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Full Length Research Paper

Biochemical polymorphism of erythrocyte potassium and glutathione protein: The relationship with some blood parameters in Kivircik sheep breed

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This paper aims to search genetic structures of purebred Kivircik sheep raised in Turkey based on erythrocyte potassium and glutathione types and also to determine the relationship between these components and some blood parameters. The phenotypic frequencies were 68% for LK and 32% for HK erythrocyte potassium types in the purebred Kivircik breed. The allele gene frequency of K^H and K^L loci were calculated as 0.56 and 0.44 respectively. There was no relationship detected between gender factor and blood parameters. But, significant relationships were obtained between erythrocyte potassium types and some blood parameters (Na_{wb}, Na_e, K_{wb}, K_p, K_e, Na_e +K_e; p<0, 05). At the same time, the correlation coefficient between K_e and Na_e was calculated as R = -0.58, (p<0.01). But, correlation coefficient was not significant between blood parameters and hematocrit value (PCV). All animals were detected as low types in terms of glutathione level (GSH^h). The mean of erythrocyte glutathione was determined as 29.79 mg/dL in red blood cell. The mean of erythrocyte potassium concentrations (K_e) were 9.22 and 23.47 mmol/L for the LK and HK types of animals, respectively and the mean differences between LK and HK types were statistically important in this study (p<0.01).

Key words: Kivircik sheep, erythrocyte potassium, glutathione, blood polymorphism.

INTRODUCTION

Blood parameters have been widely used to determine genetic diversity in animal populations. Because, blood biochemical polymorphism may be used for determining the type of twin births, identifying the parentage test and estimating the genetic distance among the animal populations. Many studies were conducted with different sheep breeds to determine the level of various blood parameters and to evaluate if any relationship between blood polymorphism and production traits existed.

Therefore, blood parameters can be used as a marker to identify relation to a production trait in domestic animals (Alpan and Ertuğrul, 1991). Biochemical components of blood groups show polymorphic structures and are determined in the early stage of an animal's life span. Also, these components are affected by environmental factors at minimum level.

Therefore, erythrocyte potassium and glutathione types may be used as indirect selection criteria to improve production and reproduction traits of livestock (Kidd et al., 1974; Kimura, 1968; Lush, 1971). On the other hand, another study did not report an important relationship with production traits (Soysal, 1983). Therefore, these blood parameters may be used as an index value for selection criteria in animal improvement programs. Potassium is one of the intracellular elements which constitute the structure of an organism. Generally, the amount of potassium concentration is fairly high at intracellular membranes. The main function of potassium is to

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Abbreviations: HK, High potassium; **LK,** low potassium; **GSH,** glutathione level; **PCV,** hematocrit value; **Na_{wb}**, whole blood sodium; **Na_p**, plasma sodium; **Na_e**, erythrocyte sodium; **K_{wb}** whole blood potassium; **K_p**, plasma potassium; **K_e**, erythrocyte potassium, regulate the intracellular density of cells (Ellory and Tucker, 1970). In opposition to the potassium concentration, the amount of sodium concentration is higher at the outside of the cell membrane.

Thus, a sodium-potassium balance is established with energy released by hydrolysis of ATP. The existence of blood potassium types in different species have been shown in different species including sheep (Soysal et al., 2003), goats (Soysal and Ülkü, 1998) for goat and cattle (Evans and Philipson, 1957; Gonzales et al., 1984). The potassium and sodium concentrations in blood are related with some economical yield traits for livestock animals (Sengupta, 1974; Antunovic et al., 2004; Milewski and Szczepanski, 2006).

In sheep, there are two potassium types, one of them is high potassium (HK), and the other type is low potassium (LK) which is dominant to the (HK) type. The HK and LK allele gene frequencies are different among sheep breeds (Pembeci, 1978). In some situations, the amount of potassium concentration in erythrocyte may change based on the hematocrit value and the amount of total erythrocyte (İçer, 2003). One of blood proteins is glutathione (GSH) formed by three amino acid; glutamic acid, cystein and glycine. In general glutathione levels in erythrocyte may be accepted as a constant among adult mammalian (Atroshi, 1979). Glutathione concentration in erythrocyte is determined by a pair of autosomal gene which is called high glutathione (GSH^H) and low glutathione (GSH^h).

