



Original Article

## Changes in the acute phase proteins and leukogram profile in Dorper lambs during the first six months of life

Alterações no perfil das proteínas de fase aguda e leucograma em cordeiros Dorper durante os seis primeiros meses de vida

Jean Silva Ramos<sup>1\*</sup> , José Tadeu Raynal Rocha Filho<sup>1</sup> , Maria Consuelo Caribé Ayres<sup>1</sup> , Alberto Lopes Gusmão<sup>1</sup> , Bruno Lopes Bastos<sup>2</sup> , José Eugênio Guimarães<sup>1</sup> 

<sup>1</sup> Escola de Medicina Veterinária e Zootecnia (EMEVZ), Universidade Federal da Bahia (UFBA), Salvador, Bahia, Brasil.

<sup>2</sup> Instituto Multidisciplinar em Saúde – Campus Anísio Teixeira (IMS-CAT), Universidade Federal da Bahia (UFBA), Vitória da Conquista, Bahia, Brasil.

### ARTICLE INFO

#### Article history

Received 04 February 2019

Accepted 29 May 2019

#### Keywords:

Ceruloplasmin

Haptoglobin

Inflammatory markers

Neonatal period

#### Palavras-chave:

Ceruloplasmina

Haptoglobina

Marcadores inflamatórios

Período neonatal

### ABSTRACT

The first months in the life of mammals are marked by many challenges, such as acquisition of maternal antibodies, immunological maturity, environmental exposure and food adaptation. These challenges may lead to changes in the concentration of inflammatory markers, as the acute phase proteins (APPs) and leukocytes. The better understanding of these markers behavior in physiological conditions is fundamental for diagnosis, monitoring, and prognosis of diseases. Therefore, the aim of this study was to determine the changes in the APPs and leukogram profile in Dorper lambs from birth until the sixth month of life. Samples were collected from 12 clinically healthy lambs at 0, 6, 12, 24, 48 hours and then at 7, 15, 30, 60, 90, 120, 150 and 180 days of age. All lambs were born with a low concentration of haptoglobin and ceruloplasmin. Significant increases occurred in the 48<sup>th</sup> hour and on the 7<sup>th</sup> day of life ( $P < 0.05$ ), respectively. The highest APPs concentration was observed on the 90<sup>th</sup> day. All leukogram cells varied throughout the experimental period. It was possible to characterize the changes in APPs and leukogram profile from birth to six months of life in Dorper lambs. This study offers new perspectives on the use of APPs in lambs during the first months of life.

### RESUMO

Os primeiros meses na vida dos mamíferos são marcados por muitos desafios, como a aquisição de anticorpos maternos, maturidade imunológica, exposição ambiental e adaptação alimentar. Esses desafios podem levar a mudanças na concentração de marcadores inflamatórios, como as proteínas de fase aguda (PFA) e leucócitos. A melhor compreensão do comportamento desses marcadores em condições fisiológicas é fundamental para o diagnóstico, monitoramento e prognóstico de doenças. Portanto, o objetivo deste estudo foi determinar as alterações no perfil das PFA e leucograma em cordeiros Dorper do nascimento ao sexto mês de vida. Foram coletadas amostras de 12 cordeiros clinicamente sadios às 0, 6, 12, 24, 48 horas e aos 7, 15, 30, 60, 90, 120, 150 e 180 dias de idade. Todos os cordeiros nasceram com baixa concentração de haptoglobina e ceruloplasmina. Aumentos significativos ocorreram com 48<sup>a</sup> hora e 7<sup>o</sup> dia de vida ( $P < 0,05$ ), respectivamente. A maior concentração de PFA foi observada no 90<sup>o</sup> dia. Todas as células do leucograma variaram ao longo do período experimental. Foi possível caracterizar as alterações no perfil das PFA e leucograma do nascimento até seis meses de vida em cordeiros Dorper. Este estudo oferece novas perspectivas sobre o uso das PFA em cordeiros durante os primeiros meses de vida.

\* Corresponding author: [jeanramos4@usp.br](mailto:jeanramos4@usp.br)

## INTRODUCTION

APPs are blood proteins that can be used as biomarkers, because their concentrations in blood changes in response to various conditions, such as inflammation, infection, stress, and neoplasia (ECKERSALL; BELL, 2010). Measurement of blood concentration of APPs can aid diagnosis and prognosis of disease due to its correlation with severity and extent of tissue damage (MARTÍNEZ-SUBIELA et al., 2001).

