubation flow cytometry assay was performed.

The stained samples were analysed by a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). At least 10.000 events (spermatozoa) were assessed in each sample. The emitted green fluorescence of Yo-Pro-1, Sytox Green or PNA-positive cells and red fluorescence of DRAQ5 and PI-positive cells were recorded in the FL-1 and FL-3 channels, respectively (Figure 1 and 2).

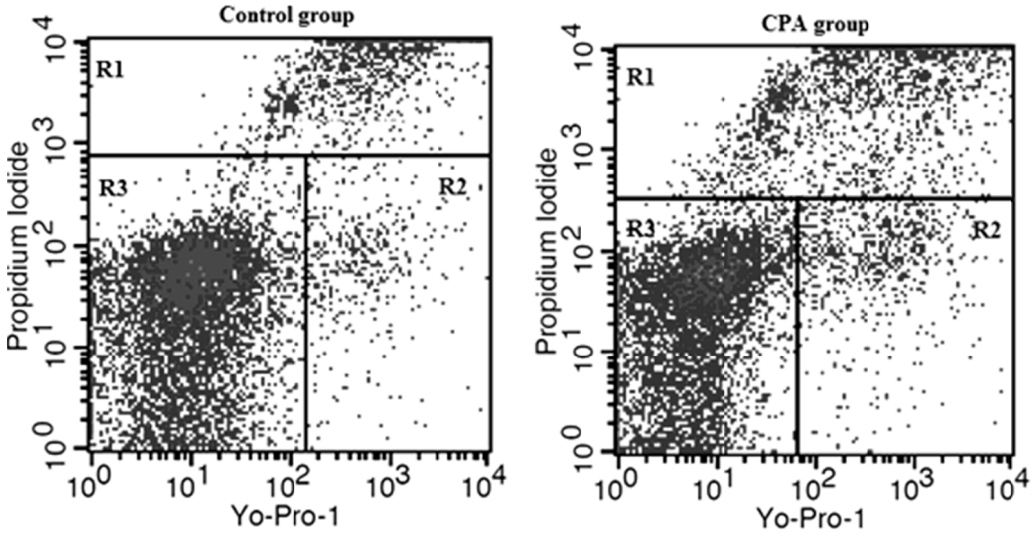

Figure 1. Representative flow cytometry dot plots for the control and experimental groups
R3 region represents viable (Yo-Pro-1^{-} and PI^{-}), R2 region - apoptotic (Yo-Pro-1^{+} and PI^{-}) and R1 - necrotic (PI^{+}) spermatozoa.

