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THE HAEMOGLOBIN, TRANSFERRIN, CERULOPLASMIN AND GLUTATHIONE POLYMORPHISM OF NATIVE GOAT BREEDS OF TURKEY, II- KILIS AND HONAMLI

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Abstract

In this research, Kilis and Honamli goats are used, which are specific local genetic resources of Turkey. The herds were independent, but they had similar care and nutrition circumstances. From each breed 30 samples were taken, in all 120 samples were collected. Erythrocyte, whole blood and serum samples were used for haemoglobin (Hb), glutathione (GSH) and transferrin (Tf), ceruloplasmin (Cp) analysis, respectively. In the analysis of these samples, Hb and Tf bands were determined by electrophoresis. However, Cp and GSH levels were analyzed by the spectrophotometer. 3 Hb phenotypes (AA, BB, AB) and 6 Tf phenotypes (AA, AB, AC, BB, BC, CC) were determined in this study. In addition, both the observed and the expected values of polymorphic characteristic for 2 characters were presented according to the Hardy-Weinberg Equilibrium (HWE). Cp levels were detected as 0.822 ± 0.055 mg/dl and 1.793 ± 0.109 mg/dl in Kilis and Honamli herds, respectively. Also GSH levels were detected as, 42,486 ± 1,034 mg/dl and 33.515 ± 0.345 mg/dl in these breeds, respectively. On the other hand, the high and low GSH levels (GSH^H and GSH^h) of herds were presented.

Key words*: electrophoresis, gene resource, goat, spectrophotometer.*

INTRODUCTION

Protecting the gene pool of breeds has a great importance, because there is a risk of genetic resources loss. In this case, the benefits of advanced breeding programs will be reduced. In studies about reclamation of livestock, the known characteristics of the population are used. The studies in order to determine the genetic quality have improved the quality in goats as in other types of genetic. Kurnianto (2009) reported that better quality breeding population through the selection of high yielding goats can be done with selection and in this way it may be possible to yield high quality animals.

Kilis goat, supply genes which are grown in Turkey (Fig. 1 a, b); occurred by crossbreeding of Hair goat with Aleppo goat originated Syrian. Kilis goats have the 25.6% of the total goat breeding presence in Turkey. This rate is quite high in terms of breeding. They are widely grown in the South-eastern Anatolia region and especially in Sanliurfa, Gaziantep, Kilis and Hatay which are borders of Syria. This area with hot weather is quite favourable for cultivation because of their vegetation (Kaymakci et al., 1997).

Figure 1. Kilis goats a) 1st herd b) 2nd herd

Honamli goats are a breed that spreading in the triangle from the lower slopes of the Taurus Mountains to the Mediterranean region and Antalya, Konya, Isparta provinces. One of purity properties of these animals is the distance between the horns on the forehead. This distance should be two centimetres for adult goats. This breed loves to play with its

caregivers. Honamli goats are highly active animals. Their eyes are large, vivid and bright.

Figure 2. Honamli goats a) 1st herd b) 2nd herd

Differences between individuals in a population were defined as polymorphism. Accordingly, alleles belong a population locus in the form of at least 5% differences are desired for balanced polymorphism by Goldstein and Schlotter (2000). These experiments are used to explain a various biochemical properties with different genetic forms and the morphological differences in chromosomes.

Polymorphism studies can be grouped under three main groups as different biochemical properties of the genetic forms (various proteins and blood group factors), the morphological differences in the chromosomes (chromosomal polymorphism) and differences in the DNA nucleotide sequence (DNA polymorphism).

Hb, one of the polymorphic characters in the blood, is frequently investigated in goats. This system is commonly controlled by two codominant alleles in the form of Hb^A and Hb^B. These two alleles have different types of electrophoretic mobility in electrophoretic analyses.

Tf availability often investigated in breeding is a polymorphic system. Tf supplies the phenotypes to be determined in the very early stages of life and allows them can be used in the indirect selection. Thus generation interval can be shortened. Especially in goats, the high correlation between Tf which in blood and mohair proceeds are noteworthy. It is possible to make a selection based on the result of the Tf polymorphism study for these proceed.

