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Full Length Research Paper

Erythrocyte potassium and glutathione polymorphism determination in Saanen x Malta crossbred goats

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This research is aimed at determining the erythrocyte potassium and glutathione polymorphisms and also to identify the relationship among the various blood parameters in Saanen x Malta crossbred goat raised in Turkey. The allele gene frequencies of K^H and K^L associated with the potassium concentration were calculated as 0.94 and 0.06, respectively. The differences between the mean values of low and high potassium concentrations in erythrocyte were statistically significant ($P < 0.01$). In addition, there were some significant relationships between erythrocyte potassium types and some blood parameters such as whole blood sodium and potassium concentrations, erythrocyte sodium and potassium concentrations and total monovalent cation concentration in erythrocyte ($P < 0.05$). The allele gene frequencies of GSH^H and GSH^h related with the glutathione concentration were calculated as 0.43 and 0.57, respectively. The difference between the mean values of low and high glutathione erythrocyte concentrations were also statistically significant ($P < 0.01$). Finally, the significant correlation coefficient between erythrocyte sodium and potassium concentrations was observed in this study ($P < 0.05$).

Key words: Erythrocyte potassium, glutathione, blood polymorphism, Saanen, Malta goat.

INTRODUCTION

There are several local goat breeds raised in different regions of Turkey. Mainly, the most common goat breed is Hair goat but Angora, Malta, Kilis and their crossbreds and few other local goat breeds are also raised. However, goat population was significantly decreased in the last decades and an approximate 5.5 million hair goat

and nearly 160 thousand Angora goat were reared in Turkey (Anonymous, 2008). Overall production level of indigenous goat breeds is lower than the level of the exotic breeds. Therefore, the crossbreeding programs are performed to increase productivity of local breeds mainly with Swiss Saanen goat in various regions of Turkey.

Blood polymorphism studies have been conducted extensively to identify biodiversity among livestock animals. Biochemical particles of blood can be determined easily at the postnatal period of young animals and these components are merely or not affected by the environmental factors. Many researches were conducted to detect the different types of blood components such as haemoglobin, transferrin, albumin, glutathione and potassium. In general, the existence of blood potassium and glutathione polymorphisms in cattle (Evans and Philipson, 1957; Gonzales et al., 1984), sheep (Soysal et al., 2003 and Gurcan et al., 2010) and goat (Soysal and Ulku,

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Abbreviations: Na_{wb} , Whole blood sodium concentration; Na_p , plasma sodium concentration; K_{wb} , whole blood potassium concentration; K_p , plasma potassium concentration; Na_e , erythrocyte sodium concentration; K_e , erythrocyte potassium concentration; Na_e+K_e , total monovalent cation concentration in erythrocyte; HK , high potassium; LK , low potassium; GSH^h , low glutathione allele; GSH^H , high glutathione allele; K^H , high potassium allele; K^L , low potassium allele;

1998) have been shown in some studies.

Several studies were also carried out to know the association between biochemical polymorphism of blood and various traits (Alpan and Ertugrul, 1991). If an association exists, then the erythrocyte potassium and glutathione types in blood can be utilized as a polymorphic marker among domestic animals (Kimura, 1968 and Lush, 1971). Even if some studies did not find any significant relationship with production traits (Soysal, 1983), a few studies displayed an important association between glutathione types and milk production traits in Finn sheep (Atroschi and Sondholm, 1982) and between the potassium and sodium types and some economical yield traits in livestock animals (Antunovic et al., 2004; Milewski and Szczepanski, 2006). On the other hand, one of the recent studies showed that there was no relationship between the haemoglobin-transferring types and milk yield in Norduz goats (Aygun and Mert, 2007). Furthermore, correlations were calculated to show the relationship between various blood parameters in some studies. For example, while a high correlation was found between erythrocyte sodium and potassium values in Turkish Hair goat (Galip and Elmaci, 2001), non significant correlation was detected between glutathione in erythrocyte and hematocrit values (Igbokwe et al., 1998).

This study aims to detect the genetic makeup of Saanen x Malta crossbred goat depending on the glutathione and potassium types in erythrocyte and also to find if the association between erythrocyte potassium and some of the blood parameters exists.

