Normal Hematology and Selected Serum Biochemical Values in Different Genetic Lines of Awassi Ewes in Jordan

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Abstract
This study was performed to evaluate the effect of intensive selection and genetic manipulation on normal hematological and blood biochemical values in healthy adult, non-pregnant Awassi ewes. Blood samples for hematology and blood biochemical analyses were withdrawn from 92 (30 Local Awassi; Lo-A, 34 Improved Awassi; Im-A, 28 Afec-Awassi; Af-A), healthy, non-pregnant ewes. Hematological analysis included total white blood cell count, red blood cell count, hemoglobin concentration, packed cell volume, platelet count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Serum biochemical analysis included total protein, albumin, blood urea nitrogen, creatinine, glucose, triglycerides, cholesterol, triiodothyronine, thyroxine, thyroid stimulating hormone, and β-Hydroxybutrate. Im-A and Af-A had significantly higher (p<.05) WBC, MCH, MCHC, and platelet counts but significantly lower (p<.05) PCV and MCV values compared to Lo-A. Hematological analysis included total white blood cell count, red blood cell count, hemoglobin concentration, packed cell volume, platelet count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Serum biochemical analysis included total protein, albumin, blood urea nitrogen, creatinine, glucose, triglycerides, cholesterol, triiodothyronine, thyroxine, thyroid stimulating hormone, and β-Hydroxybutrate. Im-A and Af-A had significantly higher (p<.05) WBC, MCH, MCHC, and platelet counts but significantly lower (p<.05) PCV and MCV values compared to Lo-A. Im-A and Af-A had significantly higher (p<.05) WBC, MCH, MCHC, and platelet counts but significantly lower (p<.05) PCV and MCV values compared to Lo-A. There were no statistically significant variation in serum T3 and T4 concentrations, however, TSH concentrations were significantly lower in Im-A and Af-A compared to Lo-A. Although, there were significant differences in some of the hematological and blood biochemical values between the local and improved lines of Awassi reported in this study, the data were within the normal ranges for healthy sheep.

INTRODUCTION
Fat-tailed, local Awassi sheep (Lo-A) is the most common breed of sheep raised in Jordan. Fat-tailed sheep is characterized by an excellent adaptability to harsh environmental conditions and resistance to common diseases (Gootwine and Goot, 1996, Gala and others, 2008). However, Awassi ewes are characterized by low milk and meat productivity, seasonality and low prolificacy rate (Gootwine and Goot, 1996, Gala and others, 2008). The demands for improved productivity while conserving its adaptability to the local conditions, several attempts have been employed to produce new breeds of improved Awassi (Gootwine and Goot, 1996, Gala and others, 2008). Intensive selection within the Lo-A breed for high milk and meat production produced a new Awassi line; the Improved Awassi (Im-A) (Gürsoy and others, 1992). Another Awassi line developed by intro-regression of the Boroola-gene into the Im-A produced the Afec-Awassi (Af-A) with high prolificacy (Montgomery and Kinghorn, 1997).

There has been an extensive amount of research evaluating different production parameters and performance of the improved breeds of Awassi sheep in recent years (Gootwine and others, 1995, Gootwine and Pollott, 2000, Kridli and others, 2006). However, information regarding normal hematological and blood biochemical values are lacking. Hematological and blood biochemical tests have been widely used for the diagnosis of various animal diseases (Bani Ismail and others, 2008). The information gained from blood parameters would substantiate the physical examination and coupled with medical history provide excellent basis for medical interventions.

Variations in blood parameters have been reported in animals due to several factors such as altitude, management, feeding level, age, sex, breed, health status, method of blood collection, hematological techniques used, diurnal and seasonal variation, ambient temperature, and physiological status of the animal (Sherman and Mary, 1994). The objectives of this study were to evaluate the effect of intensive selection and genetic manipulation on normal hematological and blood biochemical values in healthy adult, non-pregnant Awassi ewes.

MATERIALS AND METHODS
STUDY AREA AND ANIMAL MANAGEMENT

Animals involved in the study belonged to Al-Khanasry Research Station located in Eastern North of Jordan, at 32°24´N, 36.03 E at an altitude of 860 m above sea level. Annual rainfall in the region is less than 180 mm; winter temperatures may go down to 0°C during the day and -2°C at night. Summer temperatures may rise up to 42°C during the day, but the nights are relatively cool.

The study was conducted during the month of May 2008. All animals were kept in the same shed. Feedstuff was communal and consisted of hay and barely-based concentrate ration. Animals were fed 3 times per day. All sheep are routinely vaccinated for FMD, PPR and Anthrax and administered anthelmintic prophylactics 4 times per year.

SAMPLE COLLECTION AND LABORATORY ANALYSIS

A total of 92, all of them were in the same age (2.5 years), non-pregnant ewes (30 Lo-A, 34 Im-A, 28 Af-A) were used in the study. A complete physical examination was performed on all animals to detect any abnormal signs. Blood samples were only taken from apparently healthy animals.