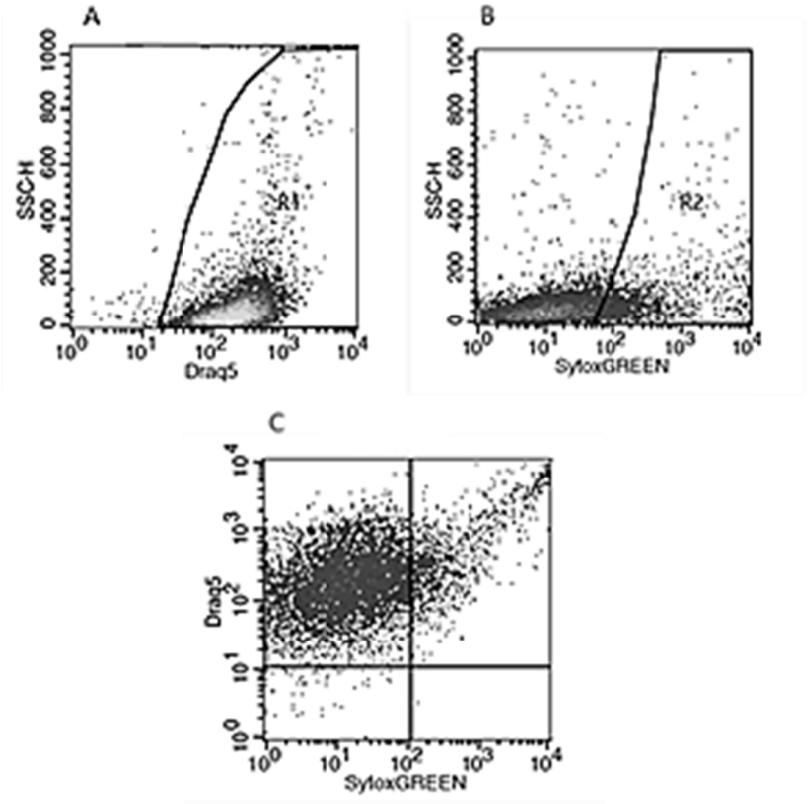

Figure 2. Representative flow cytometry dot plots for the control and experimental groups stained with DRAQ5
A). Region R1 represents DRAQ5-ositive nuclear cells (DRAQ5^{+}) B). Region R2 represents Sytox Green-positive necrotic cells (Sytox^{+}) C). DRAQ5^{+} and Sytox Green^{+} positive nucleated necrotic cells. Markers of apoptosis and acrosome damaged were evaluated by the same assay.

Statistical analysis
The experiment with samples was replicated four times (from April to May). Observed results were statistically evaluated by a one-way ANOVA (t-test) using SigmaPlot software (Systat Software Inc., Germany) and expressed as the means ± SE (standard error). *P*-value at <0.05 was considered as statistically significant.

Results and discussion

Different labelling patterns - DRAQ5, DRAQ5/PNA, DRAQ5/Yo-Pro-1, DRAQ5/ SYTOX Green, PNA, Yo-Pro-1 and PI identify seven different cell populations. Spermatozoa were classified either as acrosome-damaged ($DRAQ5^+/PNA^+$ and PNA^+), apoptotic ($DRAQ5^+$/Yo-$Pro\text{-}1^+$ and Yo-$Pro\text{-}1^+$) and necrotic ($DRAQ5^+$/SYTOX $Green^+$ and PI^+). Significant differences ($P<0.05$) among the experimental groups (with and without DRAQ5) were observed (Table 1).

Table 1. Percentages of nucleated, acrosome-damaged, dying and dead spermatozoa in the control and experimental groups, determined by flow cytometry

Parameters (%)	Control	GL/K^+
$DRAQ5^+$	96.08±1.55	95.70±2.11
$DRAQ5^+/PNA^+$	4.09±0.54	$9.76±3.35^a$
$DRAQ5^+$/Yo-$Pro\text{-}1^+$	$8.82±2.40^c$	$27.98±7.10^c$
$DRAQ5^+$/$SYTOX^+$	11.11±8.96	19.55±5.61
PNA^+	6.02±2.20	$17.31±3.30^b$
Yo-$Pro\text{-}1^+$	$4.61±2.58^d$	$8.87±0.82^d$
PI^+	9.53±1.22	29.67±3.99

Values are presented as least squares means ± SE. Experimental groups with different superscripts are significantly different ($P<0.05$; ^{a} versus ^{b}, ^{c}versus^d)

We applied flow cytometry to examine proportion of apoptotic, necrotic and acrosome-damaged spermatozoa. Many authors used flow cytometry protocols to analyse specific parameters of spermatozoa quality, such as viability, apoptosis, acrosomal status, capacitation, mitochondrial membrane potential, lipid peroxidation, reactive oxygen species generation (ROS) or DNA damage (MARTÍNEZ-PASTOR et al., 2010).
Apoptotic cells can be easy assessed using a Yo-Pro-1 fluorescent dye (Martin et al., 2004). Degradation phase is accompanied by the release of pro-apoptotic factors. During the degradation phase of apoptosis, the cytoplasmic membrane becomes slightly permeable. Apoptotic cells become permeable to Yo-Pro-1 green fluorochrome, while remaining impermeable to PI. Thus, the use of combined Yo-Pro-1 and PI dyes provides a sensitive indicator for apoptosis (IDZIOREK et al., 1995).
On the other hand, Sytox Green is a high-affinity acid stain that does not cross the membranes of viable cells, while easily penetrates the damaged plasma membrane. This dye was suggested as a good indicator for viability assessment (Roth et al., 1997). In this study we tested the efficiency of Sytox Green dye for the detection of dead cells in fresh or frozen/thawed semen samples by flow cytometry.

In order to distinguish between sperm cells, the cellular debris or other cells in the seminal plasma, DRAQ5 in combination with the markers of apoptosis (Yo-Pro-1), necrosis (Sytox Green) and acrosome damage (PNA) were used in our flow cytometry assay. DRAQ5 is considered as a novel DNA-detecting far-red-fluorescing dye, a modified anthraquinone, which has a unique combination of properties exploitable by flow cytometry (Smith et al., 2004). It provides a convenient mean of stoichiometrically labelling cell nuclei in live cells. As far as we know, this is the first study to report the detection of DRAQ5 positive cells, like nuclear marker of cock spermatozoa, which is able to recognize the cells with nucleus and separated from surrounding debris or other cells.

