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RELATIONSHIP BETWEEN THE GENETIC HEMOGLOBIN POLYMORPHISM, MORPHOMETRY AND FERTILITY OF PRAMENKA SHEEP BREED FROM CENTRAL BOSNIA

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Pramenka sheep belong to the group of primitive sheep breeds with a triple-purpose production: milk, meat and wool. None of these production directions is emphasized. Sheep of this breed are the reflection of the environment in which they are located. The characteristics of these sheep are highlighted depth measures of external appearance with modest width measures. Another weaker feature of Pramenka sheep is poor fertility. Despite the mentioned disadvantages, Pramenka sheep is the most grown sheep in Central Bosnia and sheep production is based on it. Methodical selection to improve the characteristics of Pramenka sheep has not applied. However, sheep breeders tend to improve the qualitative and quantitative characteristics of sheep, and that means more intensive work on the selection. In this sense, genetic markers are used to carry out the selection lately. Three types of hemoglobin, HbAA, HbAB and HbBB of Pramenka sheep population (at 189 male and female animals) in Central Bosnia were segregated by the method of electrophoresis. The following genotype frequencies are determined: HbAA 0.11; HbAB 0.41; and HbBB 0.48. Allele frequencies, HbA and HbB for Pramenka sheep breed (estimate based on genotype frequencies) were 0.315 and 0.685. It was found that the population of sheep was in the equilibrium of the frequency of hemoglobin genotypes. Sheep with HbAA genotype had lower morphometric measures in relation to the other two genotypes. The statistically significant difference between sheep with HbAB genotype and HbAA genotype was recorded only for the shin perimeter. The ewes that lambed one lamb have the following frequency of HbAA, HbAB and HbBB genotypes: 0.13; 0.40 and 0.47, and the ewes with twins: 0.02; 0.40 and 0.58. Fertility, as important quantitative

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characteristic of sheep, was more emphasized in genotype *HbBB*, than in *HbAA* genotype. This was confirmed by the statistical analysis.

Key words: fertility, hemoglobin, morphometric characteristics, Pramenka sheep, selection

INTRODUCTION

Sheep that have different hemoglobin polymorphisms react differently to natural conditions in which they are located. Sheep that have HbAA genotype are more tolerant to unfavorable environmental conditions than sheep with HbBB genotype. For this reason, it is necessary to examine the effect of the hemoglobin polymorphism on qualitative and quantitative characteristics, especially on morphometric characteristics and fertility of sheep. Reproductive characteristics, of which the most important is fertility of sheep, have a low heritability and therefore it is poorly repaired by selection work. Improving reproductive performance with increasing success is carried out by farmers with the family and within family selection. Fertility is quantitative characteristic with additive (cumulative) inherited, and it is greatly influenced by external factors. Genetic markers and the possibility of genetic modification have been used in recent researches in various fields of biotechnology and reproduction. This provides new approaches to characterization, conservation and sustainable use of animal genetic resources (AnGR), (GAMA and BRESSAN, 2011; IVANOV et al., 2011). Until recently, breeding and selection methods in livestock production were based mainly on the principles of population genetics. The basic principle of such selection was carried out the selection of individuals based on their phenotypic performance, or an external manifestation of some production properties (BALENOVIĆ et al., 2007). Modern selection of domestic animals most applies the genetic markers.

Using genetic markers for important production characteristics and external appearance represents a great progress in breeding of domestic animals. This is because the selection of animals can be made at an early age, before the animal begins to produce itself. If the selection is performed through the animal's progeny, it is not necessary to wait for the results of the production of offspring, in order to obtain the results of the breeding. In addition, the use of genetic markers in breeding animals provides a cheaper animal selection process. In domestic animal breeding two types of genetic markers are using: 1. determination of the polymorphism of proteins in the blood and milk, and 2. reading of the structure and function of individual genes and non-coding regions of DNA. Using techniques of molecular genetics, the preferred alleles of parents and offspring should be noted, and through, based on the results we can directly select individuals for future reproduction (IVANKOVIĆ, 2005; FADIEL *et al.*, 2005; HU *et al.*, 2007).

Genetic improvement of sheep is the base for sustainable development of this branch of animal husbandry. Phenotypic characteristics of sheep are influenced by various genetic and environmental factors (CARO-PETROVIĆ *et al.*, 2013). The aim of this study was to determine the types of hemoglobin of the Pramenka sheep breed in Central Bosnia and relationship of hemoglobin type with morphometric characteristics and fertility of sheep.