MATERIALS AND METHODS

Animal material and feeding

The animal material consisted of 42 Saanen x Malta crossbred goat which are located in the Malkara region in Tekirdag. Blood samples were obtained from only 42 females which are about 1 to 2 years old. On average, the live weight of mature animals is 45 to 50 kg; milk production is approximately 2 to 3 kg/day. All animals were fed with roughage in the morning period and roughage and mixed feed in the evening period. Animals were fed with about 1 kg mixed feed which has 18 to 24% crude protein (CP). About 600 to 800 g maize silage and 1.5 kg maize and approximately 250 g grass hay daily per animals were used for herd feeding.

Blood parameters analysis

Blood samples were taken by vacuum tubes with lithium heparin as an anticoagulant from animals before feeding in the early morning. The flame photometry was used to detect some major blood parameters including whole blood sodium concentration (Na_{wb}), plasma sodium concentration (Na_p), whole blood potassium concentration (K_{wb}), plasma potassium concentration (K_p). In addition, erythrocyte sodium concentration (Na_e), erythrocyte potassium concentration (K_e) and total monovalent cation concentration in erythrocyte (Na_e+K_e) were estimated by using the amount of sodium and potassium in plasma as well as in the whole blood and

packed cell volume values were calculated based on the following formula as mmol/l (Gonzales et al., 1984).

$$K_e = K_p + [(K_{wb} - K_p) / (PCV * 10^{-2})]; Na_e = Na_p + ((Na_{wb} - Na_p) / (PCV * 10^{-2}))$$

Where PCV, is the hematocrit value. If the K_e is below or equal to 13.00 mmol/l, then it is named as low potassium (LK), conversely; if the K_e is over this value, then it is called as high potassium (HK); Galip and Elmaci, 2001).

The erythrocyte glutathione concentrations were read by using the spectrophotometer at 412 nm (Burtis and Ashwood, 1994). Moreover, the glutathione concentrations were estimated using the following formula as mg/dL in erythrocyte.

$$GSH = (\text{observed GSH value} / (PCV * 10^{-2}))$$

If GSH concentration is below or equal to 20 mg/dL, then it is called low glutathione (GSH^l). On the other hand, if the concentration is over 20 mg/dL, then it is named as high glutathione (GSH^h) (Ekmekci and Mert, 2009). In addition, hematocrit value (%) (PCV) was identified by the microhematocrit method according to Burtis and Ashwood, (1994).

Data analysis

Since the low potassium allele (K^l) were dominant to high potassium allele (K^h) and likewise GSH^h were dominant over to GSH^l , the allele frequencies were estimated by the square root method for potassium and glutathione types (Cotterman, 1954). Based on this method, homozygote gene frequency was found by taken the square root of homozygote recessive genotype frequencies. The Chi-square test was applied to know if the deviation between observed and expected genotypic frequencies assured the assumption of Hardy-Weinberg equilibrium.

All statistical analyses were done by the Statistical Package for the Social Sciences (SPSS) statistics 18 base packed program (IBM SPSS statistics 18 2010). Some descriptive statistics and correlation coefficients between the blood parameters were calculated. The mean concentration of each parameter was compared between potassium types in animals by the student's t-test. Kolmogorov-Smirnov (K-S) test were also used to determine whether PCV, GSH, K_e and Na_e were distributed normally. In addition, histogram charts were displayed for these blood parameters in this study.

RESULTS AND DISCUSSION

In this study, glutathione polymorphism displayed a polymorphic structure. The phenotypic frequencies for the erythrocyte glutathione types were detected as 19% for low GSH and 81% for high GSH. At the same time, the allele gene frequencies of GSH^h and GSH^l loci were calculated as 0.43 and 0.57, respectively (Table 1). Besides, phenotypic frequencies of erythrocyte potassium were observed as 10% for LK and 90% for HK types of animals. Similarly, the allele frequencies were calculated as 0.94 and 0.06 for K^h and K^l , respectively. The observed frequencies of potassium and glutathione types were found to be in Hardy-Weinberg equilibrium based on Chi-square test (Table 1).

Table 1. Distribution of phenotypic and gene frequencies based on the potassium and glutathione types.