In our study, we applied DRAQ5 nuclear dye with a high affinity for DNA that rapidly penetrates the plasma membrane of both viable and nonviable spermatozoa. The use of DRAQ5 dye allows to distinguish spermatozoa from surrounding debris of the cell suspension. Subsequently, gated cell population ($DRAQ5^{+}$) was used to accurately distinguish live and dead cells using markers as Yo-Pro-1, Sytox Green and PI. For acrosome-damaged spermatozoa a PNA lectin was used.
Our study confirmed a significantly higher incidence of apoptotic, necrotic and acrosome-damaged spermatozoa after cryopreservation in the experimental groups (stained with DRAQ5) compared with the non-staining (without DRAQ5) group (Table 1).
These differences may be due to various binding mechanism of each marker tested. Therefore, the choice of proper markers for a cell viability evaluation should be done carefully.

Conclusion and recommendation

Our study showed differences in the proportion of apoptotic and necrotic spermatozoa between the staining types used. It can be concluded, that these differences may be due to various binding mechanism of each marker used. We recommend to apply the proper markers for spermatozoa quality assessment.

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Determination of mineral composition and nutritional value of snail meat for human consumption cultivated in mini-paddock pen system

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Abstract

In this study we aimed to prove the nutritional value of commercially raised *Helix lucorum* snails. The snails (N=100), collected from a farm operating by a mini-paddock pen system, were analyzed for macronutrient composition and mineral content of the edible portion. The percentage of crude protein, fat, ash and moisture, as well as levels of selected minerals were determined by automatic systems and electro thermal atomic absorption spectrometry after microwave digestion. Mean values were calculated and compared with the current dietary reference values according to EFSA. Results showed that snail meat is a good, low-fat source of protein. 100 g of snail meat can provide 17% of the daily calcium requirements, 18.5% of the phosphorus, 26% of the zinc, and double the requirements for copper. In conclusion, meat from farmed *H. lucorum* is a valuable source of essential nutrients and should be made accessible to a wider population.

Keywords: snail meat, *Helix lucorum*, macronutrients, mineral content

Introduction

Currently, 821 million people in the world are undernourished, while 672 million are obese (FAO, 2018). In view of the increasing global population (projected to rise to around 10 billion by 2050) stakeholders are constantly searching for new sources of food that are both high in nutrients and minimally damaging to the environment. Less popular sources of protein and minerals, such as edible land snails, can be adopted to address this need. Snails of the species H*elix aspersa* (*C. aspersum*) and *Helix pomatia* are a popular high-end choice in countries like France, Italy, Spain and Portugal, mainly imported from farms in Greece, Turkey, Eastern Europe and North Africa (GHOSH et al., 2016). Lack of culinary traditions and limited information are some reasons for the low consumption of snail meat in the Balkan region (ÇAĞILTAY et al., 2011).

Since snails are not widely available on the market of Balkan countries, many people resort to collecting wild species for food, thus endangering the stability of wild snail populations. Some of these wild snails have been known to contain substantial amounts of toxic metals, such as lead and cadmium (CORDA et al., 2014; CICERO et al., 2015). Raising wild snail varieties, i.e. *Helix lucorum* (Turkish snail), in farms could be a way of preserving endangered species and reducing the health risk to consumers through good feeding and farming practices. In this study we aim to prove the nutritional value of commercially raised *Helix lucorum*.

Materials and methods

Sampling
To fulfill the purpose of the study one hundred snails *(Helix lucorum)* of size fit for processing and consumption were collected from a private snail farm operating by a mini-paddock pen system. At the farm the snails had been fed Complete Feed for Snail 70/1, produced by Bonmix, Bulgaria, that included grain components, calcium carbonate, soybean meal, L-lysine, L-methionine, choline chloride, vitamin-mineral premix and specially selected sources of calcium and digestible phosphorus with specific particle size. The average consumption of feed by one snail in the growing period was 20-25 g. Average weight of a specimen in the sample group was 39.78 ± 3.82 g with shell. At the laboratory the snails were initially treated with 20% NaCl solution by a method described by ÖZOGUL et al. (2005). Afterwards internal organs and meat (the edible parts) were removed from the shells using hooks and the specimens were homogenized.