MATERIALS AND METHODS

Hemoglobin polymorphism of Pramenka sheep in Central Bosnia was determined in 189 blood samples. Blood samples were taken from different strain of Pramenka sheep (Dubska, Privorska and Kupreška) from ten locations where the sheep breed is grown. Those strains belong to the group of larger strains of Pramenka sheep. Blood samples were taken by puncture of the

jugular vein. As an anticoagulant in test tubes was used heparin. The blood plasma is separated after centrifugation of the samples at 3000 rotations/minute for ten minutes, and the erythrocytes were washed with physiological solution and haemolysed by freezing.

Determining the hemoglobin types was performed by electrophoresis on a 12% starch gel in a continuous buffer system Tris-borate-EDTA, pH 8.5. Interpretations were made based on the relative mobility of the hemoglobin bands towards the anode, with hemoglobin AA (single band) being the fastest while hemoglobin BB (single band) was the slowest and hemoglobin AB (double band) having slow and fast bands (RIKEN, 2006; AKYNYEMI, 2010).

Calculation of gene frequencies was done by forms:

Of gene A; AA + AB
$$\frac{1}{2}$$
 (1)
Of gene B; BB + $\frac{1}{2}$ AB (2)

It was determined whether the population is in equilibrium, on the basis of the calculated gene frequency, using the Hardy-Weinberg's law, according to the formula:

$$p^{2}AA + 2pqAB + q^{2}AB \qquad (3).$$

Data were grouped according the hemoglobin polymorphism after calculating the frequency of genes and genotypes and calculated equilibrium (χ^2). Sheep with a different type of hemoglobin (*HbAA*, *HbAB* and *HbBB*) were mutually compared. The eight, most important, morphometric traits were determined: withers height, body length, chest depth, shoulder width, chest perimeter, hip height, hip width and shin perimeter. Measuring of the height, length and width were taken by Lydtin's stick, and the scope was taken by ribbons. The data were analyzed using the method of variance analysis with unequal number of repetitions. Statistical Programme STATISTICA 10, STATSOFT was used for statistical analysis. After this, the sheep are assorted with one or two lambs and genotype frequencies of hemoglobin were calculated and compared using the test differences between the proportions of the two samples.

RESULTS AND DISCUSSION

Pramenka sheep breed belong to the group of primitive sheep breeds with a triple-purpose production: milk, meat and wool. None of these production directions is emphasized. Sheep of this breed are the reflection of the environment in which they are located. The characteristics of these sheep are highlighted depth measures of external appearance with modest width measures. This is the main deficiency of this breed and it should be repaired by the selection. Besides the morphometric measures of Pramenka sheep and fertility should be fixed by selection. Using hemoglobin polymorphism in the selection purpose is simply because it is the easy way to determine the type of hemoglobin and connect it with certain characteristics of animals. Hemoglobin carries out important functions in the organism of animals, and for these reasons it is essential blood protein which polymorphism can be linked with certain morphological and production characteristics of animals.

It was pointed out that one of the major blood protein is hemoglobin, constant current red (evergreen) protein (BETTATI *et al.*, 2009), which attracted attention because of its biochemical, biophysical and physiological characteristics and which is of great importance for the selection of animals (HRINCE, 2008; YAKUBU and AYA, 2012).

The frequencies of hemoglobin genotypes are shown in Table 1. The largest number of animals of Pramenka sheep population in Central Bosnia belong to HbBB genotype (frequency 0,48) and the smallest number to HbAA genotype (frequency 0,11). Sheep with homozygous HbBB genotype and heterozygous sheep with HbAB genotype caused a greater frequency of HbB gene, which amounted to 0,685, while the frequency of *HbA* genes was of 0,315. It has been shown that animals that are found at higher altitudes have a higher frequency of hemoglobin HbAA genotype. The reason for this is that HbAA genotype has higher binding affinity to oxygen as compared to other genotypes (STORZ, 2010; CHEVIRON and BRUMFIELD, 2011). The reason for the higher frequency of HbB gene compared to HbA gene in the population of Pramenka sheep in Central Bosnia can be the ways of sheep migration during the year and the influence of other breeds of sheep that in their genetic basis have a greater frequency of HbB gene. The calculated value of χ^2 test indicates that the population of Pramenka sheep in Central Bosnia is in the genetic balance or equilibrium. Pramenka sheep, according to their appearance, is the reflection of the environment, which it is located and has all the morphometric and production characteristics of primitive breed. Selection methods in order to improve its qualitative and quantitative traits have not been applied. Table 2 provides information on the sheep average morphometric indicators of different hemoglobin types.