Orally administered copper (Cu), after being absorbed from the top of the stomach and intestines, enters into blood plasma and erythrocytes. After 24 hours from the absorbance, High amount of Cu are collected in Cp (Murray et al., 1990). Besides, Cp is involved in various antioxidative and cytoprotective activities and thus helps to maintain cell integrity, on the other hand, Cp which protein facilitates binding of Fe to Tf protein.

Types of erythrocyte GSH are under genetic control and they have also inherited property. GSH levels in the blood are fairly constant for all adults (Mert et al., 2003). Decrease in intracellular GSH levels lead to cell apoptosis via oxidative stress. Additionally, apoptosis for living is a great important condition related to cell death.

MATERIALS AND METHODS

In this study 30 animals from 2 herds (which two different breeds) with a total of 120 goats were used from Kilis and Honamli goats, indigenous genetic resources grown in Turkey. In study was gone to Kilis centre for Kilis goats and Konya-Seydisehir for Honamli goats and were studied with two different herds for avoiding similar blood results in the wake of kindred.

Blood samples were taken at 8 o'clock in the morning when they were hungry. The transferred bloods in purple tube with EDTA were used for Hb and GSH analysis. On the other hand, transferred serum samples to plastic dry tube were used for Tf and Cp analysis.

Polymorph properties in the starch gel plate, direct current power with the separation on the basis of Hb type determination (Soysal, 1983), a continuous buffer system using horizontal starch gel electrophores was made according to Meyer (1963) and Braend (1971) reported. Reading the Hb types were taken into account electrophoretic rate, the faster was defined as Hb^A and the slower was described as Hb^B (Ustdal, 1976).

Transferrin type analysis, the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Zacharius et al., 1969; Racusen, 1978; Jay et al., 1990) discrete BioRad SDS-PAGE method was performed by adapting BioRad Miniprotean Tetra Cell system (Laemmli, 1970). Tf types were defined when they taken into their electrophoretic velocities. The fastest outgoing type was Tf^A and Tf^C was defined as the slow-moving (Dogrul, 1995).

Polyacrylamide gel, free functional groups which react with the acrylamide monomer

N,N'-methylene bisacrylamide-like through-linked with bifunctional compounds, are shaped to polymerize. A radiator must be used to determine the location of the protein bands on the gel under UV light.

Hb band types were directly determined on the gel at the study. The gel was photographed for Tf and the genotype of each individual was noted. Genetic variants in terms of both properties, gene and the genotype frequencies were determined by direct counting method (Nei, 1987; Russell, 1992). According to this method, related gen frequency are collected with more than two times of homozygous phenotypes and half of the number of heterozygous phenotypes. After then, the result is divided to the total number of individuals (Duzgunes et al., 1987; Nei, 1987).

Hb and Tf alleles in terms of population, in terms of Hb and Tf genetic stability control system (differences between expected and observed the importance of genotype-HWE) for identifying whether or not provided, the chi square ($\chi 2$) compliance test was used (Pembeci, 1978; Duzgunes et al., 1983; Yeh et al., 1997).

$$\chi^2 = \sum (\text{observed-expected})^2 / (\text{expected})$$

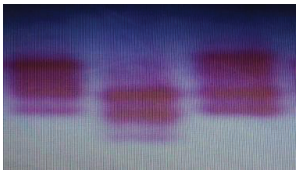
On a further analysis method, serum Cp levels, read in pH 5.2 and at 37 °C in acetate buffer, P-phenylene diamine dichloride (PPD) with serum samples formed by the colored product absorbance spectrometer at 550 nm were analysed (Ceron and Subiella-Martinez, 2004). Calculation of Cp was performed according to the method described in the literature reference (Curzon and Vallet, 1960).

Occurrence of the yellow color was measured spectrophotometrically at GSH analysis (Beutler et al., 1963; Rizzi et al., 1988). The amount of GSH in the sample, at a wavelength of 412 nm of the colored compound was assessed by determining the optical density (Burtis and Ashwood, 1999).

SAS 9.3 software package was used in the calculation of statistical analysis of Cp and GSH levels.