Cytokines, such as interleukins (IL-1 and IL-6) and tumor necrosis factor alpha (TNF- $\alpha$ ), are responsible for stimulating hepatocytes to synthesize APPs (BODE et al., 2012). Considering different species, there are different APPs, such as serum amyloid A, haptoglobin,  $\alpha_1$  acid glycoprotein, C-reactive protein, ceruloplasmin, fibrinogen and transferrin (MURATA; SHIMADA; YOSHIOKA, 2004). Clinically haptoglobin is the most important APPs in ruminants (ECKERSALL; BELL, 2010). Changes in the concentration of APPs in lambs occur as a result of diseases such as interdigital dermatitis (CARVALHO et al., 2012), scab caused by *Psoroptes ovis* (WELLS et al., 2013), and infection by *Haemonchus contortus* (ZHONG et al., 2014). It also occurs during stressful conditions including animal transport (PICCIONE et al., 2012).

There are no studies about changes in the APPs (Haptoglobin and ceruloplasmin) profile in Dorper lambs, by spectrophotometric technique, during the first months of life. Some research has been performed on calves and goat kids (KNOWLES et al., 2000; TÓTHOVÁ et al., 2015; ULUTAS et al., 2017) and low concentration of APPs was observed at first days of life. Knowles et al. (2000) observed an elevation of haptoglobin levels in calves during the first week of life. In Santa Inês lambs Ramos et al. (2018a) observed low concentration of ceruloplasmin during first days of life.

Leukogram is a hematological parameter used in diagnosis, prognosis, and monitoring of diseases. It is especially important in cases of infection and/or inflammation. There are studies showing that age does not significantly influence the total number of leukocytes (EGBE-NWIYI; NWAOSU; SALAMI, 2000; ADDASS; MIDAU; BABALE, 2010). On the other hand, leukocyte values were influenced by age according to Njidda; Shuai'bu; Isidahomen (2014). Lambs with 30, 60, 90, and 120 days showed a change on neutrophil and lymphocyte counts, however the leukocytes count in the neonatal period was not evaluated (SOUZA et al., 2018).

The interpretation of changes in the haptoglobin, ceruloplasmin and leukogram profile from birth to the sixth month of life is fundamental for a better understanding of the behavior of these markers in physiological conditions, consequently, helping in diagnosis, monitoring, and prognosis of several diseases. The objective of this study was to determine changes in serum concentration of haptoglobin, ceruloplasmin, and

leukocytes during the first six months of life in Dorper lambs.

## MATERIAL AND METHODS

The study was conducted at Nova Esperança Farm, located in Candeias County, Bahia State, Brazil (latitude and longitude of 12 ° 40 '04 "S and 38 ° 33' 02" W). The use of animals and all experimental procedures were approved by the Ethics Commission on Animal Use of the Veterinary Medicine and Animal Science School from Federal University of Bahia (UFBA), under protocol number 06/2012.

Twelve healthy lambs (six males and six females) were born to multiparous Dorper ewes of different ages. These ewes were all laparoscopically inseminated with semen from the Dorper breed. Ewes were monitored at the time of delivery and all births were eutocic. From the total number of ewes, four had twin births and four had single births. After birth, animals were kept in 3 m<sup>2</sup> bays. Blood sampling was standardized in timing and way of collection. Lambs received colostrum naturally from the ewes.

On the 15<sup>th</sup> day, commercial concentrate, Genese Ovin® (Socil by Neovia, Saint Nolf, France) and powdered cane molasses were available for consumption. Lambs remained with their mothers until weaning on day 90. Deworming was performed approximately 30 days after birth (IVOMECC®; Merial Ltd., Iselin, NJ, USA) and vaccination against clostridiosis was performed on day 90 (Poli-Star®; Vallée S.A., São Paulo, SP, Brazil).

Blood samples were obtained at the following moments: immediately after birth (without colostrum intake), 6, 12, 24, and 48 hours after birth, and then on days 7, 15, 30, 60, 90, 120, 150, and 180. All animals were born within a five-day period. Blood samples were obtained by jugular venipuncture using tubes containing ethylenediaminetetraacetic acid dipotassium (EDTA-K<sub>2</sub>) and tubes without anticoagulants (both, Becton-Dickinson Brazil, São Paulo, Brazil).

