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Erythrocyte potassium, sodium and glutathione concentrations and their relationship with reproduction, body weight and fleece weight traits in Awassi sheep

Abstract
Relationship between erythrocyte potassium (K_e), erythrocyte sodium (Na_e) and blood glutathione (GSH) concentrations and production/reproduction traits were investigated in Awassi sheep. Thirty healthy sheep subjected to analyze for phenotypic and gene frequencies of K_e, Na_e and GSH concentrations. Correlation between these parameters and production/reproduction traits such as greasy fleece weight, milk production, birth rate, single and twin birth rate and body weight were calculated. A positive correlation was found between birth rate and mean K_e concentration ($r=0.481$, $P<0.008$) and Na_e concentration ($r=-0.454$, $P<0.013$). Another correlation between GSH concentration and greasy fleece weight ($r=-0.368$, $P<0.049$) and milk production ($r=0.379$, $P<0.050$) parameters was also evidenced. These suggest that K_e, Na_e and GSH concentrations may be included in the metabolic profile testing parameters.

Keywords: Awassi sheep, erythrocyte potassium, erythrocyte sodium, glutathione, biochemical polymorphism, production traits, reproduction traits

Zusammenfassung
Titel der Arbeit: **Zusammenhang zwischen Erythrozytenkalium, -natrium sowie Glutathionkonzentration und Reproduktionsmerkmalen, Gewicht und Wollcharakteristika bei Awassi-Schafen**
Das Verhältnis zwischen den Erythrozytenkalium (K_e), -natrium (Na_e) sowie Blutwerten von Glutathion (GSH) und den Produktions- und Reproduktionsmerkmalen wurden bei Awassi-Schafen untersucht. Dreißig Tieren wurden Blutproben entnommen, um Korrelationen zwischen phenotypischen Eigenschaften und Genfrequenzen von K_e, Na_e und GSH zu bestimmen. Korrelationen zwischen den genannten Blutparametern und Produktion- und Reproduktionsmerkmalen, wie Wollgewicht, Milchproduktion, Geburtsrate, Zwillingsrate und Körpergewicht, wurden errechnet. Eine positive Korrelation wurde zwischen Geburtsrate und mittlerer K_e-Konzentration ($r=0.481$, $P<0.008$) und Na_e-Konzentration ($r=-0.454$, $P<0.013$) festgestellt. Es wurde ebenfalls eine Korrelation zwischen GSH-Konzentration und Wollgewicht ($r=-0.368$, $P<0.049$) sowie Milchproduktion ($r=0.379$, $P<0.050$) nachgewiesen. Diese Ergebnisse weisen darauf hin, dass die K_e, Na_e und GSH-Konzentrationen eventuell als metabolische Testparameter verwendet werden können.

Schlüsselwörter: Awassi-Schafe, Erythrozytenkalium, Erythrozytennatrium, Glutathion, biochemischer Polymorphismus, Produktionsmerkmale, Reproduktionsmerkmale

Introduction
Awassi sheep constitute approximately 7% of sheep population in Turkey. This breed has high adaptation capability and is the unique sheep race with high milk capacity which is able to live in flocks (AKCAPINAR, 2000).
The relation between blood parameters and production/reproduction traits may be controlled by the same genes which is called "pleitrophic effect" of genes (AYALA and KIGER, 1980). Most animal cells maintain high internal potassium and low

