

Relationships between the Polymorphism of Blood Proteins and Some Reproduction Traits in Norduz Goats

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Abstract—The aim of this study was to determine the influences of polymorph systems such as hemoglobin and transferrin types on some the reproduction traits in Norduz goats. The material of this study consisted of 65 Norduz goats. The polymorphism of hemoglobin and transferrin phenotypes of Norduz goats were determined. Blood proteins were analyzed through horizontal starch gel electrophoresis. It was found that the hemoglobin and transferrin types were controlled by two allel genes (HbA and HbB; TfA and TfB) in the goats. The phenotypes of HbAA, HbBB and HbAB; TfAA, TfBB and TfAB were observed in the population. Hemoglobin and transferrin gene frequencies were 0.73 for HbA and 0.27 for HbB; 0.80 for TfA and 0.20 for TfB. Hemoglobin and transferrin genotype percentage frequencies were 51% for HbAA, 4% for HbBB and 45% for HbAB; 65% for TfAA, 4% for TfBB and 31% for TfAB. According to the analyses of variance, the influences of hemoglobin and transferrin types on the gestation efficiency were not significant. However, the highest gestation efficiencies were obtained from HbBB and TfBB types with the averages of 16.05 ± 1.75 and 13.67 ± 3.51 kg, whereas the lowest gestation efficiencies were obtained from HbAB and TfAB types with the averages of 9.94 ± 0.82 and 9.67 ± 0.60 kg, respectively. In conclusion, there were polymorphism in hemoglobin and transferrin types in Norduz goats.

Index Terms—gestation efficiency, hemoglobin, transferrin, polymorphism, Norduz goat

I. INTRODUCTION

In animal husbandry, one of the most important ways of increasing production are studies on the improvement of genotype. However, quantitative traits such as meat, milk, fleece, reproduction *etc.* are especially difficult for the correctional and the effective study. Because these characters show polygenic inheritance, it has also been influenced by environmental factors. Also, it takes a long time to be determined by individual selection of superior genotypes. Moreover, to determine the genotype of the individual by depending on their phenotypes is impossible precisely [1].

Polymorphism is an ancient Greek word and means roughly "many forms". In goat breeding, genotypic differences and the correlations between polymorphic

biochemical properties and various yields are extremely important for animal breeding. Globally, there is effective rivalry for new technologies that may directly or indirectly affect the future of animal production and improvement.

One way to study this genetic diversity is by the determination of genetic variability through polymorphism studies. Polymorphism in a population assures a pool of genetic variability, for if none exists, there would be no progress made through selection and breeding. This accentuates the need to study polymorphism among breeds as well as within breeds. The protein variants have their use in the study of origin and the evolution of breeds of livestock. These markers have proved to be useful for parentage determination and population analysis [2]-[4].

One of the first goals of a genetic selection program is to estimate the genetic potential at the earliest age as possible. In this case, the characteristics which can be measured in the early ages are important. One of these parameters is the blood protein characters. The type of serum transferrin and hemoglobin is two of the most important blood proteins [5].

Blood proteins can be used for kinship analysis and as breed markers, a function of great importance, especially for the preservation of breeds [6], [7]. A population is said to show polymorphism when two or more distinctly inherited varieties coexist in the same individual [8]. This type of polymorphism is increasingly being used in the study of genetic variation within and between populations and to estimate genetic divergence [9]. This is because the biochemical elements (blood proteins and enzymes) can be used widely as biomarkers of corresponding structural genes. These biomarkers are not affected or do not depend on environmental factors and this makes them suitable for genetic studies. Although there are more advanced technologies for genetic studies of polymorphism in farm animals, the economic situations and inadequacy of infrastructural facilities in developing countries means that the importance of such studies using starch gel electrophoresis cannot be discountenanced [10]. Blood proteins have been used widely to characterize animal population because of their polymorphism and simple mode inheritance. Thus, blood protein polymorphisms have been studied in different species

using starch gel electrophoresis. They are useful in studies of basic genetics, population dynamics, clinical diagnosis and in gene mapping. Even though the genetic variation on the Tf gene locus depends on the geographic or natural limits, this situation forces the population to isolation groups which result a difference among populations [7], [11], [12].

Norduz goat known as a subtype of Hair Goat breed is favorably characterized by their adaptation to the harsh environmental and the feeding conditions, in addition to the potential of the milk production. Norduz goat has generally black-bodied, grey or brown hair. The Norduz goat has been maintained in Van-Norduz provinces of Eastern Anatolia in Turkey. Traditional breeders generally rear this variety of goat, which graze mostly in the range of Norduz plateaus and outskirts of the villages.

