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BLOOD PARAMETERS IN THE MORADA NOVA SHEEP: INFLUENCE OF AGE, SEX AND BODY CONDITION SCORE

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ABSTRACT

The present study was aimed at determining the serum biochemical panel and the erythocytogram of the sheep of the Morada Nova breed. Further, it was also aimed at verifying the variations of these parameters promoted by the sex, age, and body condition score. The study was conducted by sampling nine herds; two from Ceará state, five from Rio Grande do Norte state, and two from Paraíba state, Brazil. It used a sample of 249 clinically healthy sheep of the Morada Nova breed. The sex, age, and body condition score were recorded from each animal. Blood samples were collected for determining the serum levels of glucose, cholesterol, triglycerides, urea, creatinine, total proteins, albumin, globulins and thyroxine (T4), the serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), the number of red blood cells (RBCs), and the packed cell volume (PCV). It was observed that the evaluated blood parameters from the Morada Nova sheep breed did not demonstrate any variation from the reference interval established for the species, except for the lower levels of globulins and higher albumin/globulins ratio. However, it was verified that the serum biochemical panel was affected by the sex, age, and body score condition.

Key words: serum biochemistry; erythrocytes; breed; Morada Nova breed

INTRODUCTION

Determination of the blood parameters profile is used for evaluating the individual health conditions and monitoring the nutritional and metabolic conditions of the animals. However, the levels of blood parameters are influenced by several factors, such as sex, breed, age, stress, diet, level of milk production, handling, climate, physiological status (lactation, pregnancy, reproductive status), and the laboratorial methodology (González and Silva, 2006; Kaneko *et al.*, 2008). For the correct interpretation of the metabolic profiles, it is necessary to comparewith reference values appropriate for the region and the population, in particular. However, the reference values of the climatic zones and similar animal groups could also be used (González and Silva, 2006; Kaneko *et al.*, 2008). Thus, it is important to characterize the specific values for each breed and region. However, there is a dearth of data in the literature concerning the values for the sheep of some Brazilian breeds.

The Morada Nova sheep breed is one of the most important native hair sheep breed from Brazilian semiarid regions, which are bred for the production of their high quality skin and meat. This breed is characterized by small size and good adaptation to the conditions of Brazilian semiarid, and is thus very important for local farmers (Lôbo *et al.*, 2011).

The present study was aimed at determining the blood parameters from the sheep of the Morada Nova breed, and to study their variation by sex, age, and body condition score.

MATERIALS AND METHODS

Study area: The study was conducted by sampling nine herds in Brazil, two from Ceará state, five from Rio Grande do Norte state, and two from Paraiba state. The prevailing weather in the localities is classified as Tropical Semiarid, with the occurrence of two seasons throughout the year: the rainy season (usually from January to June) and the dry season (generally from July to December). In Ceará state the collections were concentrated in the municipality of Morada Nova, located at 6º 30' S and 44.4 m high altitude, with an average annual temperature of 27.1 ºC, humidity of 67.5%, and annual rainfall of 872.2 mm. In Rio Grande do Norte state the collections were concentrated around the municipality of Lajes, located at 5º 42' S and 199 m high altitude, with an average annual temperature of 27.2 ºC, mean annual relative humidity of around 70%, and annual rainfall of 414.7 mm. In Paraiba state, the average annual temperature is 26 °C, with an average humidity of 80%.

Experimental animals: The present study used 249 clinically healthy free-ranging sheep of Morada Nova breed for the biochemical and hematological evaluations. The sex, age, and body condition score of each animal were recorded. The animals were separated in age groups of up to 6 months-old, 6 to 12 months-old, and older than 12 months-old. The visual estimate of the body condition score was performed in animals older than 6 months-old according to Russel *et al.* (1969), ranging from 2.0 to 4.5. All ratings of the body condition score were performed by the same observer.

Collection of blood: Blood samples were collected at 7:00h to 8:00h after overnight fasting by jugular venipuncture, using vacuum tubes with EDTA as well as tubes without the anticoagulant. The vials containing the blood and the anticoagulant were inverted several times to ensure proper mixing. The tubes were transported to the laboratory immediately after blood collection.