sodium ion concentration 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) reported that erythrocyte potassium concentration varied in sheep and two distinct types could be found in British breed. The author demonstrated that some sheep have erythrocyte potassium values of 80-90 mmol/ L (HK-type) while others have 20-30 mmol/ L (LK-type). This difference is genetically controlled and the gene(s) determining the HK type being apparently dominant to LK type (TUCKER, 1971). It has been evidenced by several researchers that erythrocyte potassium and sodium concentrations in domestic animals are associated with some production/ reproduction traits such as milk production, body weight, mortality rate, fleece weight, fertility and adaptation capacity (RASMUSEN et al., 1974; MULEI et al., 1988; TUCKER et al., 1973; ATROSHI, 1979; KRISHNAMURTHY et al., 1978; SENGUPTA, 1974; ANTUNOVIC et al., 2004; MILEWSKI and SZCZEPANSKI, 2006). The erythrocyte cell contains relatively large amount of GSH. GSH functions by protecting the protein-SH groups of enzymes, hemoglobin or membrane from oxidation (AGAR and BOARD, 1983). Glutathione peroxidase catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. An integral component of the enzyme glutathione peroxidase is selenium. The physiological function and peroxidant role of this enzyme have been precisely determined (SZILAGYI et al., 1994; CASTILLO et al., 2001; KOLEDZIEJ et al., 2005). In some sheep breeds, levels of GSH show a distinct bimodal distribution and as potassium and sodium concentrations it is possible to divide animals into two groups according to their erythrocyte GSH concentrations. Sheep with GSH values below 55 mg/ 100 ml erythrocyte are classified as GSH low type (GSH^h) and those having higher concentration than this values are classified as GSH high type (GSH^H) (ATROSHI, 1979). GSH concentration is regulated by a pair of autosomal alleles; however this genetic effect is modulated by environmental and some other genetic factors. (CASTILLO et al., 2001; SWIDERSKA-KOLACZ et al., 2001). Several studies have been conducted to find out correlations between GSH concentration and different production/ reproduction parameters. Higher mortality (TUCKER et al., 1976) and lower body weight (ATROSHI, 1979) were reported for GSH^h lambs. On the other hand, higher body weight gain (ATROSHI, 1979) and better milk production capacity (ATROSHI and SANDHOLM, 1982) were reported for GSH^H ewes. Higher wool production capacity was also stated for Indian sheep having lower erythrocyte GSH concentration (KALLA and GHOSH, 1975).

Adaptation experiments of Awassi sheep, the unique race able to grow up in flocks, are actually carried out in our research center. Data obtained from the present work would be useful for the improvement of Awassi race since there are no detailed studies on the correlation of production/ reproduction and biochemical polymorphism characters of this race. The variability of erythrocyte K and Na concentration and blood GSH level of Awassi sheep and their relationship with some productive/ reproductive traits were investigated.

Materials and methods

Thirty Awassi sheep of sixteen month of age obtained from the "Animal Production, Research and Application Centre" of Uludag University, Faculty of Veterinary Medicine were used as research material. All procedures involving animals were approved by the Animal Care and Use Committee of Uludag University.

The sheep were fed free choice source of high quality clover hay with 300 g concentrate mixture. The concentrate mixture was composed from mainly whole barley (70 %), maize (20 %) and soybean meal (10 %) supplemented with mineral/vitamin combination. Blood samples were collected by jugular venipuncture directly into heparinized tubes. The hematocrit was determined by centrifuging the blood in heparinized capillary tubes in a microcapillary centrifuge (Nuve Laboratory Equipment, Ankara, Turkey) for 5 min at 13000× g and a reader (International Equipment Co., Needham, MA, USA). After centrifugation at 3000× g for 10 min (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 by 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. Hematocrit values were assessed by using the following formula (GONZALEZ et al., 1984):

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

where: 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 (LK) allele frequencies were calculated from the square root of the percentage of (HK) phenotype animals (SOYSAL et al., 2005). The level of reduced GSH in erythrocytes was determined by the method of BEUTLER (1971). Erythrocyte was firstly deproteinated by addition of trichloroacetic acid (TCA) and centrifuged (10 min, 3000 g/min). Thereafter, DTNB [5, 5'-dithiobis (2-nitrobenzoic acid)] was added into the supernatant and the formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm at 25 °C against reagent controls.

Shearing process was performed in the 16th month of age by means of shearing machine (Oster Professional Products, McMinnville, TN, USA). The animals were weighed at the beginning of insemination period (18 months of age). Parturition was towards the end of second age. The ewes were milked twice a day and individual milk production was recorded bi-weekly. When daily milk yield per ewe decreased to 200 ml (approximately 150 days) the animals were dried off (ICAR, 1992). Total milk production was estimated according to THOMAS et al. (2001).

Statistical analysis

Correlations between high and low type erythrocyte potassium, sodium, glutathione groups and other traits were analyzed with pair wise correlation analysis. All statistical analyses were carried out with Minitab version 12 statistical software (MINITAB 12, 1998).

Results

Data regarding to erythrocyte potassium, sodium and glutathione levels, their types, phenotypic and gene frequencies of Awassi sheep are presented Table 1. Individuals having erythrocyte potassium concentration below or equal to 50 mEq/L were grouped as LK whereas those having higher than this value were grouped as HK. The overall range of K_e and Na_e concentrations were 10.62-105.83 and 34.00-126.00 mmol/l, respectively. As it can be seen from the table, both of K_e and Na_e concentration exhibited a bimodal distribution, nonetheless HK and HNa animals were predominant.

