Hemogram of healthy sheep (*Ovisaries*) of the Santa Ines breed raised in the region of Piedade, São Paulo State: influence of age and sex

Hemograma de ovinos (*Ovis aries*) sadios da raça Santa Inês criados na região de Piedade, estado de São Paulo: influências etárias e sexuais

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Abstract

Influence of age and sex factors on blood count was investigated in healthy sheep of the Santa Ines breed, raised in the region of Piedade (São Paulo State). The hematocrit and red blood cell count presented higher values in younger females, and a gradual decrease occurred during animals' ageing. In contrast, the total number of leukocytes was lower in younger animals and showed a gradual increase with their ageing.

Keywords: Hematology. Erythrogram. Leukogram. Ovine.

Resumo

Foi investigada a influência dos fatores etários e sexuais sobre o hemograma de ovinos sadios da raça Santa Inês, criados na região de Piedade, no Estado de São Paulo. O hematócrito e o número de hemácias apresentaram maiores valores nas fêmeas mais jovens e houve um decréscimo gradual durante o desenvolvimento etário dos animais. Em contraposição, o número total de leucócitos foi menor nos animais mais jovens e apresentou elevação gradual com a evolução da idade dos animais.

Palavras-chave: Hematologia. Eritrograma. Leucograma. Ovinos.

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Introduction

After a century of studies on veterinary hematology, new research would not be justified if generalization of various hematological concepts were not questionable. There is an agreement among researchers that extrinsic (environmental, climatic, nutritional, and of differentiated managements) factors can cause significant quantitative and qualitative changes in the blood profile of these animals (BIRGEL, 1982). The Piedade region is currently known to supply Santa Ines sheep to the market of both the region and capital of São Paulo State. In recent years, a change in eating habits was observed in the southeastern region of the State where an increased consumption of meat from this animal species was recorded. Piedade (altitude: 781 m) is located about 100 Km (west direction) from the capital of São Paulo (23°42'43"S / 47°25'40"W). It is a mountainous region suitable to raise small ruminants. Other studiesthat were used as a blood count reference for this breed were conducted in different edaphoclimatic regions as those in the Amazon, a moist and flat region (LIMA et al., 2015), west of São Paulo State, a plateau region (DAVID et al., 2012), and Piauí State, a Brazilian semi-arid region (PEREIRA et al., 2015).

In 1929, Fraser studied sheep blood profile, and did not show any effect of age factors on the leukogram in ovine species. In contrast, Josland (1933) found that variations occurred in the leukogram of these ruminants. Overas (1969) used 68 animals of the

Norwegian breed, analyzed the same parameters, and noted changes in the age group of animals, mainly in relation to the number of erythrocytes and mean corpuscular volume (MCV). Ullrey et al. (1965) observed that the erythrogram values decreased from birth to the 14th day of age and then increased until three months of age. They also observed that the number of reticulocytes had a lower value after birth but increased in the first two weeks of life, as a function of a decrease in the total number of erythrocytes. Similar results were observed by Littleton et al. (1968) and Upcott et al. (1971). Some authors believe that the decline in the total number of erythrocytes in the first weeks of life is due to an inadequate iron supplementation (ULLREY et al., 1965; BOSTEDT, 1979).

Thus, a limitation was noticed in the literature regarding hematology studies in ovine (*Ovisaries, L.*). Indeed, Todd et al. (1952) found mean values for the blood count of the Southdown (9,200 leukocytes/μL) and Hampshire (7700 leukocytes/μL) breeds. The values found in theirerythrogram revealed no effect of the breed factor. Watson (1953) studied the effect of altitude on the value for hemoglobin concentration in sheep raised in Peru. They observed values in the range of 8-10 g/dL at sea level and an increase in hemoglobin to the range of 12-16 g/dL at altitudes above 3000 m. On the average, hemoglobin concentration in sheep raised in the mountainous regions of Norway was 3 g/dLhigher than those raised in low altitude regions (OVERAS, 1969).

Several authors reported the significance of seasonal changes in the sheep blood count (HOLMAN, 1944; SINGH CHAKAL; RATTAN, 1981). Animals with nutritional deficiency showed lower values for the total number of erythrocytes during winter, and these values increased in periods of food abundance (JONES; KREBS, 1972). Dantas et al. (2002) evaluated the erythrogram of sheep fed with increasing amount of alfalfa hay and the values found by them indicated a gradual decrease in the values for mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) as a function of increase in food intake. Chalhoub et al. (2000) compared the hematimetric profiles of sheep submitted to elective cesarean section and normal deliveries. The erythrogram values did not change in any of the groups studied. In the group of ewes submitted to cesarean section, the mean values found seven days after delivery were as follows: total red blood cell count (10.05 x 10^6/μL), hemoglobin (12.7 g/dL), globular volume (37%), total leukocytes (6.35 x 10^3/μL), neutrophils (3360/μL), lymphocytes (2590/μL), eosinophils (80/μL), and monocytes (130/μL). In the group of ewes with normal delivery, the average values found seven days after delivery were as follows: total red blood cell count (9.04 x 10^6/μL), hemoglobin (12.6 g/dL), globular volume (37%), leukocytes (5.88 x 10^3/μL), neutrophils (2610/μL), lymphocytes (2880/μL), eosinophils (120/μL), and monocytes (150/μL).