Clinical biochemistry panel: Blood samples without the anticoagulant were centrifuged at 2000 rpm for ten minutes. The serum samples were frozen individually at -20 °C until further use. The serum levels of glucose, cholesterol, triglycerides, urea, creatinine, total protein, and albumin, and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using specific commercial kits (Katal, Belo Horizonte, MG, Brazil) and automatic analyzer SBA-2000 (Celm, Barueri, SP, Brazil). The results of total protein and albumin levels were used for calculating globulin and the albumin/globulin (A/G) ratio. The serum levels of thyroxine (T4) were determined by a specific commercial kit (Katal, Belo Horizonte, MG, Brazil) and a semiautomatic ELISA reader (Quick ELISA, São José do Rio Preto, SP, Brazil).

Hematologic profile: After collection, the blood samples with EDTA were taken to the laboratory for determining the packed cell volume (PCV) and the number of red blood cells (RBCs), and calculating the mean corpuscular volume (MCV). The microhematocrit technique was used to determine the PCV, while the number of RBCs was determined in the Neubauer chamber, using routine laboratory techniques. Blood was diluted at 1:400 for RBCs counts. The calculation of MCV was done by dividing the hematocrit (percentage) by the number of red blood cells ($x10^6/\mu l$) and multiplying by 10, to obtain the values in femtolitres (fl).

Statistical analysis: The obtained data were grouped by sex, age, and body condition score and reported as the mean ± SEM. The sex groups included just animals up to 6 months-old, because the number of male sheep older than 6 months-old was very low. The age groups were: up to 6 months-old, 6 to 12 months-old, and older than 12 months-old. The body condition score groups were: (1) 2 to 2.25, (2) 2.5, (3) 2.75, (4) 3, and (5) 3.25-4.5.Sex groups were compared using the t test and age and body condition score groups were compared using analysis of variance (ANOVA) with separation of means by the Duncan's method (GraphPad Prism v.4 for Mac). The level of significance was set at $P< 0.05$.

RESULTS

The overall mean values of the blood parameters of the Morada Nova sheep breed are shown in Table 1, along with some reference values given by the literature.

The values of the blood parameters in relation to the age are presented in Table 2. It was found that the age of the sheep significantly affected ($P < 0.05$) the values of blood cholesterol, triglycerides, urea, creatinine, total protein, globulins, albumin/globulin (A/G), AST, ALT, and MCV. However, the values of glucose, albumin, erythrocyte, PCV, and thyroxine were not affected by the age ($P> 0.05$).

Table 1. Overall mean and SEM values for blood parameters of the Morada Nova sheep.

Parameter	Results (n=249)	Kaneko *et al.* (2008)	Radostits *et al.*(2002)
Glucose (mg/dl)	64.8±1.35	50-80	50-80
Cholesterol (mg/dl)	68.9±1.91	52-76	43-103
Triglycerides (mg/dl)	36.1±1.16	-	-
Urea (mg/dl)	55.8±1.19	36.6-92.0	17.1-42.8
BUN (mg/dl)	26.1±0.56	17-43	8-20
Creatinine (mg/dl)	1.64±0.08	1.2-1.9	1.2-1.9
Total proteins (g/dl)	5.99±0.11	6.0-7.9	6.0-7.9
Albumin (g/dl)	3.01±0.05	2.4-3.0	2.4-3.0
Globulins (g/dl)	2.70±0.11	3.50-5.70	-
A/G ratio	1.57±0.08	0.42-0.76	-
AST (U/l)	92.8±1.49	60-280	60-280
ALT (U/l)	30.3±0.63	30±4	22-38
Thyroxine (µg/dl)	4.29±0.12	-	-
(mmol/l)	55.2±1.54		

RBC (x10^6/µl)	10.9±0.36	-	9-15
PCV (%)	32.9±0.27	-	27-45
MCV (fl)	31.7±0.41	-	28-40

Table 2. Values (mean ± SEM) of the blood parameters tested in different age groups of the Morada Nova sheep.