Also the GSH^H gene is dominant towards GSH^h (Atroshi et al., 1981) Töre (1979) reported that there was a relationship between erythrocyte potassium value and glutathione types and the erythrocyte potassium level was lower in GSH^h types of animals than in GSH^H types of animal. Even if Kivircik is one of Turkish indigenous sheep breeds located in the Marmara Region of Turkey, it is very difficult to find purebred animals due to extensive crossing, mainly with Merino sheep. Therefore, the purebred Kivircik breed is raised and controlled as *in situ* not only by governmental animal research institutes but also by private farms located in that region as one of the native genetic resources of Turkey. This breed is generally characterized by their long - thin tails and white coat colour.

On average, the live weight of mature animals is 45 - 50 kg for male and 35 - 40 kg for female, lactation period is about 150 -160 days and milk production is approximately 60 - 90 kg for a Kivircik ewe. The importance of this breed is the quality of meat which is much better than that of other sheep breeds raised in Turkey.

Therefore, Kivircik lamb meat is more preferred among the sheep meat consumers. This paper aims to show the genetic structures of purebred Kivircik sheep based on erythrocyte potassium and glutathione types and to determine the relationship between potassium type in erythrocyte and some of the parameters in the blood stream.

MATERIALS AND METHODS

Animals

The animal material constituted of 50 purebred Kivircik sheep which were located in the Kirklareli region in Turkey. Blood samples were obtained from 16 males and 34 females' age 1 - 2 years old.

Blood typing

Blood samples were taken by 20 ml sterilized and labelled vacuum tubes with anticoagulant (lithium heparin) from the external jugular vein. Blood samples were divided into two parts; one part was used for determination of the erythrocyte potassium polymorphism, and the other one was for erythrocyte glutathione polymorphism. Moreover, blood samples were used to show a relationship with some blood parameters including whole blood sodium (Na_{wb}), plasma sodium (Na_p), erythrocyte sodium (Na_e), whole blood potassium (K_{wb}), plasma potassium (K_p), erythrocyte potassium (K_e), and total monovalent cation concentration in erythrocyte (Na_e+K_e). Hematocrit value (%) (PCV) was determined by the microhematocrit method which was indicated by Burtis et al. (1994). According to this method, blood samples were centrifuged at 10,000 rpm for 5 min within two hours after the blood samples were collected. The potassium and sodium concentrations in the whole blood and plasma samples were determined by flame photometry (Jenway PFP 7).

Additionally, erythrocyte potassium and sodium concen-trations were calculated by using the amount of potassium and sodium in the whole blood and plasma and hematocrit values based on the following formula as mmol/L (Gonzales et al., 1984).

$$K_e = K_p + [(K_{wb} – K_p) / (PCV /100)]$$

$$Na_e = Na_p + [(Na_{wb} – Na_p) / (PCV /100)]$$

K_p: concentrations of K in plasma, Na_p: concentrations of Na in plasma
K_{wb}: concentrations of K in whole blood, Na_{wb}: concentrations of Na in whole blood
K_e: concentrations of K in erythrocyte, Na_e: concentrations of Na in erythrocyte
PCV: packed cell volume (%).

Erythrocyte potassium types were named LK (low potassium) for animals if the concentrations of K_e were below or equal to 13.00 mmol/L and HK (high potassium) for animals if the K_e concentrations were over this value (Galip and Elmacı, 2001). The erythrocyte glutathione concentrations were determined by spectrophotometer at 412 nm (Burtis et al., 1994). Also erythrocyte glutathione concentrations were calculated using the following formula as mg/dL.

GSH, mg/dL in eritrosit **=** [observed standard GSH concentration value / (PCV/100)]

Erythrocyte glutathione types were called GSH^h (low glutathione) type for animals if the concentration of GSH^h is below or equal 50.00 mg/dL and GSH^H (high glutathione) for animals if the glutathione concentration is over this value (İçer, 2003).

Data analysis

Gene frequencies were calculated with the square root method for

Table 1. The mean and standard error of blood parameters for erythrocyte potassium types and gender factors of purebred Kivircik breeds.