Biochemical analysis
A 2.0 g sample of air-dried snail meat was weighed and rounded to the nearest 0.01 g. The sample was then placed in a round-bottomed 100 ml flask and 22.5 ml of hydrochloric acid (HCI) and 7.5 ml of nitric acid (HNO_3) were added. The flask was connected to a reflux condenser and left for no less than 16 hours at room temperature, then heated gently to boiling point for 2 hours.
After cooling and flushing the condenser with 25 ml of 12.5% nitric acid, the sample was filtered and 100 ml of 12.5% nitric acid was added to the liquid phase part. The reagents used in the analysis were qualified as pure (Merck® and Fluka®). The standard solutions for ETAAS determination of Ca, P, K, Na, Mg, Fe, Zn, Mn and Cu with concentration of 1000 $mg.l^{-1}$ were supplied by Merck (Darmstadt, Germany). Double-distilled water was used for all procedures.
The samples were analyzed with Perkin-Elmer AAnalyst 800 atomic absorption spectrometer (Norwalk, CT).

The samples were prepared according to AOAC method 983.18 (2006) and subjected to moisture analyses using air drying AOAC method 950.46 (1997). Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content was determined by the method of the Soxhlet using an automatic system (Soxtec 2050, FOSS, Sweden). Crude ash was determined by incineration in a muffle furnace (MLW, Germany) at 550^{o}C for 8 h. Crucibles were brought about the room temperature and weighted.

Statistical and nutritional analysis
Statistical analyses were performed using StatSoft STATISTICA v.12. The accuracy of the measurements was assessed by mean and standard error of mean (Mean ± SEM). The obtained nutrient values were compared to other foods from the USDA Food Composition Databases, available online. Dietary reference values were taken from the latest report by the European Food Safety Authority (EFSA, 2017).

Results and discussion

The results of the proximate analysis of snail meat are presented in Table 1. Similar to other aquatic products (e.g. fish, scallops and frogs) snail meat is a good source of protein, while simultaneously containing very low amounts of fat (ATANASOV et al., 2009; ÇAĞILTAY

et al., 2014). The obtained values are comparable with published reports on other snail species (ÖZOĞUL et al., 2005; ÇAĞILTAY et al., 2011). It has been noted in many studies that nutritional composition may change between species. Factors like location, collection methods, seasonal features and feed composition are all important contributors to nutritional value (MILINSK et al., 2006, NICOLAI et al., 2011).

Table 1. Macronutrient and mineral composition of snail meat (*Helix lucorum*)

Element	Technique	n	Mean ± SE
Moisture (%)	AOAC 950.46	20	76.10±0.31
Crude protein (%)	AOAC 988.05	20	13.56±0.22
Fat (%)	AOAC 960.39	20	0.40±0.03
Ash (%)	AOAC 942.05	20	1.61±0.05
Calcium (mg/100 g)	ETAAS	20	159.3±0.28
Phosphorus (mg/100 g)	ETAAS	20	102.2±0.18
Potassium (mg/100 g)	ETAAS	20	94.3±0.11
Sodium (mg/100 g)	ETAAS	20	87.6±0.13
Magnesium (mg/100 g)	ETAAS	20	38.0±0.11
Iron (mg/100 g)	ETAAS	20	1.43±0.04
Zinc (mg/100 g)	ETAAS	20	1.99±0.02
Copper (mg/100 g)	ETAAS	20	3.17±0.05

Similar to fish meat, our study suggests that snails are also rich in minerals. In *Helix lucorum* the highest concentration was found for calcium, followed by phosphorus, potassium, sodium and magnesium. The mineral content of snail meat is known to change based on location, season and feeding practices (ÖZOĞUL et al., 2005).

Calcium is an essential micronutrient and amounts for about 2% of the body weight in a healthy human (ÖKSÜZ, 2012). Similar amounts of calcium detected in *Helix lucorum* meat are found in cod, sesame seeds and spinach. The population reference intake (PRI) for calcium is 950 mg/day for people over 25 years of age (EFSA, 2017). The high concentrations found in all samples shows that snail meat can be a good source of calcium.

Phosphorus is a structural component of hard tissues such as bone and scales, as well as a constituent of various coenzymes, phospholipids and nucleic acids (ATANASOFF et al., 2013). Snail meat is a rich source of phosphorus, similar to the amounts found in milk and veal. The adequate daily intake of phosphorus for adults is set at 550 mg/day.