Potassium type	Phenotypic frequency (%)	Gene frequency	Glutathione type	Phenotypic frequency (%)	Gene frequency
LK	10	K ^L 0.06	High GSH	81	GSH ^H 0.57
HK	90	K ^H 0.94	Low GSH	19	GSH ^h 0.43

Table 2. Descriptive statistics for blood parameters based on the erythrocyte potassium types.

Blood parameter	LK types (n=4)				HK types (n=38)			
	$\bar{x} \pm s$	CV (%)	Min	Max	$\bar{x} \pm s$	CV (%)	Min	Max
PCV (%)	29.3 ± 8	27	21	40	26.9 ± 4	15	18	34
Na _{wb} (mmol/l)	174 ± 3**	13	153	196	150.6 ± 3**	5	148.7	191.7
Na _p (mmol/l)	157.1 ± 7	20	141	168	143.8 ± 3	12	90	182.7
Na _e (mmol/l)	217 ± 20**	24	179	240	173 ± 32**	18	79	330
K _{wb} (mmol/l)	29 ± 3*	10	26.3	32.9	35.7 ± 4*	11	28.5	43.9
K _p (mmol/l)	26.2 ± 2	6	18.1	29.5	31.1 ± 7	20	17.1	43.5
K _e (mmol/l)	11.1 ± 2**	20	8.2	13.2	49.2 ± 3**	23	26	91.3
Na _e +K _e (mmol/l)	228 ± 15*	27	214.5	242.1	221.9 ± 2*	27	144.5	360.1
GSH (mg/dl)	23.8 ± 3	12	20.3	26.4	22.3 ± 3	9	19.3	27.4

*P < 0.05; **P < 0.01.

Table 3. Correlation coefficients among blood parameters.

Blood parameter	PCV	Na _{wb}	Na _p	Na _e	K _{wb}	K _p	K _e	Na _e + K _e	GSH
PCV	1.00	-0.37*	-0.40*	0.02	0.13	0.32	-0.22	-0.24	-0.11
Na _{wb}		1.00	0.37*	0.49**	0.14	-0.14	0.16	0.55**	-0.12
Na _p			1.00	0.14	-0.19	-0.04	-0.03	0.45**	0.24
Na _e				1.00	0.17	-0.21	-0.34*	0.75**	-0.13
K _{wb}					1.00	0.23	0.51**	0.59**	0.05
K _p						1.00	0.21	0.32*	0.19
K _e							1.00	0.60**	-0.16
Na _e + K _e								1.00	-0.17
GSH									1.00

*P < 0.05, **P < 0.01.

Some basic statistical results are given in Table 2 for blood parameters based on animal erythrocyte potassium types. The mean of erythrocyte potassium concentrations (K_e) were 11.1 and 49.2 mmol/l, for the LK and HK types, respectively. The mean erythrocyte sodium concentration (Na_e) was also detected as 217 and 173 mmol/l for the LK and HK type of animals, respectively. Finally, the differences between erythrocyte potassium types were found statistically significant for blood parameters of Na_{wb}, Na_e, K_e (P < 0.01); K_{wb}, and Na_e+K_e (P < 0.05). Hematocrit values (%) of blood were calculated as 29.3 and 26.9% for LK and HK type of animals, respectively. But the observed differences of hematocrit values were not statistically important in this research. On the

contrary, the erythrocyte glutathione concentrations were detected as 18.5 and 23.3 mg/dL for GSH low and GSH high type of animals, respectively and the difference between glutathione concentrations was observed to be statistically significant (P < 0.01).

Correlation coefficients among various blood parameters are given in Table 3. PCV values were significantly correlated with Na_{wb} and Na_p as R= -0.37 and R= -0.40 (P < 0.05), respectively. Similarly, the correlation between Na_e and K_e was observed as R= -0.34 in erythrocyte which was statistically significant (P < 0.05). The important correlations between Na_e+ K_e with Na_{wb} (R= 0.55), K_{wb} (R= 0.59), Na_p (R= 0.45), K_e (R= 0.60), Na_e (R= 0.75) (P < 0.01) and K_p (R= 0.32; P < 0.05) were also detected,

Table 4. Kolmogorov-Smirnov's normality test (K-S) for blood parameters.

