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Erythrocyte Potassium, Sodium and GSH Concentrations of Saanen and Turkish Hair Breeds of Goats

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Abstract.- Biochemical variation of three genetic markers, namely erythrocyte potassium (K_e), erythrocyte sodium (Na_e) and erythrocyte glutathione (GSH) in sixty Saanen and Turkish Hair Goats was analysed. The potassium and sodium concentrations were determined by flame photometry. The level of GSH in erythrocytes was determined by the DTNB method. The frequencies of the mentioned loci have been calculated and compared with some of the goat breeds studied so far by other authors. Our results indicated that there were bimodal distributions in K_e and GSH concentrations in Saanen goat breeds, whereas there was only GSH polymorphism in Turkish Hair Goats. The distribution of K_e concentrations suggested the presence of high-K/low-K (HK/LK) polymorphism, based on the predetermined dividing value of 50 meq/L of K_e concentration in erythrocytes of Saanen Goats. Both types are shown to be controlled genetically by an autosomal locus with two alleles HK and LK, with HK being dominant over LK. While majority of the Turkish Hair Goat population (93%) had low (GSH^h) or 'deficient' erythrocyte GSH concentrations (<50 mg/100 mL erythrocyte), Saanen breeds (93%) had high (GSH^H) erythrocyte GSH levels. Relationships between the parameters were also evaluated. In Saanen Goats, erythrocyte K_e and Na_e values exhibited positive correlation with the total monovalent cation concentrations (K_e + Na_e) while in Turkish Hair Goats only K_e value showed correlation with the same monovalent cations.

Key words: Erythrocyte potassium, glutathione, Saanen goats, Turkish hair goats.

INTRODUCTION

The existence of blood potassium and GSH types in goats has been reported (Haba *et al.*, 1991; Khan and Taneja, 1983; Komatsu *et al*., 1980; Mostaghni, 2004; Tuñon *et al*., 1987a,b) in various goat populations. Most animal cells maintain high internal potassium and low sodium ion concentrations due to the activity of the sodium/potassium-ATPase (Na/K-ATPase) pump in the membrane, which uses energy derived from the hydrolysis of ATP to accumulate potassium ions and expel sodium ions. The enzyme ATPase is intimately involved in the potassium or sodium-pump mechanism (Tucker, 1971). Evans (1954) showed that erythrocyte potassium values varied in sheep and two distinct types could be found in British breed. He demonstrated that some sheep (HK-type) have red cell potassium values of 80-90 mmol/L and other sheep (LK-type) have 20-30 mmol/L. This difference is genetically controlled and the gene(s) determining the HK type being apparently dominant to that determining LK type (Tucker *et al.*, 1973).

The red cell contains relatively large quantities of GSH, a tripeptide of glutamate, cysteine and glycine. GSH functions by protecting the protein-SH groups of enzymes, haemoglobin or the membrane from oxidation (Agar *et al*.,1983). In some sheep breeds, levels of GSH show a distinct bimodal distribution and it is possible to divide animals into two groups according to their erythrocyte GSH concentration. Sheep with GSH values below 55 mg/100 mL erythrocyte are classified as GSH low type (GSH^h) and those with contents above this value are regarded as GSH high type (GSH^H). GSH concentration is regulated by a pair of autosomal alleles; however the main effect of this gene is modified by environmental and genetic factors (Atroshi and Osterberg, 1979).

To our knowledge the information about red blood cell K^+, Na^+ and GSH types of Saanen Goats

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and the Turkish Hair Goats is not yet available. In the present study we aim to investigate and to compare both breeds with regard to the above parameters. Data obtained would be useful for breeding programs.

MATERIALS AND METHODS

Animals

Thirty Saanen Goats were obtained from Balikesir Province Animal Breeding Association in Turkey and 30 Turkish Hair Goats came from a commercial farm in the same region. All procedures involving animals were approved by the Animal Care Committee of Uludag University.