Parameters	<6 months-old (n=83)	6 to 12 months-old (n=28)	>12 months-old (n=106)	*P*
Glucose (mg/dl)	64.3±2.74	67.1±3.11	62.7±19.8	n.s.
Cholesterol (mg/dl)	76.9±3.21[a]	97.3±4.87[b]	58.3±2.29[c]	<0.0001
Triglycerides (mg/dl)	40.7±2.70[a]	38.4±2.65	31.9±1.15[b]	0.0035
Urea (mg/dl)	56.8±2.43[a]	45.6±1.66[b]	57.8±1.57[a]	0.0059
Creatinine (mg/dl)	1.05±0.08[a]	1.62±0.14	2.07±0.14[b]	<0.0001
Total proteins (g/dl)	6.09±0.15[a]	7.84±0.32[b]	5.61±0.15[a]	<0.0001
Albumin (g/dl)	3.04±0.09	3.01±0.11	2.95±0.06	n.s.
Globulins (g/dl)	3.08±0.17[a]	4.83±1.42[b]	2.66±0.15[a]	0.0040
A/G ratio	1.55±0.15[a]	0.66±0.03[b]	1.62±0.09[a]	0.0001
AST (U/l)	93.7±1.72	102.9±3.07[a]	88.9±2.52[b]	0.0080
ALT (U/l)	32.6±0.91[a]	24.9±3.04[b]	30.1±0.78[a]	0.0012
Thyroxine (µg/dl)	4.61±0.20	4.37±0.28	4.06±0.17	n.s.
RBC (x10^6/µl)	11.8±1.21	10.6±0.29	10.4±0.16	n.s.
PCV (%)	32.5±0.45	33.0±0.92	33.2±0.37	n.s.
MCV (fl)	30.8±0.89[a]	31.3±0.62	32.2±0.53[b]	0.0329

[a,b,c] The mean values with the different letter represent significant difference (P<0.05, ANOVA followed by the Duncan test)
n.s.: non-significant

The blood values, according to the sex of the sheep with up to 6 months-old are presented in Table 3. The serum concentrations of glucose were significantly higher (P< 0.05) in females in comparison to males, while the concentrations of urea and thyroxine and the activity of ALT were higher (P< 0.05) in males. However, the values of cholesterol, triglycerides, creatinine, total protein, albumin, globulins, A/G ratio, AST, MCV, and red blood cells were not significantly (P> 0.05) affected by the sex.

The blood parameters according to the different groups of body condition score are presented in Table 4. There were significant differences (P<0.05) in the values of urea, albumin, ALT, hemoglobin, MCV, and thyroxine. On the other hand, there were no significant differences (P> 0.05) in the values of glucose, cholesterol, triglycerides, creatinine, total protein, globulins, A/G ratio, AST, and PCV.

Table 3. Values (mean ± SEM) of the blood parameters tested in the male and the female Morada Nova sheep of age up to 6 months.

Parameters	Females (n=64)	Males (n=19)	*P*[1]
Glucose (mg/dl)	67.1±2.81	54.1±7.25	0.0481
Cholesterol (mg/dl)	78.3±4.10	68.1±4.08	n.s.
Triglycerides (mg/dl)	40.2±3.19	42.2±5.06	n.s.
Urea (mg/dl)	53.6±2.83	67.7±3.84	0.0139
Creatinine (mg/dl)	1.09±0.10	0.92±0.08	n.s.
Total proteins (g/dl)	6.23±0.18	5.61±0.27	n.s.
Albumin (g/dl)	3.02±0.10	3.11±0.17	n.s.
Globulins (g/dl)	3.26±0.19	2.50±0.34	n.s.
A/G ratio	1.46±0.17	1.86±0.29	n.s.
AST (U/l)	94.7±2.03	89.6±2.97	n.s.
ALT (U/l)	31.4±1.05	37.0±1.46	0.0089
Thyroxine (µg/dl)	4.50±0.24	5.58±0.20	0.0200
RBC (x10^6/µl)	12.2±1.49	9.81±0.74	n.s.
PCV (%)	32.6±0.50	32.4±1.04	n.s.
MCV (fl)	31.2±0.99	28.8±2.13	n.s.

[1] *P*: Student t test
n.s.: non-significant

Table 4. Values (mean ± SEM) of the blood parameters tested in different groups of the body score condition of the Morada Nova sheep.