Blood parameters	K-S value	P-value
PCV	0.059	0.15
GSH	0.120	0.13
K _e	0.136	0.11
Na _e	0.135	0.11

respectively. On the other hand, the correlation between GSH and the other blood parameter concentrations were not important in this study. Moreover, the erythrocyte potassium, sodium and glutathione concentrations and hematocrit value (%) displayed the normal distribution based on the Kolmogorov-Smirnov's normality test (K-S) in Table 4 and given as histogram charts in Figure 1a, b, c and d, respectively.

In the present study, the mean of blood parameters were observed only for the female animals since there was no evidence for significant differences between two genders in the previous studies (Aikhuomobhogbe and Orheruata, 2006; Gurcan et al., 2010). But it should be noted that, the observed difference might be important if the number of animals from both gender could be increased. In addition, the mean of potassium concentration (K_e) were found 9.0 and 48.5 mmol/l for LK and HK types in Turkish hair goat, respectively (Galip and Elmaci, 2001). They also reported significant differences for Na_e and K_e between LK and HK types as reported in the present study. In another study conducted with Saanen and hair goats raised in Marmara region, the erythrocyte potassium concentration reported 97.6 and 82.5 mEq/l, respectively. All Saanen and 90% of hair goats were classified as high potassium type (Turkyilmaz, 2003). The breed difference in terms of HK type was also found to be statistically significant in the study. Similar results for HK type were obtained in the present study. Even if LK type of potassium is dominant to its counterpart genetically, the majority of animals were interestingly observed as HK type in goat flocks. This situation could be mean that HK-type animals might have a selective advantage in nature.

From study with Nigeria Sahel goats, the mean erythrocyte glutathione concentrations were reported as 46.5 mg/dL and 74.8% of animals were detected as the low GSH type (Igbokwe et al., 1998). They also stated that, even if there were some anemic animals in the flock, the phenomena of anemia was not directly related with the low GSH in erythrocyte, thus, the biochemical reasons of the low GSH was needed to be ascertained thoroughly. In a study with Saanen and hair goats, the difference between breeds for erythrocyte glutathione concentrations was statistically significant and most of the animals from both breeds were classified as low GSH

type, as well (Turkyilmaz, 2003). On the contrary, there was no anemic animals based on packed cell volume percentage (PCV%) value and most goats displayed high GSH type in the present study. In addition, Garcia et al. (2003) studied blood polymorphism in 3 different breeds of goat (Tinerferip, Majorero, Polmero) from Canary Islands. In their study, Na, K and GSH concentrations were calculated as 73.4, 30.3 mEq/l and 67.9 mg/dL in erythrocyte, respectively, where K and GSH values showed a normal distribution as was reported in other studies. Moreover, in the study with Norduz goat flocks raised in commercial and Agricultural Research Institution in Van, Turkey, statistically significant differences were observed for blood parameters (Hb, K and GSH) between two flocks (Ekmekci and Mert, 2009).

Turkyilmaz (2003) reported that the gene frequencies of GSH^H was higher than the frequency of GSH^h in Saanen goats, however, the frequency of GSH^H was lower than its counterpart in hair goats. In terms of the potassium types, the high frequencies for K^H were detected in both Saanen and HAIR goats (Galip and Elmaci, 2001; Turkyilmaz, 2003). Therefore, there was no contradictory for the gene frequencies among the studies conducted with Saanen goat breed. Furthermore, there were no significant differences with regard to hematocrit percentage values among the previous findings in West Africa Dwarf goats (Daramola et al., 2005), Saanen and hair goats (Turkyilmaz, 2003) and Saanen x Malta crossbred goat in the present study. In terms of the correlation, Galip and Elmaci (2001) stated a high correlation coefficient between Na_e and K_e concentrations in Turkish hair goat (Galip and Elmaci, 2001), as reported in the present study. Igbokwe et al. (1998) also calculated the correlation coefficient between GSH and PCV%, but the correlation was not statistically significant. Likewise, the correlation coefficient between GSH in erythrocyte and all blood parameters were not statistically important in the present study, either.