Blood samples were initially processed on site, at the farm. Samples without anticoagulants were centrifuged at 1000 x g for five minutes and the serum obtained was stored in Eppendorf tubes at -20 °C. Frozen samples were placed on isothermic boxes with ice for transportation to the Hematology Laboratory at the Federal University of Bahia's Veterinary Hospital (UFBA).

The white blood cell count was obtained by counting in the Neubauer chamber. Blood smears were performed and stained with Quick Diff (LaborClin, Pinhais, PR, Brazil) and leukocyte differentiation count was performed under light microscopy. These methodologies were used because the distance between the farm and the laboratory was 100 km.

Haptoglobin concentration was obtained according to the methodology of Jones; Mould (1984). A standard curve was developed with standard haptoglobin solutions diluted from 0.56 to 0.01 g/L. Fifty microliters of haptoglobin or serum sample was added to 50  $\mu$ L of 0.9% saline solution in each well. Subsequently, 50  $\mu$ L of sheep methemoglobin solution (30 mg/dL) was added and plates were incubated for 10 min at 20 °C. A blank sample (50  $\mu$ L of 0.9% saline) was processed along with each serum sample. Following incubation, a guaiacol reagent (150  $\mu$ L, pH 4.0) and 50  $\mu$ L of an H<sub>2</sub>O<sub>2</sub> solution (0.02 mol/L) were added. After 10 min, absorbance at 490 nm was measured using a microplate reader. All samples were processed in duplicates and the means of each duplicate were used to calculate final concentration based on the standard curve (BASTOS et al., 2013; RAMOS et al., 2018b).

Enzymatic activity of ceruloplasmin was measured according to the method developed by Schosinsky; Lehmann; Beeler (1974). Blood serum (50  $\mu$ L) was incubated with 750  $\mu$ L of acetate buffer (pH 5.0) in two test tubes (one marked "5 min," and the other "15 min"). The tubes were placed in a 30 °C water bath and allowed to stand for 5 min for temperature stabilization before pipetting at timed intervals. Two hundred microliters of the o-dianisidinedihydrochloride reagent (pre-incubated at 30 °C) was pipetted into each tube. The timer was started when the first substrate was added. After exactly 5 min, the "5 min" tube was removed from the water bath, and 2.0 mL of sulfuric acid (9.0 M) was added and mixed immediately. At exactly 15 min, the "15 min" tube was removed and 2.0 mL of the 9.0 M molar sulfuric acid was added and mixed immediately. The absorbance of the purplish-red solutions was measured at 540 nm, with a 1-cm light path using deionized water as blank.

The enzymatic activity of ceruloplasmin was expressed in International Units (IU/L) in terms of substrate consumed using the recommended formula [Ceruloplasmin oxidase activity = (A15 - A5) x 6.25 x 0.1 U/mL], in which A15 and A5 are the measured absorbances of the "15 min" and "5 min" solutions, respectively. The factor 6.25 X 0.1 was obtained from the pre-established formula [Concentration of substrate oxidized = (absorbance x 3 x 20) / (9.6 x 10)  $\mu$ mol / mL per minute], where the value 9.6 is the molar absorptivity of colored solutions in terms of substrate consumed (mL/ $\mu$ mol/ cm); the value 3.0 is the correction factor for the final measured solution volume; the value 20 is the correction for the serum volume used (50  $\mu$ L); and the value 10 is the incubation time (in minutes).

Data was analyzed using Statistical Package for the Social Sciences, version 19.0 (SPSS Inc., Chicago, IL, USA). Data normality was checked using the Shapiro-Wilk test at a 0.05 level of significance. Non-parametric data were submitted to logarithmic, inverse, and quadratic root transformations to obtain a normal distribution. To evaluate the effect of time on the parameters, values of

parametric data, and values normalized with the transformations were analyzed using repeated measures analysis of variance (ANOVA) followed by a Bonferroni post hoc test ( $P < 0.05$ ). Data that were non-parametric were analyzed by using the Friedman test to evaluate the variations over time. Multiple comparisons were performed using the Wilcoxon test with Bonferroni correction ( $P < 0.004$ ). The time of birth (0 hours) was used as the reference point in statistical analyses. Haptoglobin and ceruloplasmin measurement values were correlated over the experimental period by the Pearson's correlation test ( $P < 0.05$ ). For all tests, a tendency toward significance was considered when the p-value was between 0.05 and 0.1 for parametric variables and between 0.004 and 0.1 for non-parametric variables.