The adequate intake of magnesium is set at 350 mg per day for adult males and 300 mg per day for females. Magnesium levels in the studied snail meat are similar to other molluscs such as squid, and fish (carp, bass).

The zinc levels were similar to values reported by GEORGIEV and ATANASOV (2011) and ÇAĞILTAY et al. (2011) for *Helix spp.* meat, and close to the amounts found in pork, veal and chicken. Since PRIs for zinc range between 7.5 and 16.3 mg daily for adults, *Helix lucorum* meat can be a valuable source of zinc in the diet.

Iron deficiency anemia is one of the major micronutrient deficiencies worldwide, significantly affecting women and children (MCLEAN et al., 2009). Among the important nutrition policies is to provide affordable heme iron rich foods to vulnerable population groups. Snail meat can be used as an alternative source of iron in the diet, because the obtained values for iron were comparable to levels in popular meats, i.e. lean beef, lamb, turkey, herring and sardines. The population reference intake for iron varies between 11-16 mg/day depending on the physiological status. Therefore, 100 g of *Helix lucorum* meat can attribute to 9-13% of the required iron intake. Possible disparities in iron levels can be

attributed to variations in iron content of the soils in which the snails were raised (WOSU, 2003).
Copper is essential for good health but very high intake can cause adverse health problems such as liver and kidney damage (IKEM and EGIEBOR, 2005). Our results indicate that consumption of just 100 g of snail meat can provide double the daily requirements for copper (1.3 mg for adult women and 1.6 mg for men).

Conclusion and recommendation

Results of this study reconfirm the nutritional status of farmed *Helix lucorum* meat, which could be used as valuable food source for humans. Information campaigns should popularize the consumption of farmed snails instead of wild snail varieties, thus preserving wild populations at risk.

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Proximate and nutritional profile of raw and cooked of snail meat (*Helix lucorum*)

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Abstract

Proximate content (dry matter, protein, fat, ash) and nutritional profile (pH, color, cooking loss, water holding capacity, tenderness, drip loss) of the edible portion of forty Turkish snails (*Helix lucorum*) were assessed in this study. Snails growing in natural conditions were collected in May of 2017 in region of Trakia valley, Bulgaria. Samples were taken from the meaty part of snails and were investigated. Proximate analysis was carried out following the methods of the Association of Official Analytical Chemists (AOAC), while nutritional profile was determined by cooking methods. The results from the analysis showed that snail meat is rich in protein (162.3±2.8 g) and low in both fat (20.5±0.2 g) and ash (1.05±0.3 g). The present work illustrates that the water holding capacity, the cooking loss after heat processing and drip loss were decreased. The results of this study have showed that snail meat (*Helix lucorum*) is good sources of protein and traditional cooking had not considerable effect on nutritional profile.

Keywords: nutritional profile, proximate composition, snail meat

Introduction

Traditionally consumers are interested in snail meat, and in different European countries it was used as an exclusive food since ancient times. In 2012, world snail market recorded a turnover of 10 billion euro with consumption of snails of about 400,000 tons per year, most of these snails were collected from nature in Eastern European countries (Poland, Romania, Bulgaria), while only 55000 tons were produced in the snail farms (TOADER, 2011). Helix spp., has been the principal subject of studies related to snail culture methods (ÇAĞILTAY et al., 2014). Economically important and edible species (in brackets are their commercial names) are: *Helix aspersa* also known as *Cantareus aperta* (brown garden snail), *Helix pomatia* (Roman snail), and *Helix lucorum* (Turkish snail). The last of them is with a large range of distribution extending from Iran in the east to Italy in the west (KORÁBEK et al., 2014). Recently has spread over many places beyond its natural distribution, including Spain, England, Czech, Slovak Republic and Russia (ČEJKA and ČAČANÝ, 2014). Being one of the largest snail species, it is sensitive to the environment and habitat modification, who can be a good model to analyze protection effectiveness (ARZUMANYAN, 2016).
Data on the biological value of meat composition and mineral content are insufficient. Generally, proximate analyses of wild snails are considered to be highly nutritious, owing to its content of essential amino acids, proteins, rich vitamins and major minerals required for a healthy and well balanced diet (ÖZOĞUL et al., 2015).