Sampling

Blood samples were collected by jugular vein puncture directly into heparinized tubes. The hematocrit was determined by centrifuging the blood in heparinized capillary tubes in a micro capillary centrifuge (Nuve Laboratory Equipment, Ankara, Turkey) for 5 min at 3,000x g in a centrifuge (Hettich, EBA 21 Centrifuge, GMI Inc., Minnesota, USA) the cellular fraction was separated from the plasma. The potassium and sodium concentrations in the whole blood and plasma were determined by flame photometry (PFP 7 Flame Photometer, Jenway Ltd., Essex, England) in 1:200 diluted samples using the procedures explained by the manufacturer's instructions. Erythrocyte potassium and sodium concentrations were calculated using whole blood and plasma values of these electrolytes and the hematocrit values were assessed by using the following formula (Gonzalez *et al.*,1984):

$$Xe = [Xp + [(Xwb - Xp) / (PCV : 100)]$$

X, sodium or potassium; Xp, concentration of cation in plasma; Xwb, concentration of cation in whole blood; Xe, concentration of cation in erythrocyte; PCV, hematocrit.

The level of reduced glutathione (GSH) in erythrocytes was determined as according to Beutler (1971).

Statistical analysis

Statistical analysis was carried out using SPSS version 13.0 for Windows. The mean concentration and standard deviation of each parameter were calculated and compared by the Student `t' Test. For parametric values Mann-Whitney test was used. Correlations among erythrocyte potassium and sodium were analyzed with Pearson's Correlation Test.

RESULTS

The results obtained from the analyses of erythrocyte, plasma and whole blood potassium, sodium, glutathione and hematocrit levels in Saanen and Turkish Hair Goats are presented in Table I. Individuals showing erythrocyte potassium concentration below or equal to 50 meq/L were grouped as LK, whereas those reaching values higher than this one were grouped as HK. The overall range of K_e and Na_e concentrations were 72.8–129.9 meq/L erythrocyte and 67.0–105.0 meq/L erythrocyte, respectively, in the Saanen

Table I.- Mean (SEM) potassium, sodium and glutathione levels in erythrocyte, plasma and whole blood of Saanen and Turkish hair Goats and hematocrit levels.

Blood parameters	Saanen Goats	Turkish Hair Goats
K_e (meq/L)	97.6±2.3 (72.8–129.9)	82.5±4.0** (21.8–111.2)
Na_e (meq/L)	85.4±1.7 (67.0–105.0)	90.0±1.9 (72.0–114.0)
K_p (meq/L)	5.1±0.1 (3.8–6.2)	4.9±0.1 (3.9–6.0)
Na_p (meq/L)	141.8±0.7 (132.0–149.0)	141.7±1.0 (131.0–151.0)
K_{wb} (meq/L)	37.6±0.9 (30.2–47.2)	24.7±0.6** (19.0–32.2)
Na_{wb} (meq/L)	133.2±1.1 (121.0–145.0)	144.4±2.0** (129.0–170.0)
$K_e + Na_e$ (meq/L)	183.0±3.2 (144.7–235.0)	172.5±4.2 (111.8–217.2)
GSH (mg/100 mL erythrocyte)	94.3±4.8 (40.5–159.0)	30.7±3.1*** (6.7–88.7)
Hematocrit (%)	33.8±0.1 (22.0–44.0)	23.4±0.5** (18.0–28.0)

The values are presented as Mean±SEM (distribution values) of 30 animals.
The level of significance between Saanen and Turkish Hair Goats groups in the same line: **, P<0.01, ***, P<0.001.
Ke: Erythrocyte potassium; Na_e, Erythrocyte sodium; K_p,

Plasma potassium; Na_p, Plasma sodium; Kwb, Whole blood potassium; KNa, Whole blood sodium; GSH, Glutathione.

Goats and were 21.8–111.2 meq/L erythrocyte and 72.0–114.0 meq/L erythrocyte, respectively, in the Turkish Hair Goats. K_e, K_{wb}, Na_{wb}, monovalent cation concentrations, GSH and hematocrit values of both type goats were different.

The correlation co-efficiencies among several blood parameters for Saanen Goats and Turkish Hair Goats are presented in Tables II and III, respectively. K_e and Na_e values in Saanen Goats presented positive correlation with monovalent cations (Table II). In Turkish Hair Goats positive correlation was established with K_e and monovalent cations (Table III).

