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Original Article

Some hematological values and alpha-naphthyl acetate esterase (ANAE)-positive lymphocyte ratios in Jaydara sheep

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ABSTRACT

Objective: The objective of the study was to evaluate the some hematological values and ANAE-positive lymphocyte ratio of Jaydara sheep in Kyrgyzstan. The availability of information on hematological and biochemical parameters is essential to research conducted with an aim to increase yields in animal production.

Materials and methods: The investigation was carried out on 60 healthy sheep aged between 2-3 years old. Leucocyte and erythrocyte counts, hemoglobin levels, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leucocyte ratios and ANAE-positive lymphocyte ratios were investigated in blood samples using an automated cell counter machine.

Results: The leucocyte count in Jaydara sheep was found to 4.92±0.59 x 10^3/mm^3. Similarly, erythrocyte count was 11.73±0.65 x 10^3/mm^3. On the other hand, hemoglobin content in blood was 11.70±0.51 gm/dL. The hematocrit, MCV, MCH, and MCHC values were 30.02±1.70 %, 25.64±1.28 μ^3, 10.68±0.59 pg and 41.76±2.31% respectively. The differential leucocyte counts in Jaydara sheep were 42.53±1.13 (Neutrophil), 52.61±1.08 (Lymphocyte), 2.75±0.55 (Monocyte), 1.58±0.17 (Eosinophil), and 0.52±0.52 (Basophil). The ANAE-positive lymphocyte ratios in Jaydara sheep was found to be 65.00±2.55.

Conclusion: The hematological parameters in healthy Jaydara sheep can be used as reference values for this particular sheep breed in future.

KEYWORDS

Jaydara Sheep, hematological values, ANAE

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INTRODUCTION

Sheep breeding bears economic significance in that it enables the use of poor quality meadows and provides raw material for branches of industry that process various animal products, including food, textile and hides (Nisbet et al., 2006).

In Kyrgyzstan, the husbandary of cattle and sheep stockbreeding in meat production accounts for approximately 48.5% and 24.3%, respectively. One of the major indigenous sheep breeds that have value on the sheep meat market is the Jaydara. Local sheep breeds with rough wool and a fat tail are common throughout the different regions of Kyrgyzstan. The rough-woolen and fat-tailed Kyrgyz sheep is known as the Jaydara Sheep. The Jaydara sheep was formed by the crossing of the different local sheep breeds raised in Uzbekistan. The Jaydara has a rather strong body structure. It is also characterized by early development, good distribution over the grazing area, resistance to most animal diseases, and endurance to long walking distances. The mean body weight of Jaydara rams is 70-80 kg, while ewes weigh 55-60 kg. The color of its wool is brown, yellow and black (FAO, 2002). The wool yield of the Jaydara sheep ranges between 1.8-2.0 kg. At present, it is well known that the Jaydara is not a pure breed, and in fact, displays a combination of characteristics acquired from different breeds, showing a wide variation in terms of tail structure, wool characteristics, body weight and other morphological features. In recent years, Jaydara ewes have been crossed with Gisar and Edilbayev rams with an aim to increase body weight and tail weight (Schillhorn van Veen, 1995).

The availability of information on hematological and biochemical parameters is essential to research conducted with an aim to increase yields in animal production. Hematological and biochemical parameters are used in both the diagnosis of animal diseases and the assessment of the management and nutrition of animals (Soch et al., 2010). Thus, the evaluation of yield traits may identify animals that are physiologically superior at an early age. In this respect, the determination of reference values for the different regions, where these animals are raised, is important. In animals, hematological values may vary with the influence of management, nutrition, environment and climate as well as with sex, age and production traits (Çelebi and Uzun, 2000; Tschuor et al., 2008; Garba and Abubakar 2012). The availability of information on the alterations observed in the hematological values of animals under different conditions is important for the understanding of their metabolism and the reactions they give to the effects they are exposed to environment (Soch et al., 2010).

After mining, sheep and wool production are the most important activities in the Kyrgyz economy. Hematological parameter is an important and reliable medium used to monitor and evaluate health and nutritional status of animals. However, there were no literature about Jaydara's hematological values.In this study, it was aimed to present reference values for some hematological parameters of Jaydara sheep raised in Kyrgyzstan, and thereby, to contribute to further research on the diagnosis and treatment of diseases and management and nutrition disorders in these animals.

MATERIALS AND METHODS

Ethical approval and study location: This study was conducted with the approval of the Ethics Board of the Faculty of Veterinary Medicine of Kyrgyzstan-Turkey Manas University. Sixty clinically healthy Jaydara ewes aged 2-3 years and of a mean body weight of 50-56 kg, which were raised by farmers on the Susamur Plateau (altitude 2500 m) in the Karabalta region of Kyrgyzstan, were used in the study.