## RESULTS

Significant variations over time were observed for haptoglobin, ceruloplasmin, total leukocytes, segmented neutrophils, lymphocytes, monocytes, and eosinophils ( $P < 0.001$ ). There was no change in haptoglobin concentration when the concentrations at birth (0 hours; before colostrum intake) and six hours after birth were compared. However, a slight increase in the haptoglobin concentration was observed after 12 and 24 hours. Significant increase in the haptoglobin concentration was observed 48 hours after birth ( $P = 0.001$ ) and remained high on days 7 ( $P = 0.043$ ) and 15 ( $P = 0.001$ ). These elevated haptoglobin concentrations returned to baseline values on the 30<sup>th</sup> day, with no significant differences in concentrations when compared to the concentration at 0 hours (Figure 1A).

Haptoglobin concentration increased significantly on the 60<sup>th</sup> and 90<sup>th</sup> days ( $P = 0.001$ ). On the 90<sup>th</sup> day, the highest concentration of this protein was observed. On the 120<sup>th</sup> day, the concentration of haptoglobin decreased while on the 150<sup>th</sup> day it increased again ( $P = 0.003$ ), and on the 180<sup>th</sup> day it started to decline.

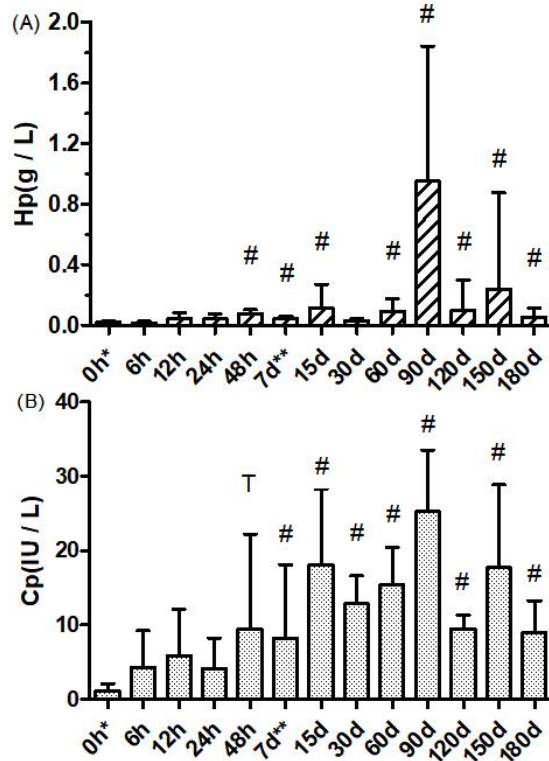
There was a low concentration of ceruloplasmin before ingestion of colostrum compared to later times (Figure 1B). A slight increase was observed 6, 12, 24, and 48 hours after birth. There was a tendency towards an increase in concentration of ceruloplasmin after 48 hours when compared to concentration at 0 hour ( $P = 0.052$ ). Ceruloplasmin concentration increased on the 7<sup>th</sup> day of life ( $P = 0.033$ ) and remained high throughout the experimental period when compared to its concentration at 0 hours ( $P < 0.05$ ). The maximum increase in ceruloplasmin concentration occurred on the 90<sup>th</sup> day. Concentrations of haptoglobin and ceruloplasmin were positively and moderately correlated ( $R = 0.5$ ;  $P = 0.001$ ).

All lambs were born with a low number of leukocytes (Table 1 and figure 2). However, slight increases in the number of leukocytes were observed after 6, 12, and 24 hours, even though these increases were not statistically

significant. After 24 hours, the number of leukocytes decreased (Figure 2A). Nevertheless, slight increases were observed on the 7<sup>th</sup> and 15<sup>th</sup> days. A significant increase occurred on the 30<sup>th</sup> day (P = 0.01) and the

number of leukocytes remained high until the end of the study (P < 0.05). The leukocyte count was highest on the 150<sup>th</sup> day.

Figure 1 – Mean and standard deviation of haptoglobin (A) and ceruloplasmin (B) concentrations of Dorper lambs (n = 12) from birth to the sixth month of life.