Table II.- Correlation among various blood parameters in Saanen.

	K_e (meq/L)	Na_e (meq/L)	GSH (mg/100 mL erythrocyte)
Na_e (meq/L)	0.281	-	-0.203
K_e (meq/L)	-	0.281	-0.120
Monovalent cations (K_e + Na_e) (meq/L)	0.863**	0.728**	-0.195
K_p (meq/L)	-0.021	-0.253	0.300
Na_p (meq/L)	0.314	0.047	0.182
Hematocrit (%)	-0.180	0.210	-0.224

(* P<0.05, ** P<0.01)

Na_e, Erythrocyte sodium; Ke, Erythrocyte potassium; K_p, Plasma potassium; Na_p, Plasma sodium; GSH, Glutathione.

Table III.- Correlation among various blood parameters in Turkish Hair Goats.

	K_e (meq/L)	Na_e (meq/L)	GSH (mg/100 mL erythrocyte)
Na_e (meq/L)	-0.137	-	-0.037
K_e (meq/L)	-	-0.137	-0.011
Monovalent cations (K_e + Na_e) (meq/L)	0.888**	0.334	-0.028
K_p (meq/L)	0.098	0.085	-0.330
Na_p (meq/L)	0.097	0.290	-0.028
Hematocrit (%)	-0.045	0.140	0.060

(** P<0.01)

Na_e, Erythrocyte sodium; Ke, Erythrocyte potassium; K_p, Plasma potassium; Na_p, Plasma sodium; GSH, Glutathione.

Distribution of K_e types were also investigated for both goat breeds. All Saanen Goats presented HK type (data not shown), while the majority of Turkish Hair Goats (90%) presented HK profile (Table IV). Thus, HK animals were predominating in both goat races. Comparison of LK and HK animals and blood parameters of Turkish Hair Goats are shown in the same table. The mean K_e and monovalent cations concentrations were found to be statistically different (P<0.01) between LK and HK animals.

Table IV.- Mean (SEM) values for characters of erythrocyte, plasma and whole blood of low (LK) and high-potassium (HK) in Turkish Hair Goats.

Blood parameters	LK	HK
K_e (meq/L)	28.2±5.7	88.6±2.5**
Na_e(meq/L)	97.3±8.4	89.2±2.0
K_p (meq/L)	4.7±0.3	4.9±0.1
Na_p(meq/L)	140.7±2.0	141.7±1.0
K_{wb} (meq/L)	24.3±0.3	24.7±0.6
Na_{wb}(meq/L)	155.0±4.1	143.2±2.1
K_e + Na_e (meq/L)	125.7±4.2	177.7±3.4**
GSH (mg/100mL erythrocyte)	31.5±8.2	23.5±0.4
Hematocrit (%)	22.7±2.7	30.6±3.4

The values are presented as Mean±SEM of 3 animals in LK group and 27 animals in HK group.

The level of significance between LK and HK groups in the same line: **, P<0.01.

Ke, Erythrocyte potassium; Na_e, Erythrocyte sodium; K_p, Plasma potassium; Na_p, Plasma sodium; Kwb, Whole blood potassium; KNa, Whole blood sodium; GSH: Glutathione.

Table V.- Mean (SEM) values of glutathione distributions in the Saanen Goats and Turkish hair Goats

Glutathione type	n	Saanen Goats	n	Turkish Hair Goats
GSH (mg/100mL erythrocyte)	30	94.3±4.8 (40.5–159.0)	30	30.7±3.1 (6.7–88.7)
GSH^h (mg/100mL erythrocyte)	2	40.5±0.1 (40.5–40.6)	28	24.4±2.2 (6.7–47.8)
GSH^H (mg/100mL erythrocyte)	28	98.2±4.2 (53.4–159.0)	2	76.6±12.1 (64.5–88.7)

The values are presented as mean±SEM (distribution values).

GSH, Glutathione; GSH^h, Low glutathione; GSH^H, High glutathione.