RESULTS AND DISCUSSIONS

Hb starch gel electrophoresis in Kilis goats, AA and AB genotypes obtained in the 1st herd, however the BB genotype was not observed. Three genotypes (AA, AB, BB) were obtained in the 2nd herd. The other hand, three genotypes (AA, AB, BB) in Honamli goats were obtained in Hb electrophoresis. Obtained bands image of Hb in starch gel electrophoresis was given in Fig. 3.



AA BB AB

Figure 3. Hb band types that appear in goat bloods

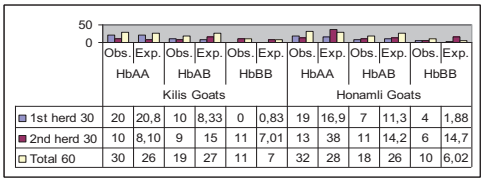
Belongings to genotypes frequencies % and homologous/heterologous genotypes were presented in Tab. 1.

Table 1. Hb genotypes, allele frequencies and homologous/ heterologous genotypes

		Kilis Goat			Honamli Goat		
		1st herd	2nd herd	Total	1st herd	2nd herd	Total
GENES \ N		30	30	60	30	30	60
GENOTYPE	HbAA	20 % 66.67	10 % 33.33	30 % 50.00	19 % 63.33	13 % 43.33	32 % 53.33
	HbAB	10 % 33.33	9 % 30.00	19 % 31.67	7 % 23.33	11 % 36.67	18 % 30.00
	HbBB	- % 0.00	11 % 36.67	11 % 18.33	4 % 13.33	6 % 20.00	10 % 16.67
FREQUENCY	Hb^A	50 0.833 3	29 0.483 3	79 0.658 3	45 0.750 0	37 0.616 7	82 0.683 3
	Hb^B	10 0.166 7	31 0.516 7	41 0.341 7	15 0.250 0	23 0.383 3	38 0.316 7
GEN	Hb AA,B B	20 % 66.67	21 % 70.00	41 % 68.33	23 % 76.67	19 % 63.33	42 % 70.00
	Hb AB	10 % 3.33	9 % 30.00	19 % 31.67	7 % 3.33	11 % 36.67	18 % 30.00

The 1st herd of Kilis goat was at HWE. In this regard, observed and expected gene frequencies difference at Hb electrophoresis was non-significant ($P>0.05$) in this herd. But the 2nd herd and intra population were not in HWE. Thus, P values were significant ($P<0.05$) in these. The situation was different for other breed. With reference to the 2nd herd of

Honamli goat was in HWE. In this regard, observed and expected gene frequencies difference at Hb electrophoresis was non-significant (P>0.05) in this herd. But the 1st herd and intra population were not in HWE. Thus, P values were significant (P<0.05) in these groups (Fig. 4).

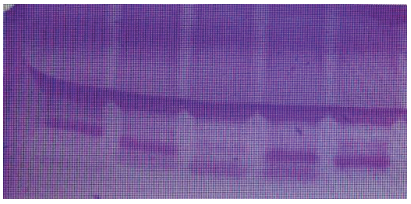


1st herd 30	20	20,8	10	8,33	0	0,83	19	16,9	7	11,3	4	1,88
2nd herd 30	10	8,10	9	15	11	7,01	13	38	11	14,2	6	14,7
Total 60	30	26	19	27	11	7	32	28	18	26	10	6,02

Figure 4. Observed and expected values of Hb frequencies in Kilis and Honamli goats

SDS-PAGE electrophoresis, 6 genotypes (AA, AB, AC, BB, BC, CC) were obtained from the 1st herd and 5 genotypes (AA, AB, AC, BB, BC) were detected from the 2nd herd in Kilis goats. Besides, SDS-PAGE electrophoresis of Tf, all 6 genotypes were obtained in Honamli goats.

Obtained bands image of Tf in starch gel electrophoresis was given in Fig. 5.



AA BB CC BC AB

Figure 5. Tf band types that appear in goat bloods

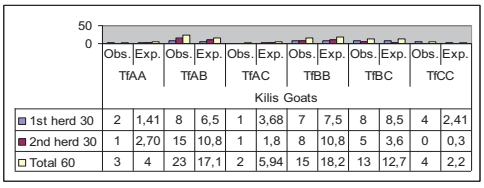
Belongings to these genotypes % frequencies and homologous/heterologous genotypes were presented in Table 2.