The aim of performed study was to enhance snail conservation included in IUCN Red List and to evaluate the proximate (dry matter, protein, fat, ash) and nutritional (pH, color, cooking loss, water holding capacity, tenderness, drip loss) profiles of the endemic snail species meat.

Material and methods

Sample preparation
The 100 snails examined came from the regions of Trakia valley, South-Eastern Bulgaria. One hundred adult snails were selected and collected in May 2017 during their activity period from May to June by hand-picking.

Proximate analyses
The samples were prepared according AOAC (2006) and subjected to moisture analyses using air drying. Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content was determined by the method of the Soxhlet using an automatic system (Soxtec 2050, FOSS, Sweden). Crude ash was determined by incineration in a muffle furnace (MLW, Germany) at 550^{o} C for 8 h. Crucibles were brought about the room temperature and weighted.

Physicochemical analyses
For evaluation of meat quality: meat pH, the colour characteristics (L*, a* and b*), drip loss percentage, hydrophilic properties of meat and cooking loss percentage were determined. The pH of meat was measured using a pH meter. (Testo 205, Testo, Germany), equipped with glass electrode and a temperature probe.
The colour of meat was determined on the 2nd post mortem hour with spectrophotometer (Lovibond SP60, X-Rite Inc., USA). The CIE L*, a* b* colour space included the following colour coordinates: lightness (L*) – ranging from 0 (black) to 100 (white); red/green coordinate (a*): + a* indicating redness and – a* indicating greenness; yellow/blue coordinate (b*): +b* indicating yellowness and – b* indicating blueness (EN 15886:2010).
Drip loss (%) was estimated as described by ROTH et al. (2006). The obtained meat samples were weighed, wrapped in aluminium foil and put in a refrigerator at 4° C. After 1 days of storage, the samples were unwrapped, cleaned of the excess fluid and weighed again.
The hydrophilic properties of meat were determined through the parameter water and cooking loss percentage. The analysis was performed by the classic method of GRAU and HAMM (1956).
Cooking loss percentage of meat was determined in a forced convection oven. The method is based on achievement of a temperature of 75-80^{o} C in the core of the sample for 15 min.
Statistical analyses were performed using STATISTICA v. 6.0 (2001). The accuracy of the measurements was assessed by mean and standard error of the mean (Mean ± SEM).

Results and discussion

The results of proximate analysis of snails are shown in Table 1. The present values of this study, in agreement with our previous published report (ZAPRYANOVA et al., 2015). NOVELLI et al (2002) reported values for *Helix lucorum* from the different regions of Italy (Cagliari, Caserta, Cuneo, Lecce, Modena, Ravenna and Verona) – 80,90% moisture, 14,90%

protein, 0,5% fat and 2,3% ash respectively. In another study with *Helix lucorum* from the regions of Kastoria, Drama, and Pella in Northern Greece and from Albania, content of protein 18,10%, and fat between 2,9-3,2% were reported (ARVANITOYANNIS et al., 2011). It has been noted in some of the studies that nutritional composition of Turkish snail may change between regions. Factors like collection method and location, season and kind of feed are important factors for the nutritional compositions of edible part of snail meat (MILINSK et al., 2006).

Table 1. Proximate composition of the flesh of snail meat (*Helix lucorum*)

Parameters	Technique	Mean±SEM
Moisture	AOAC 950.46	742.0 ± 0.3
Protein	AOAC 988.05	162.3 ± 2.8
Fat	AOAC 960.39	20.5 ± 0.2
Ash	AOAC 942.05	1.05 ± 0.3

The results of meat chemical investigation are presented in Table 2. The color of meat is an extremely important factor because it is deemed a visual measure of estimating meat quality. In general, measurement of L* value is easier and faster than pH. The obtained values of snail meat colour are comparable to those published by ZYMANTIENE et al., (2008), and considerably higher than values obtained of JUKNA and VALAITIENĖ (2012), that could be explained with influenced by age and diet.

The pH value in snails' meat is close to that in animals' immediately after the slaughter process and reserves within these limits. This is also related to the fact that when vital functions in snails are stopped fermentation processes after slaughter are not observed lactic acid (ZYMANTIENE et al., 2008). In agreement with the findings in other studies (ZYMANTIENE et al., 2008; OKONKWO and ANY AENE, 2009) the higher value of pH was found in snail meat (7.65±0.3). This result confirmed that pH is associated with meat colour in a way that lighter muscles (L*>50) have higher pH values than darker (L*<45) ones.