Blood parameters	Potassium types		Gender factor	
	LK, n = 34	HK, n =16	Female, n = 34	Male, n =16
	X ± Sx	X ± Sx	X ± Sx	X ± Sx
PCV (%)	32.79 ± 1.20 [a]	33.38 ± 1.44 [a]	33.67 ± 1.10 [a]	32.43 ± 1.69 [a]
Na_{wb} (mmol/l)	218.38 ± 2.27 [a]	210.70 ± 3.69 [b]	201.88 ± 2.76 [a]	192.63 ± 3.45 [a]
Na_p (mmol/l)	205.62 ± 2.09[a]	201.31 ± 3.31 [a]	203.92 ± 2.31 [a]	204.92 ± 2.69 [a]
Na_e (mmol/l)	245.89 ± 7.85 [a]	225.50 ± 13.5 [b]	197.60 ± 10.4 [a]	164.46 ± 7.98 [a]
K_{wb} (mmol/l)	9.97 ± 0.33[a]	13.88 ± 0.61[b]	11.34 ± 0.53 [a]	10.96 ± 0.53 [a]
K_p (mmol/l)	10.77 ± 0.37 [a]	11.25 ± 0.60 [b]	9.97 ± 0.40 [a]	10.96 ± 0.53 [a]
K_e (mmol/l)	9.22 ± 0.45 [a]	23.47 ± 1.59 [b]	14.04 ± 1.71 [a]	11.22 ± 0.38 [a]
Na_e+K_e (mmol/l)	255.11 ± 7.45 [a]	249.01 ± 12.45 [b]	211,68 ± 10,11[a]	175,68 ± 5,25[a]
GSH (mg/100 ml)	27.36 ± 1.45 [a]	25.60 ± 1.25 [a]	29.08 ± 0.90 [a]	31.30 ± 1.73 [a]

a and b at the same row are statistically significant (p<0.05)

(K_e) types, thus low potassium alleles (K^L) were dominant to high potassium alleles (K^H) (Cotterman, 1954). The deviations from Hardy-Weinberg Equilibrium were tested by the chi-square test. IBM SPSS Statistics 18 base packed program was used in all statistical analyses (IBM SPSS Statistics 18, 2010). Some basic statistical analysis and correlation coefficients were calculated for each blood parameter in this study. The mean concentration of each parameter was compared not only between genders but also between potassium types of animals by using student's t-test. Scatter diagrams were also displayed for all blood parameters in this study.

RESULTS

In this present study, phenotypic frequencies were observed at 68% for LK and 32% for HK types of animals for erythrocyte potassium. At the same time, the allele frequencies were calculated as 0.56 and 0.44 for K^H and K^L, respectively. The observed frequencies of potassium types are found to be in Hardy-Weinberg equilibrium. Even if animals have various amounts of glutathione concentration, all animals were determined as low types for glutathione protein alleles (GSH^h) since all measured concentration levels are lower than 50 mg/dL in this study. Thus, the gene frequency of GSH^h is calculated as 1.00. Table 1 describes blood parameters according to gender and erythrocyte potassium types for purebred Kivircik sheep.

Hematocrit values are observed at 33.67 and 32.43% for female and male animals, respectively. Erythrocyte sodium concentrations (Na_e) are found at 197.60 and 164.46 mmol/L for female and male animals, respect-tively. The mean erythrocyte potassium concentrations (K_e) are calculated at 14.04 and 11.22 mmol/L for female and male animals, respectively. As a result, gender is not identified as an important factor for blood parameters (Na_{wb}, Na_p, Na_e, K_{wb}, K_p, K_e, Na_e+K_e) in this study. The mean glutathione concentrations in erythrocyte calculated at 29.08 and 31.30 mg/dL for female and male animals, respectively, but this difference is also not statistically important.