Non-significant difference (P>0.05) at observed and expected gene frequencies of Tf electrophoresis were in Kilis goats. So they were in HWE in terms of Tf genotypes (Figure 6).

In Honamli goats, significance of observed and expected Tf gene frequencies was important in the 1st herd (P<0.05), as it was not in HWE in terms of Tf genotypes, while others were in equilibrium (Fig. 7).

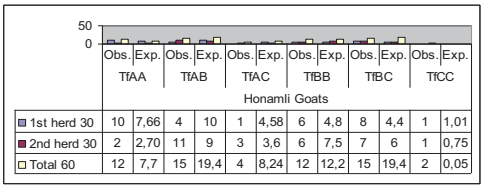
Table 2. Tf genotypes, allele frequencies and homologous/ heterologous genotypes

		Kilis Goat			Honamli Goat		
		1st Herd	2nd herd	Total	1st herd	2nd herd	Total
	N	30	30	60	30	30	60
GENOTYPE	Tf AA	2 % 6.67	1 % 3.33	3 % 5.00	10 % 33.33	2 % 6.67	12 % 20.00
	TfAB	8 % 26.67	15 % 50.00	23 % 38.33	4 % 13.33	11 % 36.67	15 % 25.00
	TfAC	1 % 3.33	1 % 3.33	2 % 3.33	1 % 3.33	3 % 10.00	4 % 6.67
	TfBB	7 % 23.33	58 % 26.67	15 % 25.00	6 % 20.00	6 % 20.00	12 % 20.00
	TfBC	8 % 26.67	5 % 16.67	13 % 21.67	8 % 26.67	7 % 23.33	15 % 5.00
	TfCC	4 % 13.33	- % 0.00	4 % 6.67	1 % 3.33	1 % 3.33	2 % 3.37
FREQUENCY	Tf^A	13 0.216 7	18 0.300 0	31 0.258 3	25 0.416 7	18 0.300 0	43 0.358 0
	Tf^B	30 0.500 0	36 0.600 0	66 0.550 0	24 0.400 0	30 0.500 0	54 0.450 0
	Tf^C	17 0.283 3	6 0.100 0	23 0.191 7	11 0.183 3	12 0.200 0	23 0.191 7
GEN	Tf AA,BB,CC	13 % 43.33	9 % 30.00	22 % 36.66	17 % 56.66	11 % 36.66	28 % 46.66
	Tf AB,AC,BC	17 % 56.66	21 % 70.00	38 % 63.33	13 % 43.33	19 % 63.33	32 % 53.33



1st herd 30	2	1,41	8	6,5	1	3,68	7	7,5	8	8,5	4	2,41
2nd herd 30	1	2,70	15	10,8	1	1,8	8	10,8	5	3,6	0	0,3
Total 60	3	4	23	17,1	2	5,94	15	18,2	13	12,7	4	2,2

Figure 6. Observed and expected values of Tf frequencies in Kilis goats



1st herd 30	10	7,66	4	10	1	4,58	6	4,8	8	4,4	1	1,01
2nd herd 30	2	2,70	11	9	3	3,6	6	7,5	7	6	1	0,75
Total 60	12	7,7	15	19,4	4	8,24	12	12,2	15	19,4	2	0,05

Figure 7. Observed and expected values of Tf frequencies in Honamli goats

There was non-significant difference (P>0.05) between Cp levels in Kilis goat herds. But on the contrary, high level significant difference (P<0.001) was detected between two Honamli herds. Analyzing Cp values of the breeds, the difference from other was fairly high level (P<0.0001) when two breeds were compared (Tab. 3). Cp level average of Honamli goats was significantly higher than Kilis goats.

There was non-significant difference (P>0.05) at GSH levels between Honamli goat herds. A low level significance (P<0.05) was detected in Kilis goats. In addition, fairly high level significant differences (P<0.0001) were found in herds and in the breed (Table 3).