Type of Hb	Number of Hb types		Frequency of Hb genotypes		χ^2		Frequency of	
	Obtained	Expected	Obtained	Expected	Calculated	Table value	Hb genes	
AA	21	19	0.11	0.09			A = 0.315	
AB	77	82	0.41	0.44	0.60	5.97	B = 0.685	
BB	91	88	0.48	0.47				

Table 1. Frequency of hemoglobin genotypes and genes of Pramenka sheep breed in Central Bosnia

Table 2. Morphometric measures of Pramenka sheep and the results of F and t – test

	Morphometric measures (average values in cm)									
Type of		Withers	Hip	Body	Chest	Shoulder	Hip	Chest	Shin	
hemoglo	bin	height	height	length	depth	width	width	perimeter	perimeter	
AA	A	71.14	72.71	73.48	32.76	21.52	20.95	91.76	8.48	
AI	3	72.77	73.04	74.69	33.75	22.05	21.31	95.15	9.06	
BI	3	72.30	73.10	74.46	33.45	21.92	21.32	94.34	8.85	
F calcula	F calculate		0.08	0.98	2.10	0.59	0.59	2.00	3.66	
E table	0,05	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	
F-table	0,01	4.61	4.61	4.61	4.61	4.61	4.41	4.61	4.61	
									1.54	
t calculat	t calculate		NS	NS	NS	NS	NS	NS	2.61**	
									1.67	
4 1	0,05	1.973	1.973	1.973	1.973	1.973	1.973	1.973	1.973	
t-value	0,01	2.603	2.603	2.603	2.603	2.603	2.603	2.603	2.603	

Data in Table 2 indicate that the hemoglobin type of sheep has a certain relationship with morphometric measures and external appearance of sheep. Five of the eight monitored measures, with *HbAB* hemoglobin type were higher compared to the other two types of hemoglobin. Sheep with *HbBB* hemoglobin had higher three morphometric measures compared to the other two types of hemoglobin (*HbAA* and *HbAB*). Sheep with *HbAA* hemoglobin type had neither bigger morphometric measure. In general, larger morphometric characteristics in heterozygote can be confirmed by the rule that heterozygous are more vital and more productive in relation to both types of homozygote. Lower morphometric measures of sheep with *HbAA* hemoglobin compared to the other two types can be found in the fact of greater resistance and adaptability of sheep. Sheep with *HbAA* hemoglobin type are found on higher altitudes in unfavorable living conditions. Those for many years shaped the look and production characteristics of sheep. Unfavorable feed and accommodation have created a smaller type of sheep, which coincides with the *HbAA* hemoglobin type.

The only morphometric measures that showed statistically significant differences between sheep of different types of hemoglobin is the shin perimeter. Through these measures can be determined the development and strength of the skeleton. Data on relatively skeletal development is obtained if the ratio of the shin perimeter and withers height is calculated. This is very important trait in the assessment of the animal. We found significant difference in shin perimeter between sheep with *HbAB* and *HbAA* hemoglobin types (Table 2). At the same time, there was no difference between sheep with the *HbBB* hemoglobin type on the one side and sheep with *HbAA* and *HbAB* hemoglobin types on the other hand. Sheep with *HbAB* hemoglobin compared to the other two types of hemoglobin have a stronger skeleton, which is very important fact for resistance and future sheep production. Other studies have shown that the hemoglobin types are linked with the certain performance of animals (BEZOVA *et al.*, 2007; BOONPRONG *et al.*, 2007). SUŠIĆ *et al.* (1993) investigated the polymorphism of hemoglobin of F2 generation crossbreeds between the Istrian and Sardinian sheep. Their results showed that genotypes AA and AB have selection advantage over the BB genotype for the body length, withers height and chest depth.

