



ISSN NO. 2320-5407

Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/7832
DOI URL: <http://dx.doi.org/10.21474/IJAR01/7832>



INTERNATIONAL JOURNAL OF
ADVANCED RESEARCH (IJAR)
ISSN 2320-5407
Journal Homepage: <http://www.journalijar.com>
Journal DOI: 10.21474/IJAR01

RESEARCH ARTICLE

PRELIMINARY INVESTIGATION OF HEMOGLOBIN POLYMORPHISM AND ITS ASSOCIATION WITH SOME MORPHOMETRIC TRAITS AND HEMATOLOGICAL PARAMETERS OF SHEEP AND GOATS IN NORTHEASTERN LIBYA.

Mabruka S. H. Sitmo¹ and Ahmad A. A. Saad².

1. Department of Pharmacology and Physiology, Faculty of Veterinary medicine / Omar Al Mukhtar University, Libya.
2. Department of Preventive Medicine and Public Health, Faculty of Veterinary medicine / Omar Al Mukhtar University, Libya.

Manuscript Info

Manuscript History

Received: 7 August 2018

Final Accepted: 9 September 2018

Published: October 2018

Keywords:-

hemoglobin, polymorphism, morphometric characteristics, blood parameters, small ruminants.

Abstract

Local sheep and goats in northeastern Libya were studied for hemoglobin polymorphism. The study was also aimed to unveil a possible relationship that might exist between hemoglobin genotypes, some selected morphometric traits, and hematological parameters. Both phenotypic data and blood samples were collected from a total of 69 adult sheep and 23 adult goats of both sexes. The blood parameters, hemoglobin concentration and packed cell volume values were determined and then the red blood cells were extracted, lysed, and subjected to agarose gel electrophoresis. Two variants of hemoglobin, Hb A and Hb B with a frequency of 0.22, 0.78, and 0.63, 0.37, respectively for pooled sheep and goats populations were observed; indicating the predominance of Hb B in sheep and Hb A in goats. These co-dominant alleles caused the presence of three Hb genotypes AA, BB and AB in both sheep and goats. The largest number of animals of sheep belong to Hb BB (62.32%) and the smallest number to HbAA (5.80%). For goats, the genotype AA had the higher frequency (52.17%) and the type AB was less spread (21.74). The body parameters, body long, shoulder height, chest perimeter, and horn length were not significantly influenced ($P>0.05$) by Hb variants in populations under the study. Hb genotypes also had no significant ($P>0.05$) effect on hemoglobin and packed cell volume values.

Copy Right, IJAR, 2018,. All rights reserved.

Introduction:-

Sheep and goats play an important role in food production in developing countries. The fat-tailed Barbary sheep is the typical sheep of Libya. It accounts for about 95 percent of Libyan sheep (FAO, 2003). Libyan local goats (Mahali) represent more than 90% of goat production and is concentrated along the coastal area. Other breeds like Targhai, Kardi, and Tibawi are limited to the southern regions of Libya (Akraim, 2012).

Libyan Sheep and goats have been variously evaluated for genetic variation based on physiological, productive, and reproductive features (Sitmo, 2018; Magid et al., 1992; Ahtash, 2006; Akraim, 2008; Hermas et al., 2010). However,

Corresponding Author:- Mabruka S. H. Sitmo.

Address:- Department of Pharmacology and Physiology, Faculty of Veterinary medicine / Omar Al Mukhtar University, Libya.

no available Data on morphological characterization of these species in Libya and no attempts have been made at their molecular characterization.

The polymorphic variants of different proteins, enzymes, mineral elements or blood group factors represent accuracy procedures for a better measurement of genetic variation in different animals species (Fésüs et al., 1983; Das and Deb, 2008; Chineke et al., 2007; Nigussie et al., 2016; Pal and Mummmed, 2016). One of the important blood proteins is hemoglobin that has attracted attention because of its biochemical, biophysical and physiological properties and its polymorphic variants have been reported to be associated with some performance traits and selection phenomenon of animals (Raushenbach and Kamenek, 1978; Yakubu and Aya, 2012). Phenotypic description with protein polymorphism could be a basis for selection and subsequent genetic improvement of farm animals (Hrinca, 2008). Polymorphism of protein variants remains useful because of their utility, ease, amount of genetic information accessed and simplicity of data interpretation and may be used first for populations whose genetic status is unknown to prioritize breeds to be analyzed using DNA-based technologies (Akinyemi and Salako, 2012; Mwacharo et al, 2002). Electrophoretic detection of polymorphism of gene products at structural loci provides a precise procedure to localize and prove their reliability as genetic markers for some economic traits and livestock diseases (Akinyemi and Salako, 2012). By the use of genetic markers the selection of animals can be made at an early age and this may represent a progress in breeding of livestock (Važić et al, 2017).