Table 3. CP and GSH values in Kilis and Honamli goats

		Kilis Goat			Honamli Goat		
		1st Herd	2nd Herd	Total	1st herd	2nd herd	Total
	N	30	30	60	30	30	60
CP	X	0.770	0.874	0.822	2.121	1.356	1.739
	±Sx	±0.05	±0.09	±0.05	±0.16	±0.11	±0.10
		7	5	5	1	1	9
	X_{min}	0.157	0.157	0.157	0.786	0.471	0.471
	X_{max}	1.571	2.986	2.986	5.186	2.829	5.186
	P	NS*			<0.001		
GSH	X	41.45	43.52	42.48	32.92	34.10	33.51
	±Sx	3	0	6	8	2	5
		±0.51	±0.79	±1.03	±0.37	±0.72	±0.34
		2	7	4	9	6	5
	X_{min}	36.53	35.46	35.46	28.80	27.20	27.20
		3	7	6	0	0	0
	X_{max}	45.60	49.60	49.60	36.53	46.66	46.66
		0	0	0	3	7	6
	P	<0.05			NS*		

*Non Significant

The scatter diagram was in Fig. 8. According to study material forming the two goat breeds belonging to the 1st and the 2nd herd's CP values were given in there.

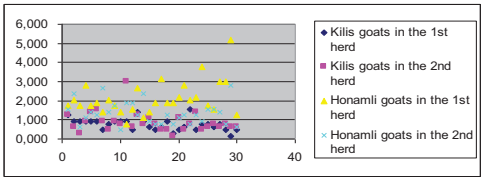


Figure 8. Cp scatter diagram

In Kilis and Honamli goats examined on the basis of GSH levels in herds, the goats above the average named as GSH^H and below average goats named as GSH^h. These two groups were analyzed independently in themselves. Value belonging to the arithmetic mean (X), standard error (Sx) and minimal-maximal values (Xmin-Xmax) were calculated. Results were given in mg/dl. The lowest GSH^h average was observed in the 1st Honamli goat herd (3.1219 ± 0.277 mg/dl); the highest GSH^H average was observed in the 2nd Kilis goat herd (4.7467 ± 0.402 mg/dl) (Table 4).

Table 4. GSH^h and GSH^H levels in Kilis and Honamli goats

		Kilis Goat		Honamli Goat	
		1st herd	2nd herd	1st herd	2nd herd
N		30	30	30	30
GSH^h	X	38.872	40.067	31.219	31.581
	±Sx	±0.440	±0.688	±0.277	±0.412
	X_{min}	36.533	35.467	28.800	27.200
	X_{max}	41.333	43.200	32.533	33.333
GSH^H	X	43.550	47.467	34.524	37.044
	±Sx	±0.327	±0.402	±0.335	±0.950
	X_{min}	41.600	44.533	33.067	34.667
	X_{max}	45.600	49.600	36.533	46.667

GSH scatter diagram of the two goat breeds' the 1st and the 2nd herds was given in Fig. 9.

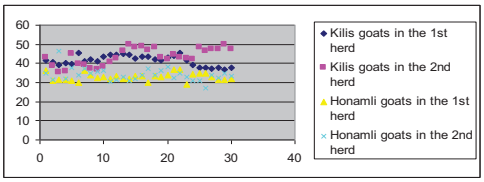


Figure 9. GSH scatter diagram

Obtaining maximum yield from living, raising resistance to various external factors and thus obtaining healthy and superior productive herds of animals is the main target from an economic standpoint.

Hb^A and Hb^B allele frequencies of Kilis goats were calculated as, 0.8333 and 0.1667 in the 1st herd, 0.4833 and 0.5167 in the 2nd herd, respectively. However in Honamli goats, Hb^A and Hb^B allele frequencies were detected as 0.7500 and 0.2500 in the 1st herd, 0.6167 and 0.3833 in the 2nd herd, respectively.

In the 1st and the 2nd herds of Kilis, Tf^A, Tf^B and Tf^C frequencies were calculated as 0.2167, 0.3000; 0.5000, 0.6000 and 0.2833, 0.1000, respectively. The other hand, Tf^A, Tf^B and Tf^C frequencies were detected as 0.4167, 0.3000; 0.4000, 0.5000; 0.1833, 0.2000 in the 1st and the 2nd herds of Honamli breed, respectively.