Sample collection: Blood samples were collected from the jugular vein into anticoagulant-coated tubes for the measurement of certain hematological parameters (erythrocyte and leukocyte counts, hemoglobin level, hematocrit value, leukocyte percentages and ANAE-positive lymphocyte rate) (Maiti et al., 1990; Soch et al., 2010). The samples were transferred to the laboratory shortly after being collected and under cold chain conditions. The hematological parameters were analysed using an automated cell counter (Mindray BC5380).

Demonstration of alpha-naphthyl acetate esterase (ANAE): For this purpose, two smears were prepared from each blood sample. After being air-dried, the blood smears were fixed in glutaraldehyde-acetone solution (pH=4.8) at -10°C for 3 min. Subsequently, the smears were dried at room temperature and were exposed to an incubation solution (prepared by adding 80 mL of buffered phosphate solution (pH 5.0), and 20 mg of the substrate alpha-naphthyl acetate, N-8505-Sigma) dissolved in 0.8 mL of acetone (Merck) by slow dripping. Later, 4.8 mL of a hexa-azotized pararosanilin mixture, obtained by incubating 2.4 mL of 4% sodium nitrite (S-3421, Merck) solution with 2.4 mL of pararosanilin (P-3750, Merck) (1 gm pararosanilin, 20 mL distilled water, 5 mL HCl concentrate) for 2 min, was added into a substrate-containing buffered phosphate solution. The solution was adjusted to a pH value of 5.8 with 1N NaOH solution and filtered. Following a 2 h period, the incubation was terminated upon the generation of red-brown granules (**Figure 1**, arrowhead). After being

washed in distilled water for 3 times, the preparations were incubated with Giemsa for nuclear staining (Maiti et al., 1990). Two hundred lymphocytes were counted from each sample under 100x objective (Leica DM2500, Switzerland), and the ANAE+ lymphocyte rates (**Figure 1**) were determined.

Statistical analysis: The statistical analysis of the data obtained was made using the SPSS software package and by applying Student's *t*-test.

RESULTS AND DISSCUSION

The data obtained from the Jaydara sheep are presented in **Table 1** and **2** and **Figure 1**. In medical practice, biochemical and hematological analyses are among the primary measurements that are complementary to clinical data obtained by means of laboratory methods which provide significant information for the clinician in regards to early diagnosis, etiology and pathogenesis of diseases and observation of the treatment applied (Soch et al., 2010). The determination of reference values for animal breeds, and thus their use in practice, is a challenging task, therefore, mostly general reference values for the particular animal species are used. However, the most rational approach remains the determination of reference ranges for animal breeds.

In sheep, the mean leukocyte count is 4.0 (2.5-7.5) 10^3/mm^3, and the percentages of the different types of leukocytes are 30 (10-50)% for neutrophils, 62 (40-75)% for lymphocytes, 5 (1-8)% for eosinophil, 2.5 (1-5)% for monocytes, and 0.5 (0-3)% for basophils (Sarıpınar et al., 2004). Research carried out on Awassi sheep has demonstrated the leukocyte count as 7.7- 11.28 10^3/mm^3, the percentages of leukocytes as 37.74-43.25% for neutrophils, 48-53% for lymphocytes, 3.0- 3.76% for monocytes, 3.5-4.94% for eosinophil and 0.23-0.60% for basophils (Yiğit et al., 2002). Further general reference values pertaining to sheep are 12 (8-15) 10^6/mm^3 for erythrocyte count, 12 (8-16) gm/dL for hemoglobin level, 38 (24-44)% for hematocrit value, 33 (23-48) μ^3 for mean corpuscular volume (MCV), 11 (9-12) pg for mean corpuscular hemoglobin (MCH), and 32 (29-35)% for mean corpuscular hemoglobin concentration (MCHC) basophils (Yiğit et al., 2002). In their research on the blood parameters of Merino sheep, Jelinek et al. (1986) reported the mean erythrocyte count as 6.5-10.3 10^6/mm^3, hemoglobin level as 101.3-121.2 g/L, hematocrit value as 32-37%, leukocyte count as 4.98-9.93 10^3/mm^3, leukocyte percentages as 25.8-60.5% for neutrophils, 0.8-8.7% for eosinophil, 0-0.33% for basophils, 30.3-71.2% for lymphocytes and 4-4.5% for monocytes, and MCV as 35.03-55.37 μ^3, MCH as 11.7-16.61 pg, and MCHC as 29.97-34.22%. In research carried out on sheep of the Tuj and Morkaraman breeds, Çelebi and Uzun (2000) reported the mean erythrocyte count as 9.40-11.49 and 8.46-10.44 10^6/mm^3, respectively, the mean leukocyte count as 7.03-7.93 and 6.29-6.31 10^3/mm^3, respectively, the hemoglobin level as 12.22-12.26 and 11.69-12.78 gm/dL, respectively, and the hematocrit value as 30.91-36.68% and 32.50-34.80%, respectively. In another research conducted by Yiğit et al. (2002) on Awassi sheep raised in the Şanlıurfa and its vicinity, some hematological parameters specific to this breed were determined. Accordingly, although having been ascertained to vary with sex, the mean erythrocyte count was determined as 7.34-8.66 10^6/mm^3, the hemoglobin level as 7.96-9.24 g/100 ml, hematocrit value

Table 1. Some hematological parameters in Jaydara Sheep (n=60).