A decrease in the total values of red blood cells, hemoglobin, and HCM was observed after delivery (ULLREY et al., 1965). Reda and Hathout (1951) found that no change occurred in the number of leukocytes at the beginning of gestation. However, leukocytosis occurred in the final stages of pregnancy. El-Sherif and Assad (2001) investigated the influence of gestation and lactation on the blood profile of Barki breed sheep and found that an increase occured in the concentration of hemoglobin, hematocrit, and MCHC during pregnancy until the 22nd week.

Batista et al. (2009) evaluated healthy sheep raised in the Piauí State and found variation in the mean values for erythrocytes, hemoglobin, and hematocrit in relation to age. In older animals, the values were higher. The averages values were calculated for red blood cells (9.06 x 10^6/mL), hemoglobin (9.24 g/dL), and hematocrit (28.38%). Madureira et al. (2013) determined the hematimetric parameters of Dorper breed animals (n = 72) raised in Brazil, which were as follows: red blood cells (13.4 ± 2.5 x 10^6/μL), hemoglobin (12.6 ± 1.3 g/μL), globular volume (37.5 ± 3.7%), total leukocytes (7,166 ± 1,967/μL), and segmented neutrophils (4,168±1,501/μL). A comparison between the values presented by males and females revealed that the values for total leukocytes (7,576±2,082/μL), segmented neutrophils (4,296±1,510/μL), and lymphocytes (2.428±1.084/μL) were higher in males.

According to Lima et al. (2015), reference values cannot be extrapolated to animals raised in geographically distinct regions. These authors attributed influence of the age factor to the number of erythrocytes, erythrocyte indices, and number of eosinophils in Santa Ines breed animals raised in the Amazon. In males, the erythrocyte values were higher than in females. Pereira et al. (2015) studied the physiological variation of hematological parameters as well and the influence of age factors of Santa Ines sheep in the final third of gestation and postpartum periods. They found higher values for red blood cells and lower values for neutrophils in lambs. These authors compared the puerperium and gestation periods and did not find any difference between the total numbers of neutrophils. David et al. (2012) studied the reference values for blood count in the western region of São Paulo State, where older animals presented higher values for erythrocytes and leukocytes, segmented neutrophils, and lymphocytes. They concluded that in ovines blood count is influenced by the animals' age range.

Objective

The objective of this study was to investigate the blood count components of healthy Santa Ines sheep, raised in the region of Piedade city, São Paulo State, and establish the reference values, taking into account the influence of age and sex factors.

Material and Methods

Whole blood samples (121) were collected from healthy female (91) and male (30) Santa Ines sheep raised in the region of Piedade city, São Paulo State. The extensive breeding system was used. General clinical examination was performed according to Antón and Mayayo (2007) aiming to select healthy animals. The study was conducted in the period Feb-Jul 2010.

The samples were distributed in age groups according to the age stratification proposed by Reichmann (1972): Group 1 (up to six months), Group 2 (from 7 to 18 months), and Group 3 (from 19 to 48 months).

The samples were collected by puncture of the external jugular vein using two-end (30 x 0.7 mm) needles. Blood samples (5 mL) were collected in siliconized (Vacutainer System®) tubes containing disodium ethylenediaminetetraacetate (EDTA; 10%; 0.05 mL) aqueous solution. The tubes were kept refrigerated until the moment of examinations, which were always completed within 24 h of conservation. Fresh blood smears (without anticoagulant) were also prepared for leukocyte differential count.

The techniques employed in the blood count followed the recommendations of Birgel (1982) and Jain (1986). Gower solution (1:200 dilution) was used for manual red cell count. The hemoglobin rate was determind by the cyanometahemoglobin method (CELM MOD. E-225D spectrophotometer). The globular volume was determined by the microtechnique using capillary tubes and a microcentrifuge (HERAEUS mod. Biofugehaemo; 11,800 rpm). The Thoma liquid (1:20 dilution) was used for total leukocyte count in a hemocytometer.