Two normal hemoglobins (Hb A and Hb B) exist in normal adult sheep and goats (van Vliet and Huisman, 1964; Hrinca, 2008; Ndamukon, 1995). Hb type A has been found to have a selective advantage in animals maintained at higher altitudes and this has been attributed to the greater oxygen affinity Hb A has over Hb type B (Evans and Turner, 1965; Dawson and Evans, 1966). Hb type B, on the other hand, has been demonstrated to be associated with better productive traits in animals (Dally et al., 1980; Aygün, 2016; Važić et al., 2017). The higher oxygen affinity of the allele A has been suggested to be due to its biophysical, biochemical and physiological peculiarities such saturation capacity with oxygen, dissociation curve of oxyhaemoglobin, erythrocyte load with hemoglobin, and metabolic profile of the erythrocyte (Raushenbach and Kamenek, 1978). These properties of the Hb A with its effects on some blood parameters such hemoglobin and packed cell volumes has been suggested to positively influence the resistance of animals carrying this type of hemoglobin against diseases such helminth infestation (Di Stasio, 1997).

Hemoglobin types have been demonstrated to have functional effects on hematological patterns in mammalian species. In sheep it has been reported that individuals carrying extra alpha-globin genes exhibit blood picture mimicking a thalassemia- like syndrome, while positively charged variants have been found to be related to a decreased mean corpuscular volume and hematocrit value. Moreover, Hemoglobin type A has been speculated to be associated with increased hemoglobin concentration and packed cell volume values (Pieragostini et al., 2006).

The present study aimed at determining the types of hemoglobin of the local sheep and goats in northeastern Libya, and to investigate the relationship if any between detected hemoglobin variants and some morphometric traits and hematological parameters.

Materials And Methods:-

Research animals:

The animals used for this study were obtained from three small holder flocks located in Sulunta locality in Al Jabal Al Akhdar district, northeastern of Libya. A total of sixty nine adult sheep (62 females and 7 males) and 23 adult goats (17 females and 6 males) were used. The animals were reared under extensive system of management.

Blood collection:

5ml of blood was collected from each of the sampled animals by jugular venipuncture into sample bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant. The test tubes were properly labeled, preserved in a cooler containing ice blocks and transported to the laboratory where the samples were analyzed.

Blood Preparation:

Blood samples were centrifuged at 3000 rpm for five minutes in order to remove the plasma. The erythrocyte stores were then washed three times with cold sterile 0.9% sodium chloride solution and centrifuged at 3000 rpm for five minutes after each washing to remove the saline solution. After removing the saline solution from the last washing, the cells were lysed with 1.5 volume of cold distilled water and 1 volume of carbon tetrachloride and centrifuged at

3000 rpm for 20 minutes. The clear supernatant hemolysates were decanted and placed into eppendorf tubes pending electrophoresis.

Laboratory analysis.

Determining the hemoglobin types was performed by electrophoresis on 1% agarose gel by the use of Tris-borate-EDTA buffer at pH 8.6. A sample size of ten microliters was loaded in the wells and run for 2 hour at 100V. The gels were stained with Coomassie brilliant blue G-250 and the results were taken by direct count (Riken, 2006; Dyballa and Metzger, 2012).

Morphometric traits and blood parameters.

Quantitative characters: body length, height at withers, chest perimeter, and horn length were recorded for each sampled Individuals. Hemoglobin concentration and packed cell volume values were determined by a hematology analyzer (Nihon Kohden, MEK 6410 K, Japan)...

Statistical analysis

Since only two alleles (A and B) were detected; then hemoglobin genotype and gene frequencies were estimated as follows:

Genotype frequency AA = No of AA/ No sampled x 100/1

Genotype frequency AB = No of AB/ No sampled x 100/1

Genotype frequency BB = No of BB/ No sampled x 100/1

For the estimation of Gene frequency the equation was adopted as thus;

$P = (NAA + 1/2NAB) / N$ and

$Q = (NBB + 1/2NAB) / N$

Where:

P = gene frequency for allele A

Q = gene frequency for allele B

N = total number of individuals sampled

The frequencies of the alleles were used in the Hardy-Weinberg equilibrium equation ($p^2 + 2pq + q^2 = 1$) in order to calculate the expected frequencies of the homozygotes and heterozygotes.