In conclusion, the erythrocyte potassium and glutathione polymorphisms were identified in Saanen x Malta crossbred goats and the important correlation coefficients were determined among various blood parameters in this study. Some significant relationships were also observed between the erythrocyte potassium types and some blood parameters (Na_{wb}, Na_e, K_{wb}, K_e, Na_e+K_e). In the future, the number of animals should be increased for better estimation of blood parameters and the blood biochemical polymorphism on the various economically important traits should be studied further especially in the indigenous breeds of Turkey.

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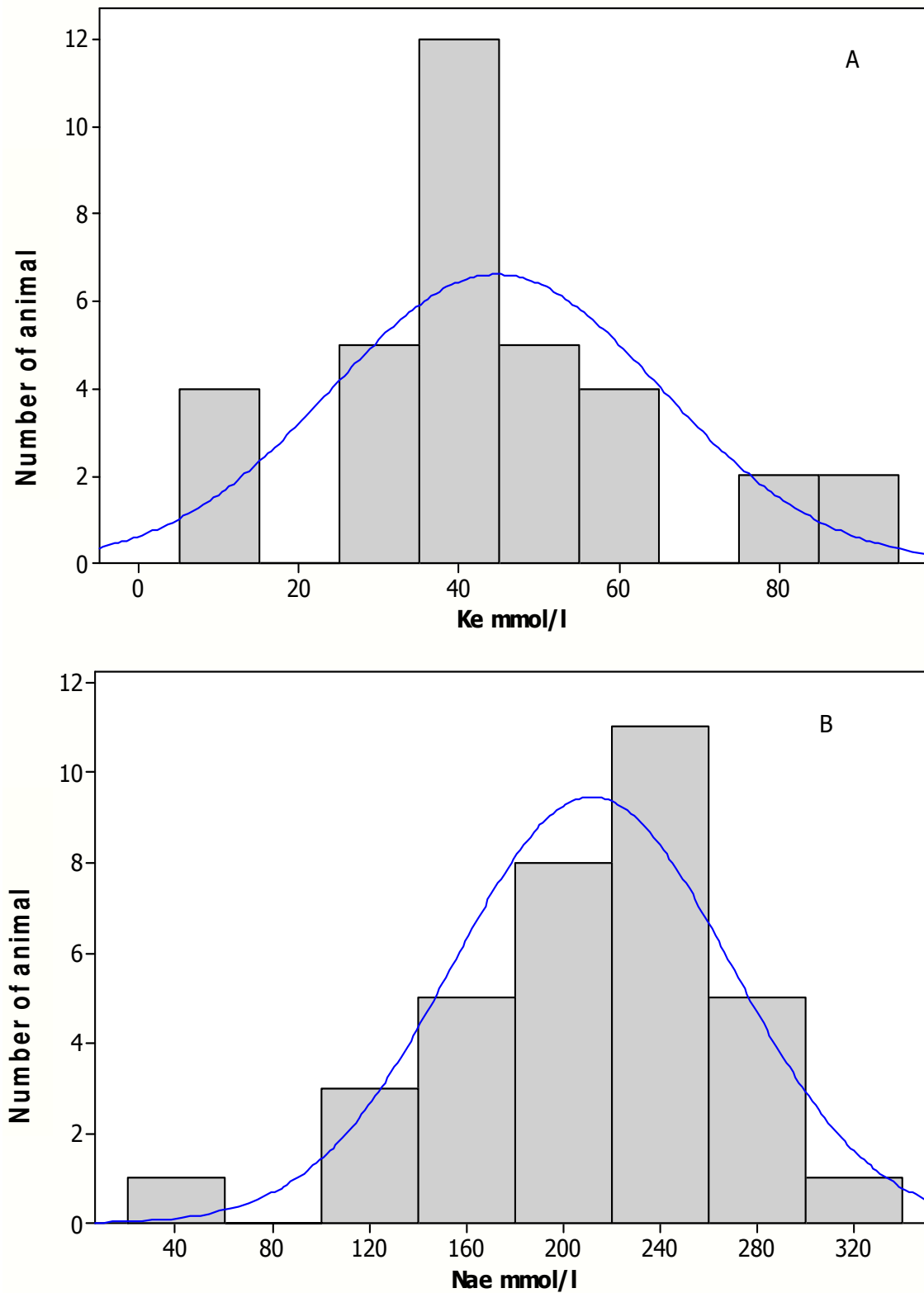


Figure 1. A to B, The distributions of animals based on the potassium (K_e) and sodium concentrations (Na_e) in erythrocyte, respectively; C, D, the distributions of animals based on GSH concentration in erythrocyte and hematocrit value (PCV), respectively.