Blood samples were taken from all animals in early morning. Five to seven milliliters of whole blood was collected aseptically from the jugular vein using disposable needles and EDTA containing vacutainer tubes (BD Vacutainer, Belliver Industrial Estate, Plymouth, UK) and another sample was placed in plain tubes without anti-coagulant. Hematological analysis including total white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was performed within 1-2 hours after collection using electronic cell counter (ABC Vet hematology analyzer, ABX Diagnostics, France).

Serum was obtained by allowing the blood to clot in room temperature for 2 hours, centrifuged, and collected in a special epindorf tubes. Serum samples were stored at -20°C until used. Serum was analyzed for total serum protein (Biuret method), albumin (BCG method), blood urea nitrogen (Colorimetric method), creatinine (Kinetic method), glucose (GOD-PAP method), triglycerides (GPO method), and cholesterol (CHOD-POD method). Commercially available ELISA kits (Biocheck, Forster City, California, USA) were used to determine serum triiodothyronine (T3) and thyroxine (T4) and thyroid stimulating hormone (TSH) levels. Serum levels of ß-Hydroxybutrate were determined using kinetic enzymatic method using commercially available kits (Randox laboratories, Antrim, UK) following manufacturers instructions.

QUALITY CONTROL

To ensure the accuracy of the test results, biochemical analyzers and reagents were checked daily with quality control kits of known values for the various constituents.

STATISTICAL ANALYSIS

Data were expressed as means±standard error of the mean and ranges. The effect of intensive selection and genetic manipulation compared to pure Awassi on various hematological and blood biochemical values were analyzed using GLM procedure of SAS (2004) program assuming the following Model: Yij= µ+ti + Eij, where Yij is the observations of each hematological and blood biochemical parameter; µ is the overall mean; ti is the effect of Awssi line (i=Im-A, Af-A and Lo-A); and Eij is the random effect of the error associated with each observation. Values were considered significant at P<0.05.

RESULTS AND DISCUSSION

Efforts to improve the productivity of local Awassi sheep have resulted in the introduction of two new lines; the Improved Awassi and Afec-Awassi. Many studies have been performed to evaluate the productivity and economic value of these breeds. However, the effects of intensive selection and genetic manipulations on individual and flock health are still lacking. In general, it has been reported that blood laboratory parameters and productive traits are essentially affected by the genetic potential of individual animals and parameters of homeostasis in the body (Alonso, 1997). This is the first report to elucidate and compare the hematological and blood biochemical parameters in 2 promising new lines of Awassi sheep in Jordan.

The least squares means and standard errors of hematological parameters are presented in Table 1. The normal values for WBC, PCV, MCV, MCH, MCHC and platelet counts were significantly different (P< 0.05) in Im-A and Af-A compared to those of Lo-A while the normal values for RBC and Hb were similar in the 3 lines (Table 1). Both Im-A and Af-A had significantly higher WBC, MCH, MCHC, and platelet counts but significantly lower PCV and
MCV values compared to Lo-A. In general, however, the hematological profiles of the lines compared favorably and were within the normal range for the ovine species (Forhead and others, 2002). The differences reported between the new lines and local Awassi sheep in some of the hematological parameters could be associated with factors that influenced RBC, Hb, and PCV. The mean total WBC values reported in Im-A and Af-A were significantly higher than that observed in Lo-A but still within the normal range reported in sheep (Forhead and others, 2002; Kramer, 2000). Breed difference for these parameters were also reported earlier (Forhead and others, 2002).

**Figure 1**

Table 1: Least square means for some hematological values of different genetic lines of Awassi sheep.

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In the serum biochemical profile, there was a significant difference (P< 0.05) between the 3 lines of Awassi sheep in total serum protein, blood urea nitrogen, cholesterol, triglycerides and ß-Hydroxybutrate values (Table 2). Serum total protein, cholesterol, triglycerides and ß-Hydroxybutrate values were significantly higher in Im-A and Af-A compared to Lo-A, while the value of BUN was significantly lower and no significant differences in creatinine values. These parameters are usually affected by the level of nutrition and closely associated with metabolic activities of individual animals. Serum albumin and glucose values were similar in the 3 studied lines. In general, however, serum biochemical parameters in the 3 lines of Awassi sheep were within the normal range reported for the ovine species (Forhead and others, 2002).

Although, it has been reported that the serum concentrations of thyroid hormones vary according to breed and are affected by genetics, in this study, there were no statistically significant variation in serum T3 and T4 concentrations between the 3 lines of Awassi sheep, however TSH concentrations were significantly lower in Im-A and Af-A compared to Lo-A. This could be due to similarities in management and nutritional status of animals and the fact that these improved lines have similar genetic make up (Bani Ismail and others, 2009).

**Figure 2**

Table 2: Least square means for some blood biochemical values of different genetic lines of Awassi sheep.

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Although, there were significant differences in some of the hematological and blood biochemical values between the local and improved lines of Awassi reported in this study, the data were within the normal ranges for healthy sheep.

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