In this study hematocrit values of blood are also evaluated based on an animal's erythrocyte potassium type. Hematocrit values (%) are found at 32.79 and 33.38% for LK and HK types of animals, respectively. But, the observed difference is not statistically significant for hematocrit values of blood. In addition, glutathione concentrations in erythrocyte are observed at 27.36, 25.60 mg/dL for LK and HK animals, respectively. Unfortunately, this difference is not important statistically, too. The mean of erythrocyte potassium concentration (K_e) is 9.22 mmol/L and it ranges from 4.23 to 11.69 mmol/L for the LK type of animals. Also, the mean erythrocyte potassium concentration (K_e) is 23.47 mmol/L, ranging from 15.59 to 37.78 mmol/L for the HK type of animals. In addition, the mean erythrocyte sodium concentration (Na_e) is 245.89 mmol/L, ranging from 66.67 to 304.97 mmol/L for the LK type of animals. Moreover, the mean value of Na_e is calculated as 225.50 mmol/L and it ranged from 144, 3 to 330, 1 mmol/L for HK type of animals. Overall, erythrocyte potassium type is statistically significant for blood parameters (Na_{wb}, Na_e, K_{wb}, K_p, K_e, Na_e+K_e) ($p<0.05$), except for (Na_p) ($p>0.05$). The distribution of potassium (K_e), sodium (Na_e), and glutathione GSH) concentrations in the erythrocyte and hematocrit value (%) (PCV) of animal are given in Figures 1a, b, c, and d, respectively. Correlation coefficients among various blood parameters in purebred Kivircik breeds are shown in Table 2. Na_e and K_e are significantly correlated with the total monovalent cation concentrations in erythrocyte (Na+K_e) as R = 0.89 and R = 0.65, respectively ($p<0.01$). Furthermore, correlation coefficients (R) were calculated at -0.58 between K_e and Na_e; at -0.54 between Na_e and Na_p ($p < 0.01$), at 0.29 between K_e and K_p ($p<0.05$) and 0.83 between K_e and K_{wb}, ($p < 0.01$), respectively. However, PCV values were not significantly correlated with erythrocyte potassium, sodium and total

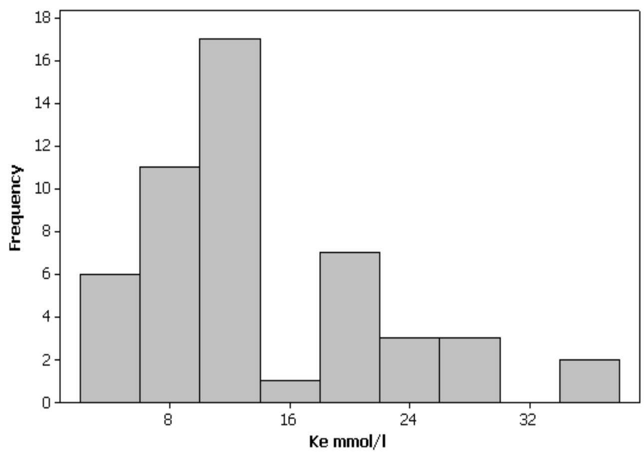

Figure 1a. The distribution of potassium concentration in the erythrocyte (mmol/l).

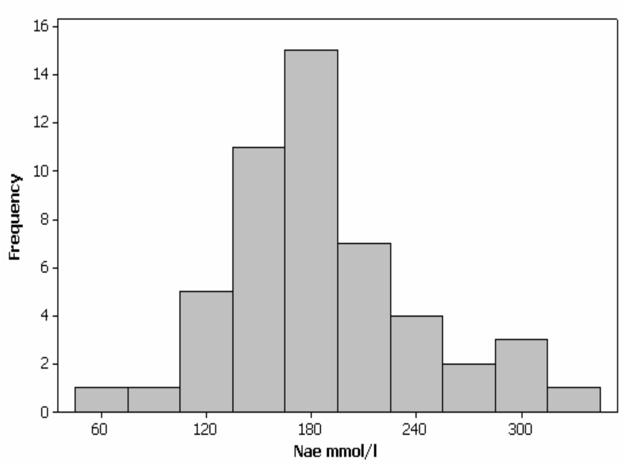

Figure 1b. The distribution of sodium concentration in the erythrocyte (mmol/l).