In Nigerian sheep breed wool fiber and horn length were higher in sheep with HbAA hemoglobin type compared to HbBB type (AKINYEMI and SALAKO, 2010). HRINCA and VICOVAN, (2011) reported that the most valuable characteristics of wool fiber: quality, shape and shine are connected with the *HbAB* hemoglobin type. At the same time, the weakest links between the characteristics of wool fibers were found for HbBB hemoglobin type. IYIOLA-TUNJI et al., (2014) reported that lambs that are born to mothers with HbAB hemoglobin type had a higher survival rate compared with lambs born to ewes with two other hemoglobin types. Those authors suggested that for further reproduction should be left the individuals with *HbAB* hemoglobin type, because the lambs of such ewes are resistant and more vital. SAM (2012) investigated the goats and reported that the Nigerian Red Sokoto goats (living in the north-eastern part of Nigeria) that have HbAA genotype have better morphological characteristics than the goats with the other two types of hemoglobin. The positive relationship of body mass and heart volume with animals that have HbAA genotype was found in West African dwarf goats in the central Nigeria Other genotypes had no significant impact on other morphological measures of the body, such as: withers height, hip height, body length, face length, ear length, horn length, the length of the pelvis and pelvic width (ABDULMOJEED et al., 2014). The mentioned African goat breed had a higher frequency of HbA gene compared to HbB gene. More favorable morphometric measures in goats with HbAA

genotype are expected because of the natural environment, which is usually harsh and which favors individuals with *HbAA* genotype.

Fertility study these sheep have shown satisfactory results. Hemoglobin genotyping was conducted on a total of 163 sheep. Out of that number 42 sheep have given birth to two lambs.

Туре		Ewe with one lamb	ıb		Ewe with two lat	bs
of Hb	No of animals	Genotype frequency	Gene frequency	No of animals	Genotype frequency	Gene frequency
AA	16	0.13	A=0.33	1	0.02	A = 0.22
AB	48	0.40		17	0.40	
BB	57	0.47	B=0.67	24	0.58	B = 0.78

 Table 3. Hemoglobin polymorphism of the lambed ewes

The ewes that lambed single lamb had a higher frequency of *HbAA* genotype and lower frequency of *HbBB* genotype compared to ewes that lambed two lambs (Table 3). The frequency of heterozygous *HbAB* genotype in both groups of sheep was similar and amounted to 0.40. In addition, the frequency of *HbA* gene was higher in sheep with single lamb. However, this was not the case for the frequency of the *HbB* gene, whose frequency was higher in sheep with two lambs. Fertility is quantitative characteristic with a low heritability, and therefore the implementation of selection on improving reproductive traits is difficult. The best results in improving reproductive traits is achieved using the family or within family selection. Males and females from families with a larger number of offspring are left for breeding. The introduction of genetic markar is a new method of selection that simplifies the selection on quantitative traits. Using of genetic markers facilitates the selection and increases the frequency of the fertility genes.

No of lambs	Genotype frequency AA	Test of proportion of AA genotype	Genotype frequency AB	Test of proportion of AB genotype	Genotype frequency BB	Test of proportion of BB genotype	Limit value of the proportion test
Single lamb	0.13	3.33*	0.40	NG	0.47	2.04*	1.96
Two lambs	0.02		0.40	NS	0.58		

Table 4. Results of the test of proportion of genotype frequency of the sheep with single and two lambs

The ewes with two lambs had a higher frequency of *HbBB* genotype in relation to ewes with single lamb (Table 4). The sheep with single lamb had higher frequency of *HbAA* genotype. Using direct and indirect genetic markers is primarily important for the implementation of an adequate selection. Providing recommendations for the use of appropriate genetic markers in breeding programs would allow choice of appropriate individuals at an early age, before the manifestation of economic important production traits. The study of genomes, particularly Pramenka sheep breed is an interesting project first of all to obtain answers to question whether the

fertility of this sheep was caused by genetic factors or the influence of external factors, primarily, nutrition. Studies in rabbits have shown that individuals with *HbAB* hemoglobin type had a better average litter weight and weight at weaning and post-weaning period than it was in animals with *HbAA* hemoglobin type (CHINEKE *et al.*, 2007). Using polymorphism of proteins in the selection purpose has been studied in other species of domestic animals especially in cattle. LUKAČ *et al.* (2014) had investigated the connection between genotypes transferrin and production traits of Holstein Friesian cattle in Vojvodina region and noticed that the cows with *AD2* transferrin genotype had better results for milk production and milk fat. Cows that are heterozygous for the transferrin gene had a higher milk yield and milk fat compared to the homozygous genotype cows. In recent years interest in the detection and characterization of genetic markers is increased, as well as studying their connection with certain quantitative characteristics, in particular with milk production in cows (BAGNATO *et al.*, 2008; MILANESI *et al.*, 2008). The situation is similar in the characterization of genetic markers in other animal species.