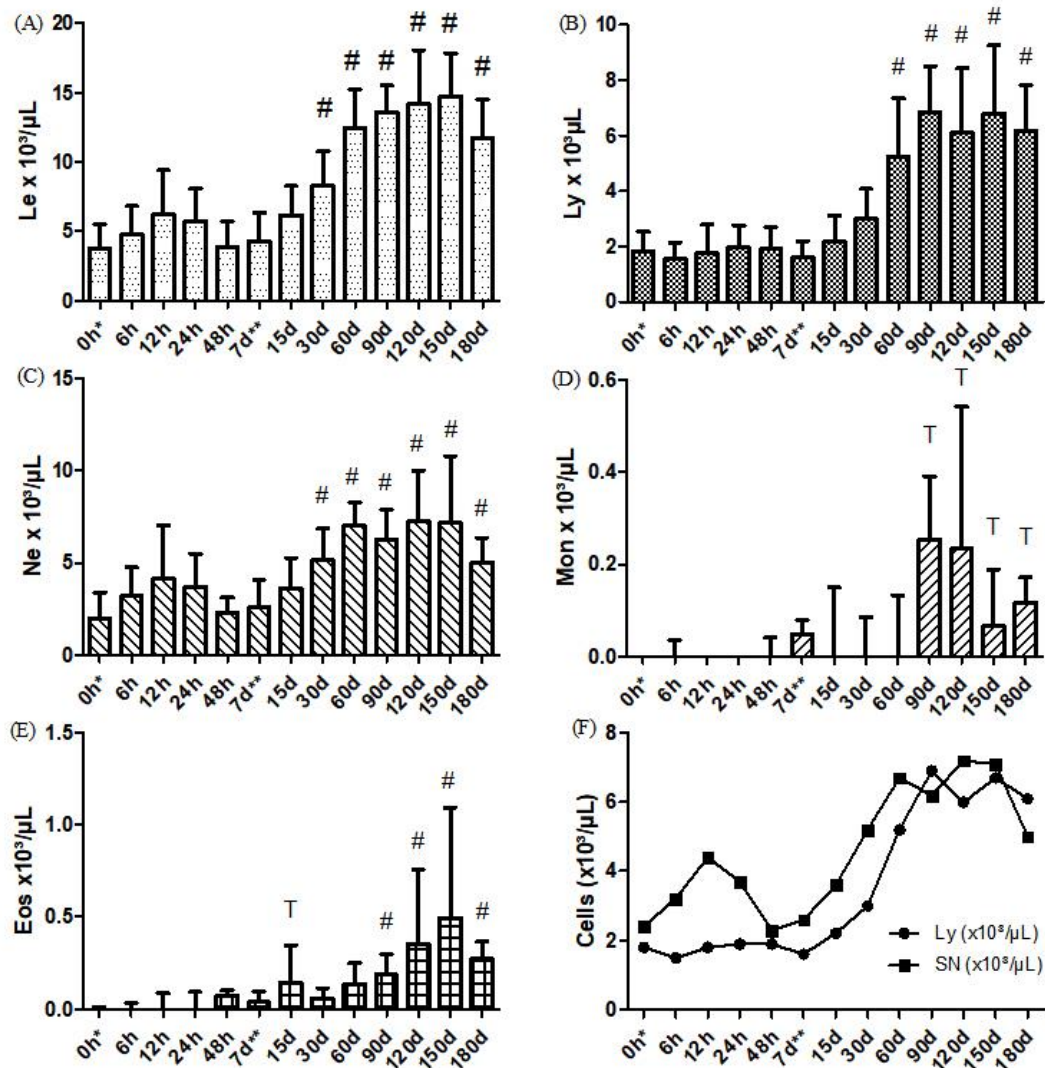
\*hours; \*\* days; Hp = Haptoglobin; Cp = Ceruloplasmin; # = P < 0.05 for multiple comparisons (Bonferroni post-hoc test). T = Tendency, p-values between 0.05 and 0.1. Compared to 0 hours.

Table 1 – Mean and standard deviation of haptoglobin, ceruloplasmin, total leukocytes, lymphocytes, segmented neutrophils and the median and interquartile range of the concentrations of monocytes and eosinophils in healthy Dorper lambs (n = 12) from birth to the sixth month of life.