Data on Hb genotype frequencies were subjected to chi-square analysis to test for goodness-of-fit for observed and expected frequencies under Hardy-Weinberg equilibrium.

The mean values of the morphometric traits and hematological parameters were compared by ANOVA and T-.test between each combination of alleles, i. e. homozygous AA, heterozygous AB, and homozygous BB, in order to check the possibility of one of the combinations of alleles being a genetic marker for one of the measured parameters. Statistical Program SPSS (2010) was used for statistical analysis.

Results:-

On electrophoresis, the hemoglobin bands showed distinct movement towards anodic end of the electrophoretogram and two electrophoretically distinct hemoglobin variants were identified. The fast moving one was designated as Hb A, while the slow moving one was Hb B. Individual animals possessed either one or both the hemoglobins were accordingly designated as Hb AA, Hb BB, or Hb AB (Hrinca, 2008). All the three hemoglobin genotypes (Hb AA, Hb AB, and Hb BB) produced by the two co-dominant alleles Hb A and Hb B were observed in the present study.

Data on Hb alleles and genotypes frequencies obtained from this study were analyzed using descriptive statistical tools. In sheep, among 69 animals typed, 4 animals were of Hb AA, 22 of Hb AB, and 43 animals were of Hb BB, with a gene frequency of 0.22 and 0.78 with respect to Hb A and Hb B. Genotypic frequencies of 5.80, 31.88, and 62.32 % were observed for Hb AA, Hb AB, and Hb BB, respectively. Out of the 7 male animals sampled, one animal was of Hb AA genotype with a frequency of 14.28 %. 3 were observed to have Hb AB, and 3 were of Hb BB with a genotype frequency of 42.86 %. The gene frequency for the two co-dominant alleles A and B was 0.36 and 0.64, respectively. In the females, only 3 out of the 62 animals sampled were observed to have Hb AA, 19 of them had Hb AB, and 40 had Hb BB with a genotype frequency of 4.84, 30.65, and 64.52 %, respectively. The gene frequency for alleles A and B was 0.20 and 0.80, respectively (Table 1).

Table no 1:-Distribution of hemoglobin genotypes and genes frequencies in Sheep

Sex	Genotype frequency			Gene frequency	
	AA(%)	AB(%)	BB(%)	A	B

Male (n=7)	1(14.28)	3(42.86)	3(42.86)	0.36	0.64
Female (n=62)	3(4.84)	19(30.65)	40(64.52)	0.20	0.80
Total (n=69)	4(5.80)	22(31.88)	43(62.32)	0.22	0.78

In goats, 17 samples of females and 6 samples of males showed hemoglobin distribution Hb AA, Hb AB, and Hb BB to be 10, 5, 2, and 2, 0 4, respectively, with genotype frequencies of 58.82, 29.41, 11.77 %, and 33.33, 0, 66.67 %, respectively; and 52.17, 21.74, 26.09 %, respectively when samples were pooled irrespective of the sex. Gene frequencies for the two alleles expressed A and B were 0.73, 0.27 and 0.33, 0.67, respectively for sampled females and males goats; and 0.63, 0.37, respectively were observed for pooled goats as shown in Table 2 below.

Table no2:-Distribution of hemoglobin genotypes and genes frequencies in Goats

Sex	Genotype frequency			Gene frequency	
	AA(%)	AB(%)	BB(%)	A	B
Male (n=6)	2(33.33)	0(0)	4(66.67)	0.33	0.67
Female (n=17)	10(58.82)	5(29.41)	2(11.77)	0.73	0.27
Total (23)	12(52.17)	5(21.74)	6(26.09)	0.63	0.37

In the whole aspect of zygosity, the hemoglobin homozygotness for sheep (68 %) and goats (78%) were much more frequent than the hemoglobin heterozygotness (32%) for sheep and (22%) for goats.

To test for the conformity of the Hb locus of the flocks under study to Hardy-Weinberg equilibrium, chi square (χ^2) analysis for the differences between the observed and expected genotype frequencies was carried out. The chi-square test yielded $X^2 = 0.27$ ($P > 0.05$) for Sheep and 6.19 ($p < 0.05$) for Goats (Table 3).