DJEDOVIĆ *et al.* (2015) investigated relationship between of polymorphism of k-casein and milk yield of cows in Serbia and they determinated that Simmental cows of AB genotype have higher milk yield (+191 kg), than AA and BB individuals. At the same time, cows with AB genotype had higher milk fat yield (+32 kg) than AA and BB animals. AB genotype crossbred animals have higher milk yield (+560 kg) than AA genotype individuals. In crossbred animals no individuals of BB genotype have been detected by BB genotypisation. The results obtained for Busha individuals showed that the BB genotype cows have lower milk yield (-149 kg) than AA and BB individuals. AB genotype cows throughout all examined breeds and crossbreds in this study had higher milk fat content in relation to AA and BB genotype, this record being confirmed by BOTARO *et al.* (2009).

CONCLUSION

An examination of the protein polymorphisms, either in blood or milk, has increasingly focused on finding relationship between genes that control protein polymorphisms and qualitative and quantitative characteristics. Determining the exact relationship between the specific protein (genotypes) with qualitative and quantitative traits improves the accuracy of the implementation of the selection process, and thus leads to improvements in livestock production. The main disadvantages of Pramenka sheep breed in Central Bosnia are poor conformation of the carcass and lower fertility. To achieve a better economic effect in sheep production, it is necessary to conduct a selection effects are obtained by combining conventional selection method with genetic marker method. The results of this study suggest that the best features of morphometric parameters had sheep with *HbAB* and the worst with *HbAA* hemoglobin type. Better fertility of Pramenka sheep breed in Central Bosnia was determined in sheep with *HbBB* hemoglobin type in relation to the other two hemoglobin genotypes. When carrying out the selection it should be taken that the quantitative characteristics are greatly influenced by external factors, which shape the activity of additive genetic components in animals.

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POVEZANOST GENETSKOG POLIMORFIZMA HEMOGLOBINA, MORFOMETRIJE I PLODNOSTI RASE OVACA PRAMENKE IZ SREDNJE BOSNE

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Izvod

Pramenka spada u grupu primitivnih rasa ovaca, nastanjuje srednju Bosnu i posjeduje trojni pravac proizvodnje: meso, mleko, vuna. Karakteristike pramenke su naglašene dubinske i skromne širinske mere trupa, kao i loša plodnost. Uprkos navedenim nedostacima, pramenka je nabrojnija ovca u centralnoj Bosni na koji se zasniva ovčarska proizvodnja. Do sada nije primjenjivana metodska selekcija na poboljšanju osobina ove ovce. Međutim, uzgajivači ovaca imaju nameru da se poboljšaju kvalitativne i kvantitativne karakteristike ovaca, a to znači da se mora intenzivno raditi na njihovoj selekciji. U tom smislu, uz primjenu poznatih vidova metodske selekcije u posljednje vreme sve se više koriste genetički markeri pri odabiru roditelja budućih generacija. Metodom elektroforeze određena su tri genotipa: HbAA, HbAB i HbBB na populaciji pramenke srednje Bosne (189 muških i ženskih jedinki). Utvrđene su sledeće genotipske frekvencije: za genotip HbAA 0,11, genotip HbAB 0,41 i genotip HbBB 0,48. Frekvencije alela HbA i HbB, koje su procenjene na osnovu genotipske frekvencije iznosile su 0,315 i 0,685. Utvrđeno je da se populacija ovaca nalazi u genetičkoj ravnoteži na frekvenciju genotipova hemoglobina. Ovce genotipa HbAA su imale niže vrijednosti ispitivanih morfometrijskih mera u odnosu na ostala dva genotipa. Statistički značajna razlika između ovaca genotipova HbAB i HbAA utvrđena je jedino za morfometrijsku meru obim cevanice. Ovce koje su ojagnjile jedno jagnje imale su frekvenciju HbAA, HbAB i HbBB genotipa 0,13, 0,40 i 0,47, a ovce sa dvoje jagnjadi 0,02, 0,40 i 0,58. Plodnost, kao važna kvanitatativna osobina kod ovaca je bila više naglašena kod HbBB nego kod HbAA genotipa.

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