The most prominent feature of Norduz goat, lots of small ruminant farming in the form of hot and dry desert conditions can be grown. Norduz goats have evolved naturally through adaptation to socio-economic and ecological conditions of Norduz region. There are many evidence of existence of Norduz goat breed in regional history. Norduz does are popular among the farmers for their appearance, body size and the characteristics of the milk yield. Further information on the characteristics of reproduction and the techniques to improve reproductive performance, and the polymorphism of blood proteins in native goat breeds of Turkey is required [13]

The objective of this study was to investigate the polymorphism of blood proteins such as hemoglobin and transferrin types in Norduz goats raised under semi-intensive conditions of Small Ruminant Farm of Agricultural Faculty of Yüzüncü Yıl University. This is an important report on the polymorphism of hemoglobin and transferrin types in Norduz goats.

II. MATERIALS AND METHODS

A. Animals and Research Area

A total of 65 Norduz goats were used in this study. Animals were maintained in the Sheep Farm of Agricultural Faculty of Yüzüncü Yıl University, Van province, Turkey. Van-Norduz district is located on the Eastern Anatolia region of Turkey. It's sea level height (altitude) is 1727 meters. Average annual minimum and maximum temperatures of the region are 5.1 °C and 14.9 °C, respectively.

Norduz goat had been brought from original habitats, Norduz region of Gürpınar district of Van city of Eastern Anatolia to the Small Ruminant Farm in Agricultural Faculty of Yüzüncü Yıl University. After the parturition, live weights of does, and birth weights of their kids were recorded. The kids were kept with their dams for three months.

Does in this study were fed twice a day with the crushed sainfoin hay (*onobrychis viciifolia*) containing 7.2% crude protein in addition to forage at lactation period (Table I). Then kids were distinguished from their dams and they were taken out to the pasture.

All does were permitted to choose their own parturition sites within the barn in which they were normally housed and to remain during the study.

TABLE I. NUTRIENT CONTENTS OF FORAGE USED IN THE FEEDING OF ANIMALS (%)

Nutrients	Crushed Sainfoin Hay
Dry matter	87.6
Crude protein	7.2
Crude cellulose	44.0
Crude fat	4.3
Crude ash	5.6

B. Blood Sampling and Assay

Blood samples were taken from *vena jugularis* of neck of does in the early morning. Animals were bled while standing. Blood samples were collected into evacuated approximately 10 ml tubes for assay of blood proteins. Blood samples were immediately chilled in an ice bath. Then, blood samples were subsequently centrifuged at 3000 rpm at 4 °C for 15 min and, they were transferred to fresh tubes, and stored at -20 °C until assayed in the laboratory.

The principle of hemoglobin-type assay is based on the separation by the direct current power in starch gel plates polymorphs properties of hemoglobin in red blood cells. Also, the principle of transferrin-type assay is based on the determination by the direct current power in the horizontal starch gel electrophoresis of polymorphic traits of transferrin in the blood plasma [14]. Hemoglobin analyzes in hemolysates were performed using the horizontal starch gel electrophoresis in *tris-boric acid buffer system*. Also, the separation of transferrin types was carried out using polyacrylamide gel electrophoresis [15]. On electrophoresis, transferrin variants performed distinct movement towards anodic end of the electrophoretogram revealing two electrophoretically different transferrin types [3], [16].

C. Statistical Analysis

In Norduz goat population, G and χ^2 tests were used to determine whether genetic balance for hemoglobin and transferrin to be or not according to Hardy-Weinberg principle. The allelic frequencies were estimated by the method of Nei [17].

Analysis of variance in relation to the effects of hemoglobin and transferrin genotype on the gestation efficiency was performed using the GLM in the Minitab statistical software package. Also, the data on the gestation efficiency was calculated for the effects of hemoglobin and transferrin using least-squares procedures [18].

The effects of hemoglobin and transferrin genotype were analyzed by the following mathematics model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$$

where is:

Y_{ijk} = Individual observation for Hb or Tf genotypes,

μ = General mean,

α_i = Effect of *i*th Hb genotype,

β_j = Effect of *j*th Tf genotype and

e_{ijk} = Residual error normally distributed with mean 0 and variance σe^2 .

III. RESULTS AND DISCUSSION

A. The View of Hemoglobin (Hb) System

It was found that types of hemoglobin was controlled by two allele genes (HbA and HbB) in Norduz goat. The frequencies for genes of HbA and HbB which were typed by the horizontal starch gel electrophoresis in tris-boric acid buffer system were calculated 0.73 and 0.27, respectively. Also, genotypes of HbAA, HbBB and, HbAB were determined. Genotypes of HbAA 33 units, HbAB 29 units and, HbBB 3 units in flock was observed (Table II).