Table no 3:-Observed and expected numbers of Hb genotypes in Sheep and Goats

Parameters		Genotype			Chi square	
		AA	AB	BB	Calculated	Table value
Sheep	Observed	4	22	43	0.27	5.99
	Expected	3.3	23.5	42.3		
Goats	Observed	12	5	6	6.19	
	Expected	9.2	10.6	3.2		

Tables 4,5 show the effect of Hb type on morphometric parameters of male and female of sheep and goats. There were no significant differences ($p > 0.05$) between morphometric parameters measured. Our results revealed that the Hb variants had no significant effects ($p > 0.05$) on horn length, wither height, body long, and chest perimeter of both Sheep and Goat populations.

Table no 4:-Effect of hemoglobin type on morphometric measurements (Mean \pm SD, in cm) of sheep

Parameter	Hb type	Female	Male
Horn long	AA	6 \pm 0.7-	68
	AB	10 \pm 2	71 \pm 1
	BB	7.5 \pm 1.3	69.3 \pm 0.5
withers height	AA	67 \pm 0.5	79
	AB	71 \pm 1	82 \pm 1.2
	BB	69 \pm 3.4	80 \pm 0.5
Body long	AA	101 \pm 2.5	130
	AB	115 \pm 8	133.5 \pm 2
	BB	110 \pm 5	130 \pm 1
Chest perimeter	AA	99 \pm 2	129 \pm 1
	AB	103 \pm 4	131 \pm 2.5
	BB	107 \pm 6	130 \pm 1

Table no 5:-Effect of hemoglobin type on morphometric measurements (Mean \pm SD, in cm) of goats

Parameter	Hb type	Female	Male
Horn long	AA	29.6 \pm 2	37.5 \pm 0.7
	AB	23 \pm 2	-

	BB	27±2	33±4.2
with height	AA	71.8±3.5	87.5±3.5
	AB	69.3±1	–
	BB	70.3±1.5	82.5±3.5
Body long	AA	111.4±10	134±19.7
	AB	98±7	–
	BB	107.3±10	112±3.5
Chest perimeter	AA	85.4±1	110±0.5
	AB	82±2	–
	BB	83.3±2	105±7

Table no 6:-shows the effect of hemoglobin genotypes on packed cell volume and hemoglobin concentration in sheep. The two blood parameters were not significantly influenced by Hb genotypes in both females and males of sheep ($P>0.05$).

Parameter	Male		Female	
	PCV (%)	Hb (g/dl)	PCV (%)	Hb (g/dl)
AA	29.9±2.38	10.23±0.45	34.6	10
AB	30.15±2.88	10.07±1.03	33.4±2.68	10.6±1.2
BB	29.56±2.97	9.85±1.1	32.4±2.06	10.1±0.7

Discussion:-

Hemoglobin polymorphism is defined by expression of the three genotypes: two homozygote, Hb AA and Hb BB, and one heterozygous, Hb AB (Hrinca, 2008). In this study, the three hemoglobin genotypes were detected. These genotypes were produced by two co-dominant alleles A and B. The Hb type BB was predominant in sheep under study. This result agrees with the reports of Templeton (1969) in different sheep breeds, Gootwine (1988) in Assaf sheep, Rodero et al. (1996) in andalosan sheep, Al-Samarrae et al. (2010) in different sheep breeds of Iraq, Nigussie et al. (2016) in indigenous sheep of Ethiopia, and Vazic et al. (2017) in pramenka sheep. While King et al. (1958), Tella et al. (2000), and Akinyemi and Salako (2010) obtained higher Hb AA genotype in Yankasa sheep and west African dwarf sheep, respectively. The predominance of Hb AA type in goats is in conformity with the observations of Bindu and Raghavan (2010) in Malabari goats, Agaviezor et al. (2013) in red Sokoto breed, Nafti et al. (2013) in Tunisian goats, and Aygün (2016) in Norduz goats. On the other hand, Hrinca (2008) reported higher Hb BB type in Carpathian goats, Yakubu et al. (2014), Musa et al. (2016), and Preedaa et al. (2016) reported higher Hb AB type in west African dwarf goats. In the present study, the gene frequency of Hb B in sheep was higher than that of Hb A. Similar findings were reported by Rodero et al. (1996), and Vazić et al. (2017). In contrast, Akinyani and Salaka (2010) reported higher Hb A frequency. In goats, Hb A frequency was higher than that of Hb B. This result is in agreement with the reports of Bindu and Raghavan (2010), Agaviezor et al. (2013), Yakubu et al. (2014), and Preedaa et al. (2016). The studies conducted by Hrinca (2008) and Musa et al. (2016) recorded higher Hb B frequencies.