Statistical analysis

The Statistical Analysis System (SAS INSTITUTE, 1985) software was used to calculate the values for mean, standard deviation, and coefficient of variation of the results in this study. Initially, the results were submitted to analysis of variance (ANOVA), and the difference between the means was analyzed by the Tukey test for parametric samples. The Kruskal Wallis test (followed by the Mann-Whitney test) was used for nonparametric samples with Bonferroni correction. Both tests had 5% significance levels ($p<0.05$), as recommended by Berquó et al. (1981). The equality of the male:female proportion, as well as the proportion of the categorized ages, were analyzed using the Chi-square test.

Results

The results thus obtained were distributed in tables 1 (erythrogram components) and 2 (leukogram).

Table 1 – Mean values and standard deviations of erythrogramdata, by sex and age, obtained from healthy Santa Ines sheep, raised in the region of Piedade city – São Paulo, 2004

	Group 1	(0-6 months)	Group 2	(7–18 months)	Group 3	(19-48 months)
	F	M	F	M	F	M
He (x 10^6/µL)	12,35 ± 3,11 A, a	11,98 ± 1,62 A, a	10,59 ± 1,60 B, a	11,79 ± 1,11 A, b	10,87 ± 2,87 A, a	11,70 ± 2,53 A, a
Ht (%)	34,41 ± 4,10 A, a	32,59 ± 3,50 A, a	32,45 ± 3,57 B, a	32,70 ± 2,95 A, a	31,42 ± 4,24 B, a	33,25 ± 2,87 A, a
Hb (g/dL)	10,70 ± 1,64 A, a	10,80 ± 1,13 A, a	10,78 ± 1,16 A, a	10,52 ± 0,86 A, a	10,26 ± 1,52 A, a	11,20 ± 1,78 A, a
VCM (fL)	29,27 ± 6,49 A, a	27,61 ± 4,61 A, a	31,07 ± 4,32 A, a	27,84 ± 2,53 A, b	29,94 ± 4,86 A, a	29,08 ± 4,76 A, a
HCM (pg)	9,04 ± 1,77 A, a	9,14 ± 1,45 A, a	10,40 ± 1,93 A, a	8,95 ± 0,70 A, b	9,82 ± 2,02 A, a	9,67 ± 0,72 A, a
CHCM (%)	31,05 ± 2,50 A, a	33,33 ± 3,53 A, a	33,37 ± 3,03 B, a	32,25 ± 1,94 A, a	32,74 ± 3,31 AB, a	33,62 ± 3,20 A, a

* Capital letters represent animals of the same sex compared with different age groups and lowercase letters represent males and females of the same age. Different letters next to the mean values indicate statistically significant difference for $P < 0.05$ He: red blood cells; HT: hematocrit; HB: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; and MCHC: mean corpuscular hemoglobin concentration

Table 2 – Mean values and standard deviations of leukogram data, by sex and age, obtained from healthy Santa Ines sheep, raised in the region of Piedade city – São Paulo – 2004

Leucocytes (x 10^3/µL)	Group 1	(0-6 months)	Group 2	(7-18 months)	Group 3	(19-48 months)
	F	M	F	M	F	M
Leucocytes	6,90 ± 3,40 A, a	6,75 ± 2,19 A, a	9,60 ± 3,10 B, a	8,38 ± 2,25 A, a	8,40 ± 3,00 B, a	8,26 ± 1,57 A, a
Linfocytes	3,66 ± 1,72 A, a	3,48 ± 1,62 A, a	4,68 ± 2,34 A, a	4,03 ± 1,89 A, a	3,91 ± 1,95 A, a	4,32 ± 1,64 A, a
Neutrophils segmented	2,90 ± 2,50 A, a	2,86 ± 1,32 A, a	4,31 ± 2,14 B, a	3,63 ± 0,79 A, a	4,06 ± 1,70 B, a	3,40 ± 0,19 A, a
Eosinophils	0,14 ± 0,26 A, a	0,11 ± 0,10 A, a	0,40 ± 0,37 B, a	0,40 ± 0,27 A, a	0,70 ± 0,77 B, a	0,17 ± 0,11 A, a
Basophils	0,02 ± 0,04 A, a	0,02 ± 0,04 A, a	0,03 ± 0,07 A, a	0,04 ± 0,05 A, a	0,02 ± 0,05 A, a	0,00 ± 0,00 A, a
Monócytes	0,20 ± 0,22 A, a	0,30 ± 0,25 A, a	0,20 ± 0,16 A, a	0,28 ± 0,20 A, a	0,23 ± 0,37 A, a	0,37 ± 0,09 A, a

* Capital letters represent animals of the same sex compared with different age groups and lowercase letters represent males and females of the same age. Different letters next to the mean values indicate statistically significant difference for $P < 0.05$. M: males; F: females

Decrease was observed in the total number of red blood cells only in the comparison between groups 1 and 2 of females, whereas decrease occurred in the globular volume of females in group 2, but not in group 3. The MCHC value increased only in females of group 2 relative to group 1. Regarding sex influence, the total number of erythrocytes was higher in males (group 2) and the values for MCV and MCHC were lower in males relative to females.