	Hp	Cp	Le	Ly	SN	Mon	Eos
0h*	0.02±0.01	1.1±1.06	3.8±1.7	1.8±0.7	2.4±2.0	0.0±0.0	0.0±0.0
6h	0.02±0.01	2.14±2.9	4.8±2.0	1.5±5.8	3.2±1.5	0.0±0.0	0.0±0.0
12h	0.04±0.03	3.98±2.45	6.2±3.2	1.8 ±1.0	4.4±2.7	0.0±0.0	0.0±0.0
24h	0.03±0.03	4.18±4.05	5.7±2.4	1.9±0.8	3.7±1.7	0.0±0.0	0.0±0.0
48h	0.07±0.02#	6.19±6.88 <sup>T</sup>	4.0±1.5	1.9±0.7	2.3±0.8	0.0±0.0	0.1±0.1
7d**	0.05±0.01#	8.28±9.82#	4.3±2.0	1.6±0.6	2.6±1.5	0.1±0.1	0.0±0.1
15d	0.11±0.2#	18.12±10.14#	6.2±2.0	2.2±0.9	3.6±1.5	0.0±0.2	0.1±0.3 <sup>T</sup>
30d	0.03±0.01	12.9±3.8#	8.3±2.4#	3.0±1.0	5.2±1.6#	0.0±0.1	0.1±0.1
60d	0.09±0.08#	15.4±5.03#	12.4±2.8#	5.2±2.0#	6.7±0.5#	0.0±0.1	0.1±0.3
90d	0.95±0.9#	25.3±8.2#	13.6±1.9#	6.9±2.3#	6.2±1.6#	0.2±0.3 <sup>T</sup>	0.2±0.2#
120d	0.09±0.2#	9.5±1.9#	14.2±3.9#	6.0±2.3#	7.2±2.8#	0.2±0.5 <sup>T</sup>	0.4±0.6#
150d	0.23±0.63#	17.7±11.1#	14.7±3.1#	6.7±2.4#	7.1±3.6#	0.1±0.2 <sup>T</sup>	0.5±0.8#
180d	0.06±0.06#	9.01±4.27#	11.7±2.8#	6.1±1.6#	5.0±1.3#	0.1±0.0 <sup>T</sup>	0.3±0.2#

\*hours; \*\* days; Hp = Haptoglobin (g / L); Cp = Ceruloplasmin (IU / L); Le (Total leukocytes x 10<sup>3</sup>/μL); Ly (Lymphocytes x 10<sup>3</sup>/μL); SN (Segmented neutrophils x 10<sup>3</sup>/μL); Mon (Monocytes x 10<sup>3</sup>/μL) and Eos (Eosinophils x 10<sup>3</sup>/μL); # = P<0.05 for multiple comparisons (Bonferroni post-hoc test), and P<0.004 for the Wilcoxon test with Bonferroni correction. T = Tendency, p-values between 0.05 and 0.1. for parametric variables and between 0.004 and 0.1 for non-parametric variables. Comparisons made to 0 hour.

Figure 2 – Mean and standard deviation of the concentrations of leukocytes, lymphocytes, and segmented neutrophils and the median and interquartile range of the concentrations of monocytes and eosinophils in healthy Dorper lambs (n=12) from birth to the sixth month of life.



\*hours; \*\* days; A = Le (Total leukocytes x 10<sup>3</sup>/μL); B = Ly (Lymphocytes x 10<sup>3</sup>/μL); C = SN (Segmented neutrophils x 10<sup>3</sup>/μL); D = Mon (Monocytes x 10<sup>3</sup>/μL); E = Eos (Eosinophils x 10<sup>3</sup>/μL) and F = Lymphocytes and neutrophils over time; # = P<0.05 for multiple comparisons (Bonferroni post-hoc test), and P<0.004 for the Wilcoxon test with Bonferroni correction. T = Tendency, p-values between 0.05 and 0.1. for parametric variables and between 0.004 and 0.1 for non-parametric variables. Comparisons made to 0 hour.