From earlier reports it has been documented that the frequencies of the two hemoglobin alleles vary from breed to breed within the same species and seem to be correlated with particular environments (Evans and Blunt, 1961; Evans and Turner, 1965). Evans and Blunt (1961) after investigating the frequency of Hb A and Hb B in 33 breeds of British sheep, revealed that the breeds of sheep that were common to higher altitudes had a significant higher frequency of Hb A, and the breeds that were common to lower altitudes had a significant higher frequency of Hb B. Templeton (1969) found this association between altitude and the frequencies of hemoglobin alleles only in some sheep breeds and attributed this to other environmental factors that may exert selection pressures on animals or to the small size of populations used in his study. Hrinca (2008) suggested that the extreme temperatures (acute cold or sultry heats), the extreme forms of relief (dessert or mountain) or precarious nutrition and breeding conditions favor the fixing of the allele Hb A and the moderate temperatures, the moderate forms of relief (forest steppe, hill) or the optimal breeding technologies are correlated with the fixing of allele Hb B. Other authors attributed the adaptive advantage of certain alleles to differential mortality or to the reproduction isolation phenomenon (Buvanendran et al., 1981; Hrinca, 2008). Bindu and Raghavan (2010) considered this as an indication of species characteristic. Vazić et al. (2017) attributed the reason for the higher frequency of Hb B gene compared to Hb A gene in Sheep to the animals migration during the year and the influence of other breeds of sheep that in their genetic basis have a greater frequency of Hb B gene.

Hb A has been reported to confer a genetic resistance to some parasites as for helminth and to have beneficial effects on health related traits as for mastitis (FAO, 1988; Dally et al., 1980). Hence the local goats of Libya should be more resistant to helminth and mastitis disease than sheep. This assumption needs to be further investigated.

As above mentioned the Hb BB genotype was dominant in sheep in the present study. The effects of Hb BB and Hb AB on reproductive performance in sheep have been reported. Ewes carrying these types of hemoglobin have been repeatedly documented to have a better fertility than ewes with Hb AA type (Dally et al., 1980; Iyiola-Tunji et al., 2014; Važić et al., 2017). The relationship between Hb genotypes and productive traits in local sheep needs to be investigated.

In this study, it was found that the Hb type BB was predominant in female sheep compared to the males, which showed equal frequencies of Hb BB and Hb AB types. The genotype frequency decreases from the BB through the AB and then to the AA in female sheep. In goats, the genotype frequency decreases from the Hb AA through the Hb AB and then to the Hb BB in females, while in males no Hb AB type could be observed. Further study using large number of animals is needed to investigate the effect of sex on the distribution of Hb types in sheep and goats.

The calculated values of X^2 test indicate that the sheep population may be in the genetic balance, while the flock of Goats may be deviated from Hardy-Weinberg equilibrium for the Hb locus (Table 3).

Data in Tables 4 and 5 indicate that the hemoglobin type of sheep and goats has a certain relationship with morphometric measures and external appearance of animals. There was an obvious tendency for the sheep heterozygous and for the goats carrying Hb AA genotype to have higher mean morphometric values.

Our study revealed that the ewes with Hb AB type had higher body long, wither height, and horn long than the ones with Hb BB and Hb AA, respectively, while the chest perimeter values were higher in ewes with Hb BB type. In males the effect of Hb AB type was the highest on all morphometric characteristics measured. This is in agreement with the result of Važić et al. (2017), who tested these measures in Pramenka sheep and recorded higher values in animals with Hb type AB. The findings of Sušić et al., (1993); Akieme and Salako (2010) differed from our results, they reported an association between higher morphometric characteristics and Hb AA type in sheep. Važić et al. (2017) considered the heterozygous sheep to be more vital and productive with higher phenotypic characteristics compared to other types of homozygotes, while Sheep with Hb AA seem to be more tolerant to unfavorable environmental conditions but may have lower morphometric measures.