Influence of age factor was observed in relation to the total numbers of leukocytes, segmented neutrophils, and eosinophils, which increased in females (group 2) and remained higher in group 3. Differences were not observed between the leukogram components in relation to sex.

Discussion

Some hemogram values ound in the present study revealed the influence presence of the age factor. This fact is in agreement with the results obtained by Josland (1933); Ullrey et al. (1965); Littleton et al. (1968); Overas (1969); Upcott et al. (1971); David et al. (2012); Pereira et al. (2015) and Lima et al. (2015).

The values found for hemoglobin rate were similar to those obtained in sheep raised in Peru at sea level (WATSON, 1953), where it was shown that the variability in hemoglobin concentration may be directly related to the O_2partial pressure factor.

The values for hematocrit ($p < 0.0153$) and MCHC ($p < 0.01$) were influenced by the age factor, being lower in females and higher in young animals, respectively, as observed by Ullrey et al. (1965) and Madureira (2013). The red cell total count ($p<0.01$) obtained in the present study was higher in young animals (Group 1), agreeing with the results obtained by Littleton et al. (1968), Upcott (1971), and Madureira (2013). This behavior may reflect the higher metabolism rate exhibited by younger animals in the growth phase; However, Ullrey et al. (1965) and David et al. (2012) observed that the erythrogram values decreased from birth to 14 days of age increasing later at three months of age. It is known that the bone marrow of neonates is unable to produce large amounts of erythrocytes. In fact, Ullrey et al. (1965) and Bostedt (1979) stated that inadequate iron ion supplementation is a possible explanation for such decline.

The values for total leukocytes found in Santa Ines sheep in the present study were different from those reported by Todd et al. (1952). The difference may have been due to the breed factor, since Todd et al. (1952) evaluated Hampshire animals, whose values were smaller than those of the Santa Ines sheep. When the values for total leukocytes obtained in the present study are compared with those of Dorper sheep raised in São Paulo State (MADUREIRA et al., 2013), the results of the present study were similar to those of Madureira, suggesting that the raising and management systems can influence the hematological parameters of the animals.

According to the results shown in table 2, influence of the age factor was also present in the total number of leukocytes. This result does not agree with those obtained by Fraser (1929) and Lima et al. (2015), because the animals' ages were different. In turn, Josland (1933); Reda e Hathout (1951); Batista et al. (2009); David et al. (2012) and Pereira et al. (2015) observed the influence of age factors in relation to the total number of leukocytes. However, these differences may occur by action of extrinsic (such as environmental, climatic, nutritional, and differentmanagement) factors, as highlighted by Birgel (1982).

It was also observed that the eosinophil and neutrophil values followed the changes in the total number of leukocytes, and increased with age. However, this fact was not observed by Batista et al. (2009). The average values for differential leukocyte count found in this study partially followed the reference values found by Chalhoub et al. (2000), who also studied Santa Ines sheep in the region of Botucatu.

As for the leukogram, it should be emphasized that significant differences were not observed regarding sex when the absolute values of different cell types are considered. However, the values for segmented neutrophils and eosinophils, were lower in younger females due to age influence. In contrast, Madureira et al. (2013) found higher values for segmented neutrophils and lymphocytes in males. In the studies of David et al. (2012) and Pereira et al. (2015), as well as in the present study, differences were found in segmented neutrophils in relation to the age factor, and higher values were observed in older animals. This study showed differencebetween eosinophils and higher values in older animals. However, significant differences were not observed in lymphocytes. The greater exposure of these animals to allergens would be a hypothesis for the increase in the number of eosinophils in older animals. On the other hand, Lima et al. (2015) found no difference between different types of leukocytes. The fact that the sheep presented a predominantly lymphocytic profile also deserves attention.

Conclusions

It was observed that in the hemogram of female Santa Inêz sheep the total number of leukocytes, segmented neutrophils, and eosinophils was influenced by the age factor and were lower in younger animals. The hematocrit and total number of red blood cells displayed the highest values in the youngest animals, and an initial decrease and subsequent stabilization were observed in the values with ageing of the animal. As for sex, differences were not observed in relation to the animals' leukogram components. In the erythrogram, the males presented a higher total number of erythrocytes and lower MCV and MCH values than females.

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