Table 2. Nutritional profile of snail meat (*Helix lucorum*)

Physicochemical parameter	Mean±SEM
Colour L*	53.10 ± 1.83
Colour a*	5.97 ± 0.11
Colour b*	16.38 ± 0.13
pH	7.65 ± 0.30
Water-holding capacity, %	71.64 ± 5.10
Drip loss, %	0.85 ± 0.23
Cooking loss, %	41.63 ± 4.90

The relationship among pH and snail meat quality has been reported by several researchers (GRUJIĆ et al., 2010; EBUNOLUWA et al., 2015). Water-holding capacity and drip loss of meat is greatly affected by pH. Water-holding capacity is defined as the ability of meat to retain its water during application of external forces. The meat with very high value of pH (> 6.3) tends to have a very high water-holding capacity. The water-holding capacity of collected snail was about 71.64±5.1 %, similar to cultured and wild snails reported by EBUNOLUWA et al., (2015).

One of the most important technological parameters is cooking loss. However, heat treatment can lead to undesirable modifications, such a decrease in the nutritional value, mainly due to vitamin and mineral losses, and changes in the fatty acid composition due to lipid oxidation (GERBER and SCHEEDER, 2009). The cooking loss in the samples analyzed ranged from 39.34% to 46.20% with a mean of 41.63±4.9% within the range reported for *Helix spp.* (JOKANOVIĆ et al., 2006).

Conclusion and recommendation

This investigation provides practical and useful information on the chemical composition for one of most popular breed of snail in Bulgaria. It can be concluded that this study contributes to a description of the proximate and nutritional profil of snail meat which could be use to extend existing information. These results will be important for the nutritionists and researchers for improving processing. It is also helpful for similar academic studies and to prepare tables of compositions of food.

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Phenotypic characteristics of the Tsigai sheep breed in Vojvodina - North Serbia

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Abstract

Tsigai is an indigenous breed of sheep in AP Vojvodina (northern part of Serbia), with combined capacities, used for milk, wool and meat production. It originates from the Asia Minor sheep, and it has spread towards Eastern Europe. It is believed that first animals came to Vojvodina from Romania (also part of Austro-Hungarian Empire), in the eighteenth century, (KRAJINOVIĆ, 2006). Tsigai is a big breed of sheep, characterized with strong body consistency. The trunk is medium in size but deep, narrow and rectangular. The chest is deep and narrow. The withers are bit shorter than the loins and the spine line is straight. The head is medium in size, but quite narrow. Tsigai sheep have no horns (male animals rarely). The ears are large and often clumpy. The head and ears are covered with black or brown hair. The legs are long and strong, covered with black or brown hair. The body weight of adult ewes is 70-75 kg, while rams weigh 110-120 kg on average.
Research was conducted on 95 sheep (46 animals of regular variety (T) and 49 of Čoka variety (ChT)) on two farms. Following measurements were done: body mass, withers height, back height, rump height, length of the trunk, cannon bone circumference, length of the chest, depth of chest, width of the chest, chest circumference, length of the head, width of the forehead and length of the ears. The following indexes were calculated: Format index (length of the trunk/withers height) x 100 (111.67 for T and 110.53 for ChT; Chest index (chest width/depth of chest) x 100, (68.53 T and 62.13 ChT); Chest depth index (depth of chest/withers height) x 100, (45.75 T and 51.90 ChT); Body Compactness Index (chest circumference/length of the trunk) x 100, (124.24 T and 126.58 ChT); Massiveness index (chest circumference/withers height) x 100, (138.76 T and 139.77 ChT); Body mass index (body mass/withers height) x 100 (110.78 T and 90.33 ChT); Body height index (rump height/withers height) x 100, (96.13 T and 98.58 ChT); Leg length index (withers height - depth of chest)/withers height)) x 100, (54.24 T and 48.09 ChT); Bone mass index (cannon bone circumference/withers height) x 100, (12.76 T and 11.67 ChT); Head width index (max. head width/head length) x 100, (79.96 T and 62.12 ChT).
By observing all the absolute measures and calculations of the Tsigai, we can say that breed characteristics are: big size body, straight dorsal line, with chest relatively wide and long but not very deep, elongated and large head with big clumpy ears. Typical exterior is inclined to the type of sheep for meat production. Considering the fact that milk yield of that breed can reach 150 liters in the 6 months lactation, some varieties with lower bone mass index are suitable for dairying.