In Does, the body long, wither height, and horn long were higher in animals with Hb AA compared to Hb BB and Hb AB, respectively, while the chest perimeter was higher in females with Hb BB type. Hb type BB as shown in Tables 3 and 4 seems to have in both female sheep and goats the intermediate effect on all body measurements tested except for chest perimeter. In Bucks the four morphometric traits measured were higher in males with Hb AA than Hb BB. Similarly, Sam (2102); Yakubu et al. (2014); and Musa et al. (2016) showed that Hb AA genotype had a selective advantage over other Hb types for some morphometric measures in goats.

There were no significant differences between sheep of different types of hemoglobin in packed cell volume and hemoglobin concentration values (Table 5). This finding is in contrast with earlier reports made by FAO (1988) and Di Stasio (1997).

Although sheep and goats breeding is an ancient tradition in Libya, genetic improvement program has been not applied for these species. Hermas et al. (2010) suggested, after investigating some productive measures in libyan goats, that their productivity and efficiency need to be improved. Genetic variation has been the objective tool traditionally used for improving of animal species. Selection on a certain trait can be applied when basic information on genetic variation of a population is available (Balenočić et al., 2007; Melus et al., 2009). More effort should be done to elucidate the genetic variation of local sheep and goats and to unveil the link between their phenotypic traits, that of an economic importance, and a number of biochemical variants which can be useful genetic markers for selection and subsequent genetic improvement.

Conclusion:-

The study revealed the existence of three Hb genotypes from two co-dominant allele (A and B) with the predominance of Hb B in sheep and Hb A in goats. Ewes and Rams with Hb AB seem to have higher morphometric traits compared to those with Hb BB and Hb AA. Does and Bucks with Hb AA type tend to have the highest measures tested. No association between hemoglobin genotypes and blood parameters tested could be observed in the present study. The association between hemoglobin genotypes and morphometric and other productive traits of small ruminants in Libya is a subject to confirmation from further work using larger sample size.

References:-

1. Agaviezor, B.O., Ajayi, F.O., Benneth, H.N. (2013):haemoglobin polymorphism in nigerian indigenous goats in the Niger delta region, Nigeria. Intern. J. Sci. Nature, 4(3):415-419.
2. Ahtash, A. (2006): Goat production in Libya: Proceeding workshop on recent advances in goat production under arid conditions held at Cairo, Egypt, pp. 303-308.
3. Akinyemi, M.O., Salako, A.E. (2012): Genetic relationship among Nigerian indigenous sheep populations using blood protein polymorphism. Agricultural Science and Technology, 4(2):107-112.
4. Akinyemi, M.O., Salako, A.E. (2010):Haemoglobin polymorphism and morphometrical correlates in the West African Dwarf sheep of Nigeria. International Journal of Morphology, 28:205-208.
5. Akraim, F. (2012): Goat production in Libya: Current state and production constraints. Krmiva., 54:189-194.
6. Akraim, F., Milad, F.S., Abdulkarim, A.A., Ganem, M. (2008): Wool characteristics of Libyan Barbary sheep in north-eastern Libya: I. fiber diameter and staple length. Livestock Research for Rural Development.,20(8).
7. Al-Samarrae, S.H., Al-Bayaati, A.J., Al-Murrani, W.K. (2010): Hemoglobin polymorphism in different animal species in Iraq. Al-Anbar J. Vet. Sci.,3(2):73-77.
8. Aygün, T. (2016): Relationships between the Polymorphism of Blood Proteins and Some Reproduction Traits in Norduz Goats. Journal of Advanced Agricultural Technologies, 3(4):247-251.
9. Belanović, T., Ekart, K.A, Belanović, M., Sušić, B., Štoković, I., Pavičić, Ž. (2007): Application of molecular genetics in advanced selection of chicken (in Serbian Primjenamolekularnegenetike u unapređenjuuzgojno-selekcijskogradakokošaka). Stočarstvo.,61(4):291 – 299.
10. Bezova, K., Rafay, J., Mojto, J., Trakovicka, A. (2007): Analysis of genetic polymorphism of blood proteins and selected meat quality traits in rabbits. Slovak J. Anim. Sci., 40:57-62.
11. Bindu, K.A., Raghavan, K.C. (2010):Haemoglobin Polymorphism in Malabari Goats. Veterinary World., 3:74-75.
12. Buvanendran, V., Sooriyaamoorthy, T., Ogunsusi, R.A., Adu, I.F. (1981):Haemoglobin polymorphism and resistance to helminthes in Red Sokoto goats. Tropical Animal Health and Production., 13:217-221.
13. Chineke, C.A., Ologun, A.G., Ikeobi, C.O.N. (2007): Haemoglobin types and production traits in rabbit breeds and crosses. Journal of Biological Sciences., 7:210-214.
14. Dally, M.R., Hohenboken, W., Thomas, D.L., Craig, M. (1980): Relationships between Hb type and reproduction. Lambs, wool and milk production and health-related traits in crossbred ewes. J. Anim. Sci., 50:418-427.
15. Das, A.K., Deb, R. (2008): Biochemical polymorphism and its relation with some traits of importance in poultry. Veterinary World., 1:220-222.
16. Dawson, T.J., Evans, J.V. (1966): Effect of hypoxia on oxygen transport in sheep with different hemoglobin types. The American Journal of Physiology., 210:1021-1025.
17. Dyballa, N., Metzger, S. (2012): Fast and sensitive coomassie staining in quantitative proteomics. In: K. Marcus, ed., 'Quantitative Methods in Proteomics'. Humana Press., 893:47–59.
18. Evans, J.V., Blunt, M.H. (1961): Variation in the gene frequencies of potassium and hemoglobin types in Romney Marsh and Southdown sheep established away from their native environment. Australian Journal of Biological Sciences., 14:100-106.
19. Evans, J.V., Turner, H.N. (1965): Haemoglobin type and reproductive performance in Australian Merino sheep. Nature., 207:1396-1397.
20. FAO. (1988): Animal genetic resources conservation by management, data banks and Training. FAO Animal production and health. paper 44/1. Rome. 1988;p.186.
21. Di Stasio, L. (1997): Biochemical genetics. In: Genetics of sheep. Piper, L.,Runvisky, A. (Eds), Oxon, CAB International, pp. 133-48.