Erythrocyte GSH values ranged between 13.05-75.67 mg/100 ml with a mean of 41.70±3.36 mg/100 ml. According to these results we classified the animals as GSH-low type ($GSH^h \leq 50$ mg/100 ml) and GSH-high type ($GSH^H > 50$ mg/100 ml). A bimodal distribution was also noted for this parameter even a significant proportion of individuals were GSH^h.

Table 1
Distribution of erythrocyte potassium, sodium and glutathione groups and their phenotypic and gene frequencies in Awassi sheep (Verteilung von Erythrozytenkalium, -natrium und Glutathiongruppen und deren Phänotypen und Genfrequenzen bei Awassi-Schafen)

Genotype	n	Distribution values	Mean ± SEM	Phenotypic frequency	Gene frequency
K_e (mmol/l)	30	10.62-105.83	73.43±5.10	–	–
LK (mmol/l)	6	10.62- 30.24	23.91±3.04	0.20	0.447
HK (mmol/l)	24	64.04-105.83	85.81±2.69	0.80	0.894
Na_e (mmol/l)	30	34.00-126.00	74.05±4.14	–	–
LNa (mmol/l)	4	34.00- 47.25	43.34±3.12	0.13	0.36
HNa (mmol/l)	26	50.67-126.00	78.78±4.01	0.86	0.93
GSH (mg/100 ml erythrocyte)	30	13.05- 75.67	41.70±3.36	–	–
GSH^h (mg/100 ml erythrocyte)	21	13.05- 46.32	31.80±2.40	0.70	0.836
GSH^H (mg/100 ml erythrocyte)	9	54.27- 75.67	64.79±2.83	0.30	0.547

K_e = erythrocyte potassium; LK = low potassium; HK = high potassium; Na_e = erythrocyte sodium; LNa = low sodium; HNa = high sodium; GSH = glutathione; GSH^h = low glutathione; GSH^H = high glutathione

Some productive/reproductive traits such as fleece yield, milk yield, reproductive efficiency parameters and body weights of sheep having with low and high K_e, Na_e and GSH values are presented in Table 2. Correlations between these parameters are shown in Table 3.

Table 2
Productive/reproductive traits and K_e, Na_e and GSH groups of Awassi sheep
(Produktions- und Reproduktionsmerkmale sowie K_e, Na_e, GSH-Gruppen der Awassi-Schafe)

Genotype	Greasy fleece weight (kg)	Milk yield (kg)	Fertility (%)	Single birth rate (%)	Twin birth rate (%)	Body weight (kg)
LK (mmol/l)	4.9±0.4	134.4±3.6[a]	67	75	25	66.8±4.3
HK (mmol/l)	5.2±0.2	112.1±7.5[b]	100	48	52	64.5±1.4
LNa (mmol/l)	5.3±0.3	85.0±19[c]	100	75	25	69.5±3.1
HNa (mmol/l)	5.1±0.2	120.1±7.9[d]	92	43	57	64.2±1.5
GSH^h (mg/100 ml erythrocyte)	5.3±0.2	111.5±8.4	95	50	50	65.9±2.9
GSH^H (mg/100 ml erythrocyte)	4.6±0.3	123.4±17	88	57	43	64.5±1.6

LK = low potassium; HK = high potassium; LNa = low sodium; HNa = high sodium; GSH^h = low glutathione; GSH^H = high glutathione
a, b = means in the same column with different superscripts were different ($p<0.05$); c, d = means in the same column with different superscripts were different ($p<0.05$)

Table 3
The correlation coefficients (r) between K_e, Na_e and GSH groups and productive/ reproductive traits
(Korrelationskoeffizienten [r] zwischen K_e, Na_e, GSH-Gruppen und den Reproduktionsmerkmalen)

Genotype	Greasy fleece weight	Milk yield	Birth rate	Body weight
K_e (mmol/ l)	0.105	−0.031	0.481**	−0.069
LK (mmol/ l)	0.586	−0.253	0.178	0.718
HK (mmol/ l)	−0.169	0.011	−0.228	0.054
Na_e (mmol/ l)	−0.155	0.189	−0.454*	−0.022
LNa (mmol/ l)	0.955*	0.891	0.574	0.748
HNa (mmol/ l)	−0.150	0.168	−0.437*	0.152
GSH (mg/ 100 ml erythrocyte)	−0.368*	0.379*	−0.269	0.148
GSH^h (mg/ 100 ml erythrocyte	−0.011	0.358	0.014	0.125
GSH^H (mg/ 100 ml erythrocyte	0.714	0.596	−0.400	0.200

*P<0.05, **P<0.01

K_e values positively affected parturition rate (r=0.481, P<0.01) whereas this parameter was negatively influenced from Na_e concentration (r=−0.454, P<0.05). Furthermore, greasy fleece weight was negatively influenced from GSH concentration (r=−0.368, P<0.049). A positive correlation was also calculated between milk yield and GSH concentration (r=0.379, P<0.05).