Parameters	Values
Leucoyte (10^3/mm^3)	4.92±0.59
Erythrocyte (10^6/mm^3)	11.73±0.65
Hemoglobin (gm/dL)	11.70±0.51
Hematocrit (%)	30.02±1.70
MCV (μ^3)	25.64±1.28
MCH (pg)	10.68±0.59
MCHC (%)	41.76±2.31

Table 2. Differential leucocyte counts and ANAE-positive lymphocyte ratios in Jaydara Sheep (n=60).

Parameters	Mean leucocyt count
Neutrophil	42.53±1.13
Lymphocyte	52.61±1.08
Monocyte	2.75±0.55
Eosinophil	1.58±0.17
Basophil	0.52±0.52
ANAE+ lymphocyte	65.00±2.55

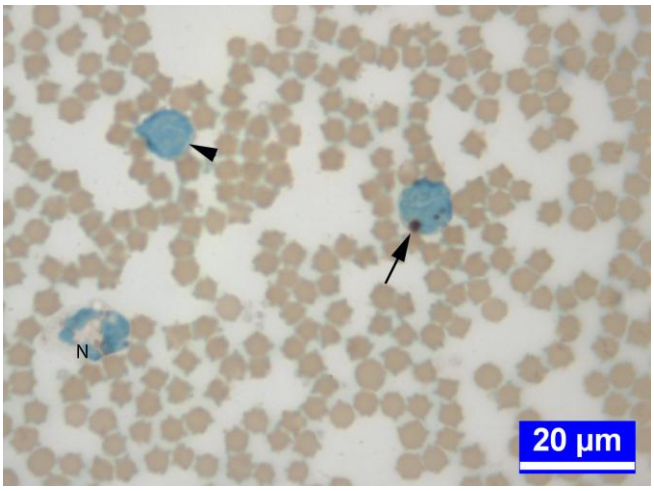

Figure 1. Neutrophile granulocyte (N) and ANAE positive (arrow) and negative (arrowhead) lymphocytes in Jaydara Sheep, ANAE demonstration.

as 22.25- 26.50%, MCV as 21.05- 34.98 μ^3, MCH as 10.66-12.10 pg , and MCHC as 32.65-39.62%.

Alpha-naphthyl acetate esterase (ANAE) is a lysosomal enzyme (Zicca et al., 1981), which is used to differentiate T lymphocyte, B lymphocytes and monocytes in humans and some animal species (Yang et al., 1979; Donmez and Sur, 2007; Donmez and Sur, 2008) and is known to be acquired by T lymphocytes during maturation (Basso et al., 1980). In a study performed on Merino sheep by Yang et al. (1979), the percentage of ANAE-positive lymphocytes was determined as 73%, and it was observed that this value decreased during the different stages of gestation. In 52-week-old male Merino lambs, the rate of ANAE + lymphocytes was determined as 67.7% (Donmez and Sur, 2008). In the present study, the rate of ANAE-positive lymphocytes was determined to be 65% in Jaydara sheep (**Table 2**). It has been reported that the percentage of ANAE-positive lymphocytes in the periphery blood circulation can be affected by management and nutrition conditions, factors influential on the health status of animals, and particularly by alterations in the immunity levels of animals (Sur, 2004).

In the present study, reference ranges were determined for some hematological parameters measured in blood samples from the Kyrgyz Sheep, referred to as the Jaydara Sheep, which is raised throughout Central Asia. The analyses performed demonstrated that, in the Jaydara ewes included in the study, the mean erythrocyte and leukocyte counts, hemoglobin level, hematocrit value and leukocyte percentages (neutrophils, lymphocytes, monocytes, eosinophil and basophils) were within the physiological ranges determined in general for sheep (**Table 1** and **2**) (Sarıpınar et al., 2004; Tschuor et al., 2008). The data obtained in the present study for the Jaydara sheep displayed similarity to the parameters determined in sheep of the Merino, Tuj and Morkaraman breeds (Jelinek et al., 1986; Çelebi and Uzun, 2000). The mean leukocyte count determined in Jaydara sheep in the present study was found to be lower than that previously determined in Awassi sheep (Yiğit et al., 2002). It is known that, even if they belong to the same breed, in healthy animals, hematological and biochemical parameters may vary with multiple factors, including sex, season, climate, nutrition, altitude, gestation, stress and age (Çelebi and Uzun, 2000).

CONCLUSION

The hematological parameters determined in healthy Jaydara sheep in the present study can be used as reference values for this particular sheep breed, and may contribute to future research to be carried out on these animals.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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