22. FAO. (2003): A Report on the State of Animal Genetic Resources in the Jamahiriya. ;http://fao.org/docrep/fao/011/a1250f/annexes/CountryReports/Libya_E.
23. Fésüs, L., Várkonyi, J., Ágnes A.T.S. (1983): Biochemical polymorphisms in goats with special reference to the Hungarian Native breed. *Anim. Genetics.*, 14(1):1-6.
24. Gootwine, E. (1988): Haemoglobin type frequencies in the Assaf dairy sheep 20 years after its formation. *Genet. Sel. Evol.*, 20(1):99-104.
25. Hermas, S.A., Ahtash, A., Majid, A. (2010): Growth measures of Libyan goat breeds and their crosses. In: proceeding of the 3^{er} international scientific conference on small ruminants development, held at Hurghada, Egypt.
26. Hrinca, G.H. (2008): Haemoglobin types in the Carpathian breed and their relevance for goat adaptation. *LucrăriȘtiințifice (SeriaZootehnie.)*, 54:110-114.
27. Iyiola-tunji, A.O., Akpa, G.N., Nwagu, B.I., Adeyinka, I.A. (2014): Survivability of lambs in relation to their dams haemoglobinvariants. *Biotechnology in Animal Husbandry.*, 30(2):215-223.
28. King, J.W.B., Evans, J.V., Harris, H., Warren, F.L. (1958): The performance of sheep with differing haemoglobins and potassium blood types. *Journal of Agriculture Science.*, 51:342- 348.
29. Magid, A.F., Zaied, A.A., Shariha, A.M. (1992): The Libyan Barbary sheep: Reproductive traits. *Libyan Journal of Agriculture.*, 13:5-12.
30. Melus, V., Kasarda, R., Kadlecik, O., Trakovicka, A. (2009): Breeding potential of the Slovak Pinzgau cattle: seeking biochemical and molecular biologic traits. *ArchivaZootechnica.*, 12:34-37.
31. Musa, J., Garba, H., Egena, S.S.A., Aremu, A. (2016): genetic variation of different phenotypes of west african dwarf goat based on haemoglobin polymorphism in Gulu, Niger state, Nigeria. *Nigerian Journal of Agriculture, Food and Environment.*, 12(3):108-113.
32. Mwacharo, J.M., Otieno, C.J., Okeyo, A.M., Aman, R.A. (2002): Characterization of indigenous fat-tailed and fat-rumped hair sheep in Kenya: diversity in blood proteins. *Tropical animal health and production.*, 34(6):515-524.
33. Nafti, M., Khaldi, Z., Haddad, B. (2013): Protein polymorphisms of Goats in Tunisian Oases. *BIOMIRROR.*, 4:5-12.
34. Ndamukong, K.J.N. (1995):Heamoglobin polymorphism in Grassland Dwarf sheep and goats of the North West province of Cameroon. *Bulletin of Animal Health and Production in Africa.*, 43:53-56.
35. Nigussie, H., Pal, S.K., Diriba, S., Mekasha, Y., Kebede, K., Abegaz, S. (2016): Phenotypic variation and protein polymorphism of indigenous sheep breeds in eastern Ethiopia. *Livestock Research for Rural Development.*, 28(8).
36. Pal, S.K., Mummed, S.Y.Y.(2014):Investigation of haemoglobin polymorphism in Ogaden cattle. *Veterinary World.*, 7:229-233.
37. Pieragostini, E., Rubino, G., Bramante, G., Rullo, R., Petazzi, F., Caroli, A. (2006): Functional effect of hemoglobin polymorphism on the hematological pattern of Gentile di Puglia sheep. *J. Anim. Breed. Genet.*, 123:122-130.
38. Preedaa, M.G., Selvara, J.P., Ramavathi, J., Yazhini, P.,Thanabal, C. (2016): preliminary investigation of hemoglobin and transferrin polymorphisms in indigenous goat breed (Salem black) of Tamil Nadu. *Journal of Cell and Tissue Research.*, 16(3):5857-5861.
39. Raushenbach, Y.O., Kamenek, V.M. (1978): The role of biochemical polymorphism in ecogenetic differentiation of animals and its signifiante for selection for rezistence to extreme environmental conditions. *Proceeding of VI-th Intern. Conf. on Anim. Blood Groups and biochem. Polymorph.*, held at Leningrad, pp. 128.
40. Riken (2006): Genetic quality monitoring by biochemical isozymes. Riken BioresourcesCentar.; <http://www.riken.go.jp/eng/>.
41. Rodero, E., de la Haba, M.R., Rodero, A., Herrera, M. (1996): Genetics and phenotypic profile of endangered Andalusian sheep and goat breeds. Rome, FAO,
42. Sam, I.M. (2012): Relationship of haemoglobin and potassium polymorphism with conformation, milk production and blood biochemical profiles in agropastoral goat. PhD thesis, Ahmadu Bello University, Zaris, Nigeria. <http://kubanni.abu.edu.ng:8080/jspui/handle/123456789/3157>.
43. Sitmo, M.S. (2018): Influence of Age and Gender on Heamatological Parameters of Apparently Healthy Barbary Sheep in AljabalAlakhdar, Libya. *International Journal of Science and Research.*, 7(2):1278-1282.
44. Sušić, V., Mikulec, K., Šerman, V., Bencetić, S., Matičić, D. (1993): Influence of selection on genotype and gene frequency on hemoglobin of sheeps (in Serbian). *Stočarstvo.*, 47(9-10):347-352.