Parameters	2 – 2,25 (n=34)	2,5 (n=47)	2,75 (n=45)	3 (n=38)	3,25 – 4,5 (n=53)	*P*
Glucose (mg/dl)	63.9±3.17	64.7±2.16	66.6±3.22	60.4±3.18	64.5±4.14	n.s.
Cholesterol (mg/dl)	74.2±4.04	63.4±4.89	76.2±5.50	70.9±5.16	64.0±4.51	n.s.
Triglycerides (mg/dl)	37.8±5.26	31.3±1.74	34.7±2.54	38.9±2.82	33.9±2.00	n.s.
Urea (mg/dl)	49.5±2.87	53.7±2.73	50.8±3.27	61.4±2.47	58.2±3.11	0.0377
Creatinine (mg/dl)	1.82±0.20	1.86±0.20	1.77±0.21	1.82±0.27	1.41±0.21	n.s.
Total proteins (g/dl)	6.48±0.34	5.83±0.30	6.13±0.29	6.02±0.25	6.02±0.23	n.s.
Albumin (g/dl)	2.71±0.14	2.88±0.11	3.16±0.11	2.92±0.09	3.13±0.10	0.0298
Globulins (g/dl)	3.88±0.32	2.95±0.29	2.97±0.27	3.10±0.26	2.90±0.24	n.s.
A/G ratio	1.02±0.14	1.69±0.21	1.57±0.15	1.41±0.20	1.43±0.14	n.s.
AST (U/l)	96.7±3.48	97.1±4.78	87.4±2.82	84.2±2.54	96.0±4.27	n.s.
ALT (U/l)	29.8±1.53	27.5±1.86[a]	28.1±1.31	29.4±1.59	33.6±1.43[b]	0.0324
Thyroxine (µg/dl)	3.72±0.20[a]	3.64±0.23[a]	4.51±0.27[a]	3.32±0.15[b]	5.92±0.35[c]	<0.0001
RBC ($x10^6$/µl)	14.1±2.58[a]	10.7±0.25	10.6±0.25	10.1±0.36	9.94±0.39[b]	0.0308
PCV (%)	32.1±0.83	33.7±0.66	33.2±0.51	32.2±0.62	33.3±0.75	n.s.
MCV (fl)	28.0±0.40[a]	32.0±0.50	30.1±0.63[a]	34.2±1.47[b]	33.8±1.25[b]	<0.0001

[a,b,c] The mean values with the different letter represent significant difference (P<0.05, ANOVA followed by Duncan test)
n.s.: non-significant

DISCUSSION

Most parameters showed results within the reference values given by the various authors (Radostits *et al.*, 2002; Kaneko *et al.*, 2008). However, the levels of globulins and the A/G ratio were exceptions. The globulin levels were observed to be lower than the reference values (Radostits *et al.*, 2002; Kaneko *et al.*, 2008). On the other hand, the A/G ratio was observed to be higher than the reference value. In pathological conditions, some increase in the serum globulin can be noticed as a result of increased production, especially in the inflammatory processes (Braun *et al.*, 2010; Diógenes *et al.*, 2010). The reduction in the concentration of serum globulin is mainly related to the non-transfer of passive immunity in young animals (Patt Jr, 1977; Brujeni *et al.*, 2010). However, deficiency of immunoglobulins production may occur (Brujeni *et al.*, 2010; Cavalcante *et al.*, 2012). It is likely that the Morada Nova sheep have a lower production of some globulin in comparison to most other sheep breeds. It is necessary to conduct studies for evaluating the serum proteins of the Morada Nova sheep, especially by the electrophoretic separation of the protein fractions for better understanding of the changes occurring in the globulin levels.

Several factors can interfere in the values of the various blood parameters in animals. In the present study, the effects of age, sex, and body condition score were assessed. It was found that the age of the sheep affected the values of blood cholesterol, triglycerides, urea, creatinine, total protein, globulin, A/G ratio, AST, ALT, and MCV.

The average concentration of the total protein in the present study was higher in animals aged 6 to 12 months in comparison to other ages. The influence of age on total serum protein was also observed in the sheep breeds, Merino landschaf (Antunović *et al.*, 2004) and Chios (Roubies *et al.*, 2006). These findings differ from those obtained in the Santa Ines sheep, which showed lower concentrations in 7 to 18 months-old in comparison to other ages (Meira Jr *et al.*, 2009). The variation in the levels of globulins in the Morada Nova sheep was also observed in the Chios breed (Roubies *et al.*, 2006). Furthermore, concentrations of albumin showed no significant difference between the different age groups, which was similar to that found in the Merinolandschaf sheep (Antunović *et al.*, 2004). However, it differed from the breeds Santa Inês (Meira Jr *et al.*, 2009) and Chios (Roubies *et al.*, 2006). These differences could be owing to physiological variations between the different breeds. Furthermore, these variations in the obtained results were probably owing to the different management, climatic conditions, and nutrition level of the animals.