TABLE II. THE DISTRIBUTION OF THE ALLELE FREQUENCIES AND THE GENOTYPES OF HEMOGLOBIN IN NORDUZ GOATS

n	Genotypic frequencies of hemoglobin			Allele frequencies	
	HbAA	HbBB	HbAB	HbA	HbB
65	33 (51%)	3 (4%)	29 (45%)	0.73	0.27

As shown in Table II, the frequency of the hemoglobin A (HbA) gene is high (0.73). The genotypic frequencies and the rates for HbAA, HbBB and HbAB were found 51%, 4% and 45%, respectively.

In a study on same breed by Yur *et al.* (1998), it was identified that were 11 HbAB, 4 HbBB and, 5 HbAA type of 20 Norduz goats [19].

There are different findings about the genetic view of the hemoglobin system in goats. Some of the research, it was found monomorphism for the hemoglobin locus [20]-[22]. However, there are the researches which reported the polymorphic for the same loci [23], [24]. Specially, a lot of reasons why a monomorphic structure in terms of hemoglobin loci are that the populations were mated in a closed manner and, that the sample size examined is small. Hemoglobin system in some populations with the small sample size has been identified as monomorphic. Moreover B-type hemoglobin in goats is rare [1].

Ref. [25] reported as HbAA = 0.50, HbAB = 0.48 and, HbBB = 0.2 of genotypic frequencies in Hair goats. In same research, it was observed that gene frequencies for Hb type are 0.74 for HbA and 0.26 for HbB have identified [25].

In the study on crossbred Maltese goat population in Ezine district, it is determined that frequencies of HbA and HbB allele are 0.876 and 0.124, respectively [26].

B. The View of Transferrin (Tf) System

In this present study, we observed polymorphism for transferrin. Transferrin types in Norduz goats have been found to be controlled by two allele genes (TfA and TfB). In this study, TfAA, TfBB and TfAB genotypes were detected. As a result of electrophoretic analysis, it was identified that were 42 TfAA, 3 TfBB, and 20 TfAB genotypes of 65 Norduz does. Transferrin genotype percentage frequencies were 65% for TfAA, 4% for TfBB and 31% for TfAB (Table III).

TABLE III. THE DISTRIBUTION OF THE ALLELE FREQUENCIES AND THE GENOTYPES OF TRANSFERRIN IN NORDUZ GOATS

n	Genotypic frequencies of transferrin			Allele frequencies	
	TfAA	TfBB	TfAB	TfA	TfB
65	42 (65%)	3 (4%)	20 (31%)	0.80	0.20

As seen in Table III, the frequency of the transferrin A (TfA) gene is high (0.80). The genotypic frequencies and the rates for TfAA, TfBB and, TfAB were determined as 65%, 4% and 31%, respectively. The frequency TfAA genotype is higher. All the Tf genotypes were analyzed for Hardy-Weinberg equilibrium. The distribution of gene frequencies in this herd was not confirmed to means predicted on the basis of Hardy-Weinberg equilibrium. It is understood that this population is not in balance in terms of this gene locus.

Many studies conducted on goats determined that transferrin allele was monomorphic or predominant allele in most goat breeds [1], [21]. In a study on Spanish native goats, it was determined that a monomorphic structure in terms of the alleles Tf was observed in Guadarrama, Andaluza and, Canaria goat breeds [27].

C. The Relationships between the Genotypes of Hemoglobin and, Ttransferrin and Some the Reproduction Characteristics

Descriptive statistics for the gestation rate, the birth rate, the twin rate, the fecundity, and the litter size in Norduz does by hemoglobin (Hb) and transferrin (Tf) are summarized in Table IV and Table V, respectively.

The number of researches investigating the relationship between the system of hemoglobin or transferrin and the reproductive traits is very limited and very old. For example, Ref. [28] suggested that Merino ewes with HbB gene have a higher fertility than others.

The frequencies of Hb genotype in Norduz goat population did not show the distribution according to the Hardy-Weinberg equilibrium. So, it is not in balance for the Hb locus of the population. It can be expected to be such results in self-closed small populations [1], [29].

In this study, the effect of transferrin types on the reproduction traits were not significant. This present study identified that the results on the reproduction traits examined in Norduz goat are in agreement with one obtained from other studies.

To compare the averages of reproduction characteristics in this present study to the values determined in other studies is impossible and not true too. Indeed, there are many factors that determine this situation. These factors are the genotype, the age, the live weight, the season, the conditions of management, the standard of hormone and so on [29].

Least-squares means and standard errors for the gestation efficiency in Norduz does by Hb and Tf are shown in Table VI. The type of hemoglobin and transferrin have no significant effect on gestation efficiency ($p > 0.05$).