The number of lymphocytes was constant from birth until the 15<sup>th</sup> day. A slight increase was observed on day 30 but a significant increase (P<0.05) was observed after 60 days (Figure 2B). The maximum number of lymphocytes was observed on the 90<sup>th</sup> day. The numbers of segmented neutrophils increased on the 6<sup>th</sup>, 12<sup>th</sup>, and 24<sup>th</sup> hours, but without statistical difference. After 24 hours, the number of neutrophils slightly decreased and remained low until day 7 (Figure 2C). On the 15<sup>th</sup> day after birth, a slight increase on neutrophils occurred. Similarly, to total leukocyte count, a significant increase (P=0.001) in number of neutrophils occurred only on day 30. Neutrophils remained high until the end of the study (P<0.05).

No significant differences were verified in multiple comparisons on monocyte counts (P>0.004). Compared to the beginning of the study, a tendency towards an increase in the concentrations was observed on days 90 (P=0.012), 120 (P=0.028), 150 (P=0.068), and 180 (P=0.028) (Figure 2D). Low numbers of eosinophils were observed at birth compared to the numbers at other times after birth. Based on multiple comparisons, a tendency towards an increase in the number of eosinophils was observed after 15 days (P=0.008). Compared to the number of eosinophils at birth, significant increases were observed on day 90 and remained elevated until day 180 (P=0.002). Maximum number of eosinophils was observed on the 150<sup>th</sup> day (Figure 2E).

## DISCUSSION

This study evaluated the changes in serum concentration of haptoglobin, ceruloplasmin, and leukocytes during the first six months of life in healthy Dorper lambs. In general, the animals were born with low concentrations of APPs, total leukocytes, segmented neutrophils, lymphocytes, monocytes, and eosinophils. However, their concentrations increased over time.

Metabolites present in colostrum, such as leukocytes and cytokines, can enter the newborn's circulatory system through breastfeeding (REBER et al., 2006) and can influence the hepatic production of haptoglobin and ceruloplasmin during the first hours of life. Other studies have also suggested that inflammatory mediators present in colostrum can induce acute phase response in neonates (ORRO et al., 2008; KILPI, 2015).

In lambs, levels of serum amyloid A and fibrinogen increased after colostrum ingestion (HERNANDEZ-CASTELLANO et al., 2014). A slight increase in haptoglobin and ceruloplasmin levels was observed at the beginning of the study. These increases were significant for haptoglobin after 48 hours, and on the 7<sup>th</sup> day for ceruloplasmin. These results can be attributed to factors such as the synthesis of APPs by the mothers' breast parenchyma and the presence of proteins in colostrum and milk (ECKERSALL et al., 2001; HISS et al., 2004). The adaptation mechanism to the extrauterine environment can influence serum concentration of both proteins in neonates during the first hours of life (KILPI, 2015; RAMOS et al., 2018a).

Changes in haptoglobin, ceruloplasmin, and leukocyte concentrations during the initial period of life may be associated with bacterial colonization in the gastrointestinal tract. In neonates, this period is a challenge because bacterial types influence the type of immune response (KOCH et al., 2016). On the other hand, the liver may be responsible for these changes in animals during early development. Some speculations can be made about the changes in APPs concentrations identified on the 15<sup>th</sup> and 30<sup>th</sup> days of life. First, it is possible that lambs were undergoing immune adaptation. The environment is the major challenge at this stage of life. Second, dietary supplementation with vitamins, minerals, and ionophores may have influenced the increase in haptoglobin and ceruloplasmin concentrations. Both protein levels increased significantly on the 15<sup>th</sup> day compared to their levels at 0 hour, and decreased on the 30<sup>th</sup> day.

Haptoglobin concentration showed a 10-fold increase compared to days 60 and 90. On these days, the haptoglobin concentration changed from 0.09 g/L to 0.95 g/L (Figure 1A). The concentration verified on day 60 was suggested by Lopherd et al. (2009) to be within the normal range of 0.06 and 0.12 g/L for sheep. Ceruloplasmin concentration increased slightly from 15.4 IU/L on day 60 to 25.3 IU/L on day 90. Although

ceruloplasmin may be considered a moderate protein, it is important for the detection of haptoglobin. Both ceruloplasmin and haptoglobin were at their highest concentrations on days 15, 90, and 120.

Challenges such as vaccination, weaning, and exposure to a new environment may have affected the values of haptoglobin and ceruloplasmin on the 90<sup>th</sup> day of life. On the 90<sup>th</sup> day, the highest concentrations were statistically different from those obtained at 0 hour. According to Tadich et al. (2009), after a few hours of weaning an increase in APPs concentration occurs. This phenomenon was also reported in calves (ARTHINGTON; SPEARS; MILLER, 2005). Stress may stimulate the synthesis of APPs. However, it is important to emphasize the need for further studies to confirm this premise.