GSH values ranged between 40.5–159.0 mg/100 mL erythrocyte (94.3±26.1) for Saanen Goats and 6.7–88.7 mg/100 mL erythrocyte (30.7±17.2) for Turkish Hair Goats. Goats with GSH values below 50 mg/100 mL erythrocyte were classified GSH-low type (GSH^h) and those with above this value were regarded as GSH-high type (GSH^H). As shown in Table V, there is a bimodal distribution. Twenty eight goats (93%) of Saanen were GSH^H type, while twenty eight goats (93%) of Turkish Hair were GSH^h.

DISCUSSION

Potassium, sodium and GSH concentration of mammalian erythrocytes and their regulation constitute characteristics specific to these cells and also a consequence of their high specialization. Electrolyte polymorphism studies were undertaken for several animal species. Polymorphism of erythrocyte potassium content was first described in sheep (Evans, 1954). A pair of alleles at one locus was proposed as being the cause of such variation, the gene for LK behaving as if dominant over that for HK type. Polymorphic cells allowed the establishment of pattern of ionic and volume regulation in erythrocytes. LK cells are the consequence of low active cation-transport and high passive membrane permeability; whereas the HK cells display reverse of this behaviour (Tosteson and Hoffman, 1960).

The inheritance of potassium type in goats is similar to that in sheep; in fact there is a bimodal distribution in Turkish Hair Goat. The difference is genetically controlled and the gene determining HK type being apparently dominant to that determining the LK type. Absence of a clear bimodal distribution in Saanen goats might be caused by the small number of LK animals found generally in that breed. Previous studies performed on goats revealed that the distribution of red cell potassium concentrations can be variable. The mean K_e concentrations in LK and HK-type Turkish Hair Goats obtained in the present study are higher than those found by Galip and Elmacı (2001), who reported a mean of erythrocyte potassium levels in the LK and HK groups of 9.0 and 48.5 meq/L, respectively in the Turkish Hair Goats. Nevertheless, Boztepe *et al.* (1993) reported higher K_e values for the Turkish Hair Goat where the values were ranged 50.9-60.4 meq/L for LK animals and 74.9-113.5 meq/L for HK animals. These discrepancies obtained by authors for the same breed could be linked to fairly wide geographic area in which the animals were originated and to the fact that the animals are brood generally under reproductively isolated conditions. On the other hand, K_e concentrations were similar to those found in Spanish goat breeds (24.8, 71.4 meq/L) (Tuñon *et al*., 1987b) and those reported by Khan and Taneja (1983) for Marwari goats.

As regard erythrocyte sodium concentrations, both breed were of high sodium type (HNa). In the total population the mean sodium concentration were 85.4 meq/L in Saanen samples and 90.0 meq/L in Turkish Hair samples, and no significant difference was found between these values.

GSH levels showed clear bimodal distribution, both in the Saanen and Turkish Hair Goats. The frequency of GSH^h was fairly high and the predominant type among the Turkish Hair Goats. There is no detailed information about the pathophysiological effect of erythrocyte GSH deficiency in goats. The genetic control of GSH concentrations seemed to be complex in sheep breeds. A number of GSH deficiency type was described in Tasmanian Merino sheep (Tucker and Kilgour, 1972), Finnish Landrace sheep (Young *et al.*, 1976) and Awassi sheep (Tucker *et al.*, 1973). It has been reported that GSH deficiency in sheep could be resulted in impaired permeability of the cells to certain amino acids and reduction of γ-glutamyl cysteine synthetase activity (Young *et al*., 1976; Young and Nimmo, 1975)

The role of GSH in the potassium transport system in the red cells of goats is poorly understood (Tucker, 1971). However, in sheep, glutathione might be involved in potassium transport. Dick *et al.* (1969) suggested that sodium-potassium activated ATPase activity is inhibited by oxidized glutathione. Tucker and Kilgour (1970) reported that sheep with genetically determined deficiency of reduced GSH in their red cells had lower than normal red cell potassium, and probably also lower red cell sodium

concentrations.

By the present study, K, Na and GSH values of Saanen and Turkish Hair Goat were compared. The underlying cause of the blood biochemical polymorphism in Turkish Hair Goat is still to be ascertained. Further studies on better understanding of interrelationship between metabolism and GSH and cation levels in the red cell are being planned in our laboratory.

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