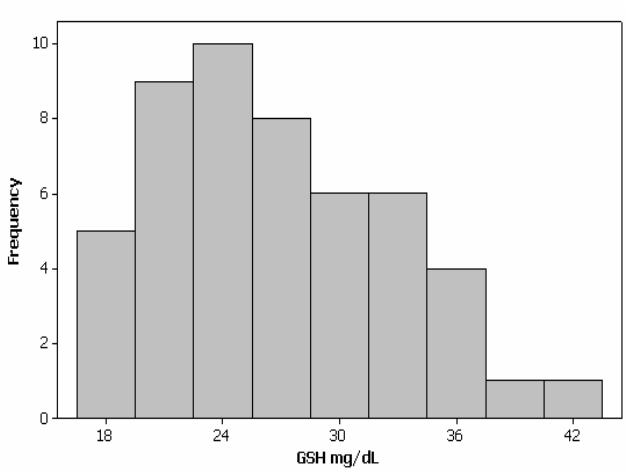

Figure 1c. The distribution of GSH concentration in the erythrocyte (mg/dL).

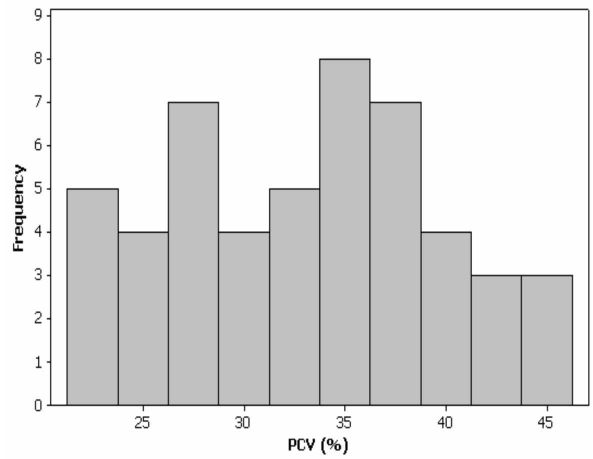

Figure 1d. The distribution of packed cell volume (PCV [%]) in the blood stream.

monovalent cation concentrations in this study. Also, there is no significant correlation between hematocrit value and blood parameters.

DISCUSSION

The potassium and glutathione polymorphism have been investigated in various studies for 5 - 6 decades. In one study which also included Turkish native sheep breeds, the K^H and K^L gene frequencies were detected for Imroz, Kivircik and Merino breeds as 0.86 and 0.14; 0.77 and 0.23; 0.19 and 0.81, respectively (Töre, 1979). Similarly, in the present study, gene frequencies of potassium types, (K^H and K^L) were also found as 0.56 and 0.44, respectively. On the contrary, the gene frequencies were calculated 0.19 and 0.81 for K^H and K^L alleles from Kivircik sheep respectively (Soysal et al., 1998). At the same time, K^H and K^L frequencies were determined at 0.77 and 0.23 in Karacabey Merino x Kivircik crossbred sheep (Gurcan and Cobanoglu, 2009). In addition to these studies, K^H and K^L frequencies were also detected in some other sheep breeds in Turkey. Soysal (1989) were calculated KH and K^L frequencies as 0.22 and 0.78 in Türkgeldi sheep, respectively. But, K^H and K^L frequencies were determined as 0.31 and 0.69 in Karayaka sheep (Soysal et al., 2003).

Hematocrit values are measured for female and male animal but the difference between genders is not important statistically. Similarly Gurcan and Cobanoglu (2009) detected 31.04 and 32.06% for female and male animals, respectively and this difference was not significant either. This value was also investigated among various sheep breeds in other studies. In these studies, it was reported that the hematocrit values were 41.0 and 38.14% for males and females in Hamdani sheep (Eksen et al., 1992); 31.67% for both genders in Karagül (Belge et al., 1997); 30.91 and 38.61% for males and females in

Table 2. Correlation coefficients among various blood parameters in purebred Kivircik breeds.