45. Tella, M.A., Taiwo, V.O., Agbede, S.A., Alonge, O.D. (2000): The influence of hemoglobin types of the incidence of babesiosis and anaplasmosis in West African Dwarf and Yankasa sheep. *Trop. Vet J.*, 18:121-7.
46. Templeton, J.W. (1969): Genetic Polymorphism of Sheep Hemoglobin. PhD thesis, Oregon State University, USA.1969;<https://ir.library.oregonstate.edu>.
47. Yakubu, A., Aya, V.E. (2012): Analysis of genetic variation in normal feathered, naked and Fulani-ecotype Nigerian indigenous chickens based on hemoglobin polymorphism. *Biotechnology in Animal Husbandry.*, 28:377-384.
48. Yakubu, A., Abimiku, K.H., Musa-Azara, S.I., Bardem, E.R., Raji, O.A. (2014): Preliminary investigation of haemoglobin polymorphism and association with morphometric traits in West African dwarf goats in north central Nigeria. *Mljekarstvo.*, 64(1):57-63.
49. van Vliet, G., Huisman, T.H.J. (1964): Changes in the hemoglobin types of sheep as a response to anemia. *Biochemical Journal.*, 93:401-410.
50. Važić, B.S., Rogić, B.S., Drinić, M.S., Pržulj, N.M. (2017):relationship between the genetic hemoglobin polymorphism, morphometry and fertility of pramenka sheep breed from central bosnia. *GENETIKA.*, 49(1):151-160.