TABLE IV. DESCRIPTIVE STATISTICS FOR THE GESTATION RATE, THE BIRTH RATE, THE TWIN RATE, THE FECUNDITY, AND THE LITTER SIZE BY HEMOGLOBIN (Hb) IN NORDUZ DOES

Traits	Hemoglobin genotype							
	HbAA		HbBB		HbAB		Total	
	Numeric	%	Numeric	%	Numeric	%	Numeric	%
Gestation rate	29/33	88	3/3	100	29/29	100	61/65	94
Birth rate	29/33	88	2/3	67	29/29	100	60/65	92
Twin rate	10/29	35	2/2	100	7/29	24	19/60	32
Fecundity	39/33	1.18	4/3	1.33	36/29	1.24	79/65	1.22
Litter size	39/29	1.35	4/2	2.00	36/29	1.24	79/60	1.32

TABLE V. DESCRIPTIVE STATISTICS FOR THE GESTATION RATE, THE BIRTH RATE, THE TWIN RATE, THE FECUNDITY, AND THE LITTER SIZE BY TRANSFERRIN (Tf) IN NORDUZ DOES

Traits	Transferrin genotype							
	TfAA		TfBB		TfAB		Total	
	Numeric	%	Numeric	%	Numeric	%	Numeric	%
Gestation rate	40/42	95	3/3	100	18/20	90	61/65	94
Birth rate	39/42	93	3/3	100	18/20	90	60/65	92
Twin rate	15/39	39	2/3	67	2/18	11	19/60	32
Fecundity	54/42	1.29	5/3	1.67	20/20	1.00	79/65	1.22
Litter size	54/39	1.39	5/3	1.67	20/18	1.11	79/60	1.32

TABLE VI. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR THE GESTATION EFFICIENCY BY Hb AND Tf IN NORDUZ DOES

Traits	n	Gestation Efficiency (kg)		
		$\bar{X} \pm S_{\bar{x}}$	Minimum	Maximum
Hb type				
HbAA	29	10.65 \pm 0.69	5.30	19.40
HbBB	2	16.05 \pm 1.75	14.30	17.80
HbAB	29	9.94 \pm 0.82	4.80	23.30
Tf type				
TfAA	39	10.62 \pm 0.73	4.80	23.30
TfBB	3	13.67 \pm 3.51	7.30	19.40
TfAB	18	9.67 \pm 0.60	5.70	15.10

It has been shown in the Table VI that HbBB genotype does (16.05 \pm 1.75kg) have higher than other Hb genotypes for serum gestation efficiency. Likewise it has been understood that TfBB genotype does (13.67 \pm 3.51kg) have higher than other Tf genotypes for serum gestation efficiency. Whereas the lowest gestation efficiencies were obtained from HbAB and TfAB types with the averages of 9.94 \pm 0.82 and 9.67 \pm 0.60kg, respectively. However, this differences were statistically not significant ($p > 0.05$). According to the analyses of variance, the influences of hemoglobin and transferrin types on gestation efficiency were not significant. In conclusion, there were polymorphism in hemoglobin and transferrin types in Norduz does. These averages in Norduz goats are similar to the reported ones for Norduz breed reared in the same conditions [1].

In Turkey, the detailed information regarding the reproduction techniques in small ruminant breeding according to the regions and districts is limited. In addition, the overall performances of Turkey's breeds of native small ruminants kept in native conditions of information regarding detailed descriptions are also insufficient. However, the development of more efficient livestock programs is due to conducting research on direct growers overall the yield performance under the

conditions of the populations of native breeds, the morphological and the physiological characteristics. By obtaining the more synthesis of this information, the more sensitive animal breeding programs and the policies can be developed [1], [5], [29].

The findings of this present study to the results of other studies in literature have suggested that the polymorphism of blood proteins depends not only on the breed but also on the breeding and the mating methods. In particular, the variation between the introductory values considerably seems to be broad level. By using of these variations, it will be possible that the reproductive performance of Norduz goats increases satisfactory level.

IV. CONCLUSION

Studies about Norduz goat breed have been performed in the border area of Eastern Anatolia of Turkey. However, very little research was conducted on the reproduction and the polymorphism of blood proteins in Norduz goat. The results of this study would be the basis for the scientific studies taking into account the relationships between the yield and the blood proteins and the reproduction problems in the region.

In this study, the relationship between the phenotypes observed in locus of hemoglobin or transferrin and the reproductive traits are statistically not significant. These findings may be an indication that remained under pressure from the breeding method and the selection applied in Norduz goat herd. In this case, it has been understood that loci examined in this study can never be used as indirect selection criteria. However, it is concluded that there were the polymorphism for the hemoglobin and the transferrin types in Norduz goat. Even so, in order to obtain a more definite conclusion is required to detailed works in native goat populations in Eastern Anatolia region of Turkey.

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