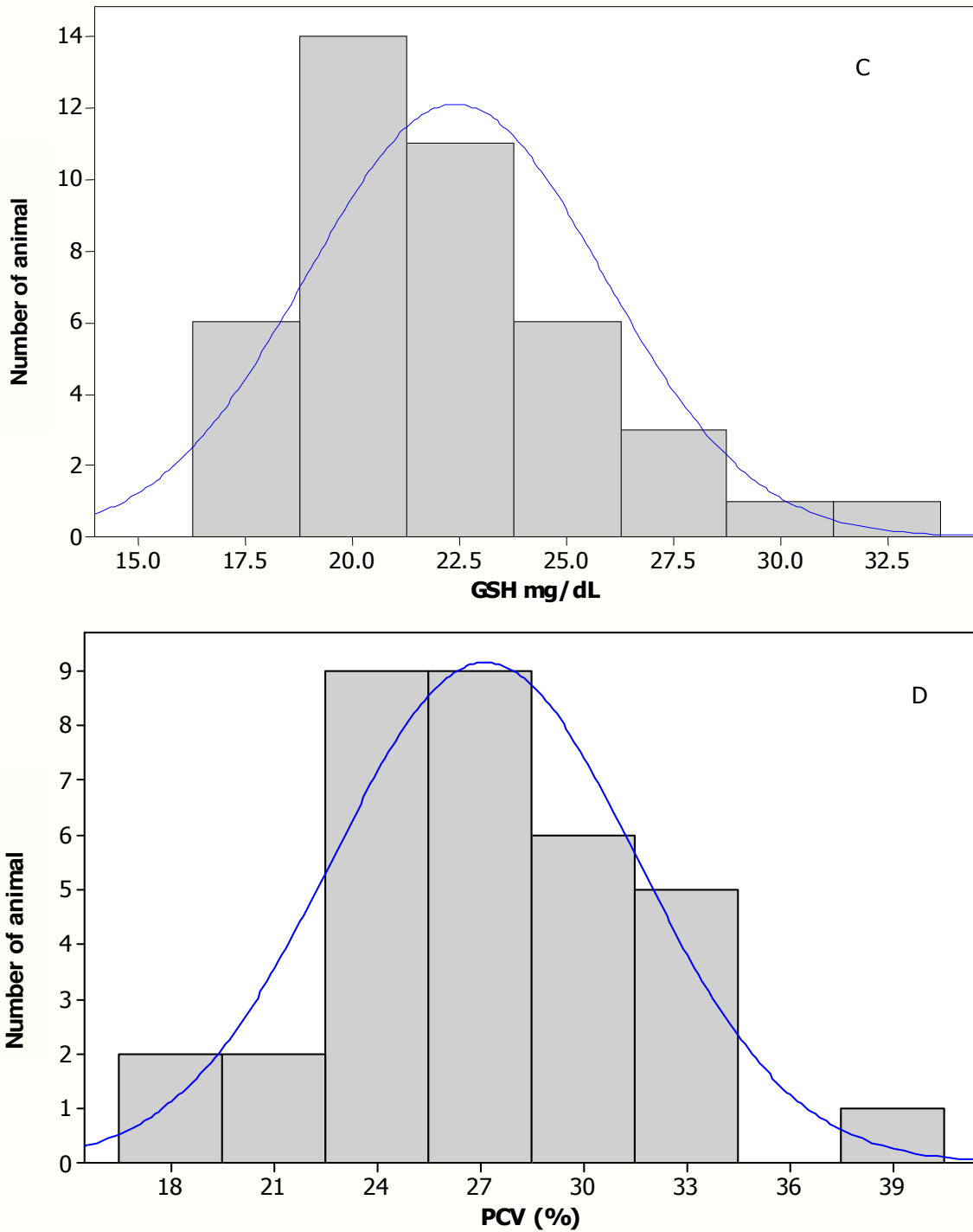


Figure 1. Contd.

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