Key words: Tsigai, exterior, indexes

Early determination of gender in Siberian sturgeon (*Acipenser baerii*) using ultrasound and biopsy techniques

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Abstract

In total there are only 6 different species of sturgeon in the Danube River as 5 of them are critically endangered. Traditionally, the caviar on the market is comes from Beluga, Russian or Stellate sturgeons in compare with caviar from aquaculture which is often yield from Siberian sturgeon or hybrids. Determination of the gender in sturgeon is very important in fish farmers, as sex is one of the main factors that determine cultivating them for caviar or meat the future. One of the best noninvasive technique for maturation monitoring is ultrasound. In combination with invasive methods such as gonads histology and sex hormone analysis, it is possible with great credibility to be identify sex. On this matter we set an aim to study the early and precise gender determination of Siberian sturgeon. Gender of 600 sturgeon were identified through non-invasive examination was conducted in farm conditions. To avoid additional injury and to obtain normal ultrasound image, fish were manual fixed. The abdominal exams were done in dorsal recumbency using transabdominal two-dimensional echography (UProbe 3C Series, Sonostar Co., China). Gonadal biopsy was performed with ultrasonography guidance making it possible to perform the biopsy with one hand while holding the ultrasound probe with the other. Materials for histological observation were fixed in 10% neutral formalin solution and processed by classical histological techniques. Determination of gender was successfully performed in all sturgeons. The testis exhibited a moderate homogenous density, appearing echographically with moderate rough granular hypoechoic pattern. The ultrasound image of ovaries were distinguished from those of male sturgeon with the strongly heterogeneous and hypoechogenicity structure. Histological section of Siberian sturgeon testis' showed maturity stage V and female were at the pre-vitellogenic stage (Stage I). In conclusion, ultrasound examination, can be a powerful tool in early determining the gender of Siberian sturgeon (*Acipenser baerii*) and could help sustainable protection the species on future.

Key words: biopsy, caviar, determination of gender, sturgeon, ultrasound

In memoriam Professor Condrea Crisante Valeriu Drăgănescu

(1927-2018)

Prof. Drăgănescu was born in December 2nd, 1927 in a small village in the Meridional Carpathian Mountains (Traisteni-Prahova). He graduated the Secondary school in Brasov and Military School Predeal, and Bucharest Zootechnical Faculty (1953, PhD 1970). A fortunate context allowed him to continue his studies at the Timiryazev Academy in Moscow (3 months in 1962), and further at the Animal Breeding Research Organization (ABRO) in Edinburgh (1966-1967). In 1955 he was appointed research assistant at the Department for Animal Husbandry of Bucharest Agricultural University where he continued to work until his retirement, as professor, in 1992. However, he continued his research and teaching activities after retiring. He was founder and representative of the new school in the field of animal husbandry higher in education and scientific research in Romania.

Prof. Condrea Drăgănescu, Honorary Professor at the Bucharest Agricultural University, was a full member of the Romanian Academy of Agricultural and Forestry Sciences. Previous positions included a visiting professorship at the Lubumbashi-Zaire University (1974-1976), a FAO National Coordinator for the Conservation of AnGR (1994-2008), and a member of the National Commission for Agricultural Academic Evaluation (1993-2001).
From 1955 to 1964 his work was based on the genealogical method of Lehndorf. After 1964 he used the research methodology established by Wright and Lush (1923-1931), including the identification of the breeds' reproductive isolation, the interval between generations, relationship, inbreeding, selection effects etc.
While science has been his main passion, Prof. Drăgănescu has always had a great interest in sustainable education, agriculture, social, even rural development. His publication record includes over 115 research articles and 19 book (e.g. Genetic History of Animal Breeds). In these papers he proposed ways for improving the breeds and for conserving them as well.

He played a significant role in the restoration of the genetic sciences in Romania following the years of Soviet-imposed Lysenko-Michurinism, but his role in this change has only been recognized in 1996, with a prize of the Romanian Academy for his "Work on Animal Genetic Resources". He was an active cooperator of Balkan Scientific Association and, thanks to Professor Imre Bodó, an active cooperator of DAGENE (1994-2010).

Professor Drăgănescu passed away in December 23rd, 2018. The lively man, our friend, who was driven by his quest to understand the mechanism of genetic evolution is no longer among us, but his attitude lives with us for a long time.

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