Nowroozi-Asl; Nazifi; Bahari (2008) reported values of  $0.11 \pm 0.06$  g/L for haptoglobin concentrations in healthy Iranian fat-tailed lambs of both sexes, which were less than a year old, whereas Dorper lambs in our study had lower haptoglobin concentrations during the initial period of life (0, 6, 12, 24, and 48 hours and 7 days). However, during the period between days 60 and 180, the values of the haptoglobin concentration were similar to those of the Iranian fat-tailed lambs, with the exception of that on the 90<sup>th</sup> day.

All the animals in this study were born with a low number of leukocytes. Their leukocyte numbers had a slight increase 6 and 12 hours after birth (Figure 2A). This may be due to stress at the time of delivery and/or the passage of maternal cells into the neonate's circulatory system during birth. Novo et al. (2015) confirmed that the increase in the number of leukocytes in the first hours of life was due to the effects of cortisol. The period after birth is very challenging for lambs because adaptations occur in both the respiratory and circulatory systems. Indeed, high levels of cortisol were found in calves soon after birth (KNOWLES et al., 2000; PAIVA et al., 2006). It is important to note that the increase observed in leukocyte counts from lambs and calves was due to the number of neutrophils, supporting the hypothesis that stress is involved (NOVO et al., 2015).

On the other hand, it is known that maternal leukocytes can enter the neonates' circulatory system (REBER et al., 2006). They provided colostrum containing labeled cells to the neonates and observed that the labeled cells remained in the circulatory system for up to 36 hours after ingestion. These findings may also justify the increase and decrease in the number of leukocytes and neutrophils observed in Dorper lambs during the initial period of life (Figure 2A, and 2C). Mean number of leukocytes was high in Dorper lambs 90 days after birth (SCHALM; JAIN; CARROL, 2010), remained high until 150 days after birth, and stabilized 180 days after birth. Using thirty-five  $\frac{1}{2}$  White Dorper x  $\frac{1}{2}$  Suffolk female lambs Souza et al. (2018) reported the highest moment for white blood cell was at 90 days of life. Factors such as

farm management, weaning, and contact with the environment may have influenced these findings. Egbe-Nwiyi; Nwaosu; Salami (2000) observed high numbers of lymphocytes compared to that of neutrophils in sheep. These results differed from those obtained in our study. We observed a high number of neutrophils. On the 90<sup>th</sup> and 180<sup>th</sup> days, the total number of lymphocytes was superior to that of neutrophils (Figure 2F). On the 90<sup>th</sup> day, the increase in number of lymphocytes may be due to vaccination and weaning.

Monocytes showed significant increases on the 90<sup>th</sup> and 120<sup>th</sup> day of life (Figure 2D). The increase in monocytes, which are important cells in the immune system, may be due to vaccination stimulus and the exposure of the animals to environment. The number of eosinophils increased after 60 days. The exposure to environment and the post-weaning that occurred 90 days after birth may have been stimulation factors in the increase of the number of eosinophils to its maximum value on the 150<sup>th</sup> day. The ingestion of helminths eggs during exposure to the environment may have contributed to the increase in number of these cells (BALIC; BOWLES; MEEUSEN, 2000), regardless of the deworming which was performed around the 30<sup>th</sup> day of life.

This study clarifies more precisely the variations of haptoglobin, ceruloplasmin and leukocytes throughout the initial period of life. Some hypotheses were generated in this research and new studies should be carried out to better understand the important changes observed in the parameters evaluated. It is important to highlight the need to work out the clinical importance (sensitivity and specificity) of APPs in disease detection in Dorper lambs.

## CONCLUSIONS

This longitudinal study provided a better understanding of the changes in concentrations of haptoglobin, ceruloplasmin, and leukocyte counting throughout the first six months of life in Dorper lambs. This study provided new perspectives on the factors that may be associated with the observed changes. Furthermore, the concentration of APPs in physiological condition is an important tool in diagnosis, prognosis, and monitoring of disease in Dorper lambs.

## ACKNOWLEDGMENTS

The authors are grateful for the funds provided by Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) studentships.

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