In this study, TfCC genotype could not be determined in the 2nd herd of Kilis breed. At that rate, only 1 of 4 from the studied herds was not obtained this genotype. The lack of Tf^C allele might be caused this situation. On the

other hand, especially the chance of choosing animals is considered as the main factor.

In this study Kilis and Honamli goat blood samples examined in terms of Hb locus and common allele frequencies were not exceeded 95%. So they have been considered as Hb polymorphic system. The other hand, results correspondingly examined in terms of Tf locus, common allele frequencies were not exceeded 95%. Also, Tf system Yuce ver. Bilgen's study (2004) have been reported a similar manner observed as in the polymorphic. Moreover, also coincides with the rare allele frequency was not less than 5% in this study when Hb and Tf were analyzed. It is desirable in such genetic studies.

In research material, frequencies of Hb genotype could not be provided HWE for herds and populations (Kilis goats: the 2nd herd, intra-population and Honamli goats: intra-population). Panmixi (random mating without selection) of genes and genotypes structure could be said that retains invariance under conditions. In this case the use of a small number of male goats for reproduction was known to be effective. When it comes to selection in goat breeds studied according to whether the reason for departing from the genetic stability of the herd was small and their causes can be connected to use the male. The reverse of this condition was detected in HWE for other populations and herds (Kilis goats: the 1st herd and Honamli goats: the 1st and the 2nd herds). Population size and such as chance factors could be effective on this desired conditions.

Tf results were obtained in analogy to Hb. In research material, Tf genotype frequencies for herds and populations which Kilis goats: the 2nd herd and Honamli goats: the 1st herd were not in the HWE. Genes and genotypes structures could be said that retains invariance under Panmixia conditions. In the case of a small number of male goats in reserched herds were likely inability to maintain the equilibrium. An investigated goat has no selection from breeds in practice. In this case, the main reasons of getting away for departing from the genetic equilibrium were effect of breeding systems, genetic mutations and inadequate herd size. Also, lots of high mating, fertility and reproductive performance of male goats can cause lead to problems in terms of polymorphic studies. This case brings genetic problems due to close kindship. On the other hand, HWE was provided in some herds and populations (Kilis goats: the 1st herd, the 2nd herd and intra-population and Honamli goats: the 2nd herd and intra-population). High level of chance factor can be caused on observed HWE. This equilibrium is desirable genetically.

Observed diversity of Hb and Tf genotypes were interpreted to animals have adapted to different environmental conditions. Additionally this can be adapted to the different environmental conditions of the various animals. If the genotype is very common in a region, environment X genotype interactions presence and indicate living things have a selective advantage. Disease resistance and yield characteristics on the other terms are important in the presence of homozygous and heterozygous genotypes. Also, impact of breed resistance and yield characteristics cannot be ignored.

The weak pasture could be caused significant differences between the obtained Cp results in this study. Furthermore, blood samples were taken in February; after pregnancy and in the first month of lactating period. On the other hand, pasture's weak mineral composition could be caused significant impact on blood levels. In addition, almost all of serum Cu in mammals and birds are in the structure of Cp.

Cobanoglu et al. (2011) have used a total of 15 goats to compare to genetic polymorphisms of Saanen, Malta and Hair goats. They figured out GSH levels on the basis of Emekci and Mert's study (2009) and found GSH^H and GSH^h alleles. Cobanoglu et al. (2011) have viewed the erythrocyte GSH polymorphism in Saanen and Malta goats. They have found GSH^h average as, 13.5 mg/dl and 13.0 mg/dl, respectively. But in Turkish Hair goats, average GSH^H level was measured as 8.9 mg/dl in same study. In these goats GSH^H levels were calculated as 44.6 mg/dl, 39.5 mg/dl and 48.5 mg/dl, respectively while there was a similar results with this study. GSH^h levels were significantly lower than the determined average values.

CONCLUSIONS

At the results of this study conducted on Kilis and Honamli goat herds, the polymorphism results were found to be useful in animal breeding and animal selection. Discussed polymorphism results may be beneficial in animal breeding for population polymorphic studies conducted in various goat breeds.

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