Discussion

The potassium, sodium and GSH concentrations 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. (SZILAGYI et al., 1994; SWIDERSKA-KOLACZ, 2001; BARANOWSKI et al., 2004; KOLEDZIEJ and JACYNO, 2005). Polymorphism of erythrocyte potassium content was first described in sheep (EVANS, 1954). A pair of alleles replaced at one locus was proposed as being the cause of such variation, the gene for HK behaving as if dominant over that for LK type. Polymorphic cells allowed the establishment of pattern of ionic and volume regulation in erythrocytes. It has been reported that cation pump transfers one sodium ion from outside the cell, but the pump works four times faster than in the HK cell. Also HK cells are relatively permeable to sodium compared to potassium than are LK cells and sodium exchange diffusion is more rapid in LK than in HK animals (TUCKER, 1971). Moreover, strongly evidenced that ATPase activity is involved in the potassium pump mechanism and the activity of this enzyme is 3-4 times greater in HK than LK membranes (TOSTESON, 1963). In LK group animals, positive correlation between levels of ATP and potassium is exist (EATON et al., 1967). LK cells are the consequence of low active cation-transport and high passive membrane permeability; whereas the HK cells display reverse of this behavior (TOSTESON and HOFFMAN, 1960).

EVANS (1957) suggested four distinct groups named $K_{e\gamma}$, $K_{e\alpha}$, $K_{e\lambda}$ and $K_{e\delta}$ of sheep that can be distinguished as to their erythrocyte K concentrations plotted against erythrocyte Na concentrations. $K_{e\delta}$-type sheep are apparently quite rare, but relatively common in Awassi sheep in Israel (EVANS, 1966).

A correlation between erythrocyte potassium and GSH type was evidenced for Finnish Landrace sheep and lower K_e values were obtained with the lower GSH type

animals (TUCKER and KILGOUR, 1973). Our findings are not in agreement with these findings but parallel to other study performed with $K_{e\delta}$-type Awassi sheep having HK, GSH^h type erythrocytes (TUCKER and KILGOUR, 1970).
In the present study, Awassi sheep were classified as high and low Na_e, K_e and GSH types. Distribution of K_e concentrations is similar to previous results obtained for Awassi sheep (EVANS, 1955; EVANS, 1957) with individuals' falling into similarly HK and LK types. HK type was predominant for this race and we have found statistically important correlation with the birth rate and HK concentrations. Similar observations were reported for different sheep races (BARANOWSKI, 2002; ANTUNOVIC et al., 2004; MILEWSKI and SZCZEPANSKI, 2006). BHASKAR et al. (1978) and YATSENKO (1973) reported higher fertility rate for the Mandya and Kırgiz wool sheep respectively having higher K_e concentrations. On the other hand, in a study performed on Black Bengal goats authors did not find correlation with reproductive traits and K_e concentration (MANDAL et al., 1988).
Concentration of GSH has also important role on some productivity traits (CASTILLO et al., 2001; BALICKA-RAMICZ et al., 2006). In the present experiment, GSH^H type Awassi sheep had higher milk production capacity and lower fleece weight. In contrast, milk production was reduced in GSH^h type animals while fleece quantity was augmented. GSH^h concentration seems to be caused by a defect in the amino acid uptake mechanism at the erythrocyte membrane (YOUNG and ELLORY, 1977). The hypothesis that GSH is involved in milk synthesis comes from the finding that the sheep with high GSH produce more milk than GSH^h sheep (ATROSHI and SANDHOLM, 1982). Glutathione concentration in erythrocytes may also be correlated with some other production/reproduction traits. For example MABON (1969) found that change in glutathione concentrations was related to feed efficiency in neonatal calves. Studies to find out correlation between GSH types and birth rate in sheep revealed that GSH^H animals had higher birth rate and advantageous fleece weight compared to other group animals (MERT et al., 1987).
These findings could suggest that since some production/reproduction traits are correlated to Na_e, K_e and GSH groups, selection of animals having superiorities for the desired characters may be done by taking into account these genetic polymorphic characters. Obtained results may be useful in the characterization and improvement of production and reproduction traits in Awassi sheep.

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