Blood parameters	PCV (%)	Na_{wb} mmol/l	Na_p mmol/l	Na_e mmol/l	K_{wb} mmol/l	K_p mmol/l	K_e (mmol/l)
Na_{wb} mmol/l	0.07						
Na_p mmol/l	0.02	-0.10					
Na_e mmol/l	0.10	0.86**	-0.54**				
K_{wb} mmol/l	0.11	-0.61**	0.05	0.45**			
K_p mmol/l	0.09	-0.12	0.62**	-0.38**	0.23		
K_e mmol/l	0.01	0.58**	-0.26	-0.58**	0.83**	0.29*	
Na_e+K_e (mmol/l)	0.10	0.86**	-0.53**	0.89**	0,53**	-0.38*	0.65**

[1]*= p<0.05, ** = p<0.01

et al., 1997); 30.91 and 38.61% for males and females in Tuj and 34.80, 32.50% for males and females in Morkaraman breeds (Çelebi and Uzun, 2000); and 35.70% for both genders in Ivesi sheep (İçer, 2003). Additionally, hematocrit value was investigated in pre and post feeding period for some breeds. It ranged from 29.79 to 33.19% in Merino; from 28.60 to 32.96% in Morkaraman; from 26.52 to 32.18% in Ivesi and from 27.57 to 32.14% in Tuj respectively (Dayioğlu and Doğru, 1996). In the same study, the hematocrit values between pre and post feeding periods among various breeds were found to be important statistically. Also, they detected a significant relationship between hematocrit value and live weight at the pre feeding period in Morkaraman breed. In the present study, the mean of blood parameters and PCV concentrations are not different between male and female animals. On the other hand, the mean of blood parameters for LK types of animals are not similar to those which are observed in HK animals (Na_{wb}, Na_e, K_{wb}, K_p, K_e, Na_e+K_e) (p<0.05), except for (Na_p, GSH, PCV). There are significant differences for the mean K_e and Na_e concentrations between LK and HK types of animals (p<0.01).

Soysal et al. (1998) have reported that the mean of potassium and sodium concentrations were found to be between 26. 31 and 290.7 mg/dL for LK type of Kivircik sheep and between 92.95 and 420 mg/dL for HK types of animals, respectively. While the LK values ranged from 10.62 - 30.24 mEq/L, the HK values were observed to be between 64.04 and 105.83 mEq/L in the İvesi breed (İçer, 2003). In another study, the whole blood sodium concentration amount was found to be between 20 and 540 mg/dL and the whole blood potassium amount were reported to be between 10 and 140 mg/dL in the Türkgeldi breed (Soysal, 1989). Likewise in the present study, the erythrocyte glutathione level was 37.80 and 67.26 mg/dL for GSH^H in the Ramlıç breed (Çamaş et al., 1987) and 9.3 and 38.18 mg/dL for GSH^H in the Morkaraman breed (Çetin and Mert, 1993). Glutathione concentration was observed at 41.70 mg/dL in erythrocyte in general but this value was 31.80 and 64.79 mg/dL erythrocyte for GSH^H in Ivesi (İçer, 2003). In terms of the relationship between sodium and potassium concentrations, a significant correlation has been found in the present study; as in other studies where the correlation coefficient was found to be important for Morkaraman (R = -0,49) and for Ivesi (R = -0.50), (p<0.05) except for R = 0.037 in Merino sheep (Doğru et al., 1991). Some previous studies were designed to see the effect of blood potassium and glutathione types on production traits. In one study with Konya Merino sheep, erythrocyte potassium and glutathione types were observed to be significant factors affecting the growth performance of animals (Serpek et al., 1993). However in another study, there was no relationship found between whole blood potassium types and fleece traits for Karagul and Karayaka sheep (Soysal et al., 2003).

Conclusion

Although many scientists are working intensively to see the effect of polymorphism at DNA level in various species, it is important to know genetic structure of animals in terms of blood protein polymorphism. Thus, several studies are being conducted to observe the effect of blood polymorphism on production and reproduction traits in livestock. In this study, first of all, erythrocyte potassium and glutathione polymorphism were identified in purebred Kivircik sheep breed and also the correlation coefficients were calculated among blood parameters. No significant relationship was observed between gender and blood parameters, but the effect of potassium types were detected on different blood parameters, except for Na_p, GSH, and PCV. In the future, this type of study should be designed with more animals of both genders.

In conclusion, these blood parameters may be used to compare the genetic structures of different sheep breeds, and also it might be utilized as an indirect selection criterion to increase the overall sheep production level.

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