The present study showed that the age factor also affected the serum cholesterol levels, which was similar to that observed in sheep breeds, Merinolandschaf (Antunović *et al.*, 2004). Further, the interference of age was also observed in the serum activities of ALT and AST in the Morada Nova sheep, which was similar to the Ethiopian breeds Barki (Anwar *et al.*, 2012; Abdel-Fattah *et al.*, 2013) and Rahmani (Anwar *et al.*, 2012), but no such interference was observed in sheep from Southern Punjab, Pakistan (Kiran *et al.*, 2012). These interferences can be attributed to the differences in the hepatic activity in the different age groups. In fact, the serum activities of

ALT and AST are often routinely used for assessing the liver function (Braun *et al.*, 2010). As cholesterol is synthesized in the liver and then released into the bloodstream (Min *et al*., 2012), it is also another good indicator of the hepatocytes function (Soto-Blanco *et al.*, 2001;Luo *et al.*, 2010).

It was found that the variation of creatinine levels (which increased with increasing age in the present study) did not occur in Chios breed (Roubies *et al.*, 2006). Creatinine is formed by the degradation of phosphocreatine for energy release in the skeletal muscle. Serum creatinine is proportional to the muscle mass (Kreider, 2003; Cirillo, 2010; Samra and Abcar, 2012). The present study demonstrates that the increasing levels of serum creatinine, with increasing age are a result of the development of animals. An alternative hypothesis is that the levels of creatinine might have been increased owing to the reduction of serum thyroxine levels, as noted earlier in the Sakis-Awassa cross-bred sheep (Yokus *et al.*, 2006). This effect would be promoted by a reduction in the glomerular filtration rate resulting from a decrease in the serum thyroxine level.

In the Morada Nova sheep, the age affected the serum levels of urea, which differed from the Merinolandschaf (Antunović *et al.*, 2004) and Chios (Roubies *et al.*, 2006) sheep breeds. As urea is a good indicator of the dietary protein intake (Schroder *et al*., 2003), it is also possible that interference in this parameter could be a consequence of the different protein nutrition statuses. However, the urea levels in the present study could be affected by variations in the glomerular filtration rate, as speculated for the creatinine levels.

The present study also evaluated the influence of sex on the blood parameters. This evaluation was performed only in animals up to 6 months-old, because the farms from where the blood was collected had very few males older than 6 months. The serum concentrations of glucose were significantly higher in females in comparison to males, while the concentrations of urea and thyroxine and the activity of ALT were found to be higher in males. In the Menz, Tukur, and Wello sheep breeds, there were no sexual differences in the activity of ALT; however, the serum activities of AST of the Menz sheep were higher in males in comparison to females. On the other hand, it was observed that the serum activity of AST of the Santa Ines sheep breed was higher in females in comparison to males (Meira Jr *et al.*, 2009). Thus, it is likely that the observed differences in the activities of ALT in the present study were the result of variations in the hepatic activity between the sexes.

The Morada Nova males had higher concentrations of thyroxine in comparison to females. This result is similar to observed in humans, which exhibited higher thyroxine levels in men than women (Ahmed *et al.*, 2009). This was probably owing to a greater activity of the gland in males promoted by sexual hormones. Thus, sex is a factor that interferes on secretion of thyroxine. The body condition score interfered in the levels of blood urea, albumin, ALT, thyroxine, RBCs, and MCV. These variations were probably related to the differences in the basal metabolism rates. In fact, several other conditions could also affect the metabolism, especially the reproductive status (El-Barody *et al.*, 2002; Antunović *et al.*, 2004; Roubies *et al.*, 2006; Karapehlivan *et al.*, 2007; Novoselec *et al.*, 2009). Furthermore, the nutrition and health conditions are also responsible for a change in the body condition score and the blood parameters.

In summary, the serum biochemical panel and the erythocytogram measured in the Morada Nova sheep showed no variation from the normal values for the species. However, a lower concentration of globulins and a higher A/G ratio was observed. It was also verified that the serum biochemical panel was affected by the sex, age, and body condition score.

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