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# Dynamics of biochemical parameters in lambs during the first four months of life

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**ABSTRACT** - We aimed to verify whether age influences the biochemical profile of healthy lambs during the first four months of life by characterizing the dynamics of its parameters and verifying whether there are differences between the profiles of growing animals and the reference values established for adults. Blood samples of 34 ½ White Dorper × ½ Suffolk female lambs were collected at 30, 60, 90, and 120 days of age, and the serum concentrations of total proteins, total globulins, albumin, urea, creatinine, bilirubins (total, conjugated, and unconjugated), total cholesterol, triglycerides, glucose, gamma-glutamyltransferase, aspartate aminotransferase, and creatine kinase were determined. Descriptive statistics of these parameters were performed to compare them with the reference intervals used for adult sheep. Age influenced the dynamics of all the biochemical parameters between 30 and 120 days of age. Moreover, except for the total, conjugated and unconjugated bilirubins, total cholesterol, and aspartate aminotransferase means, the values of the lambs differed from the reference intervals established for adults. In conclusion, there is an influence of age on biochemical parameters in healthy female lambs in the first four months of life, and often the biochemical parameters of young animals differ from those of adults. Therefore, the interpretation of tests performed on growing animals should be made based on specific reference intervals for this age group.

**Keywords:** age, blood, clinical biochemistry, metabolic profile, sheep

## 1. Introduction

Examination of the biochemical profile is an important clinical tool that assists in the differential diagnosis of various diseases (Russell and Roussel, 2007). In ruminants, a biochemical analysis can also be applied to populations for the evaluation of so-called metabolic profiles, mainly evaluating the nutritional management, reproductive performance, and incidence of diseases in livestock (Peixoto and Osório, 2007).

For this to be possible, prior knowledge of the reference values in healthy animals and of the functioning of metabolic dynamics in normal situations and in cases of physiological, nutritional, and/or pathological alterations is essential (Mohri et al., 2007; Lima et al., 2015). For correct laboratory interpretation, factors that act on metabolic mechanisms and those that interfere with the analysis of results, such as species, breed, sex, production system, feeding, reproductive stage, climate, and differences in techniques used in each laboratory must be considered (Meira Jr et al., 2009; Braun et al., 2010).

In recent years, there has been an increase in researchers' interest in establishing reference intervals for different breeds, sexes, and age groups of the Brazilian sheep flock (Batista et al., 2009; Meira Jr et al.,

2009; Santana et al., 2009; Madureira et al., 2013; Carlos et al., 2015; Lima et al., 2015; Souza et al., 2016). However, even in these studies, animals in the growth phase are categorized as a generalized age group, without a specific assessment of the changes in metabolic dynamics within the neonatal and growth periods. In the literature, there are still few studies covering this phase in detail (Devendran et al., 2009; Lepherd et al., 2009; Silva et al., 2010; Antunović et al., 2012; Souza et al., 2014; Cruz et al., 2017); thus, often the reference intervals used in the evaluation of lambs are still based on values determined for adults.

However, the highest mortality rates are observed in lambs, and metabolic disorders in the first months of life, if not properly treated, will impair productivity in the following periods (Magalhães et al., 2016). There is a consensus that age has an important effect on the concentration of many metabolites, especially before puberty (Lepherd et al., 2009; Antunović et al., 2012). This is already well established in calves (Feitosa et al., 2007; Delfino et al., 2014); however, a better understanding of the influence of this factor on the biochemical profile of lambs is required.

Therefore, the objective of this study was to verify whether there is indeed an influence of age on the biochemical profile of healthy lambs during the first four months of life, by characterizing the dynamics of its parameters during this period. Moreover, we aimed to verify whether there are differences between the profiles of growing animals and the reference values established for adults.

## 2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (case no. 016/2011 of August 04, 2011). The study was carried out in Pinhais, Paraná State, Southern Brazil (25°38' S, 49°14' W, and 953 m altitude).

Thirty-four ½ White Dorper × ½ Suffolk female lambs, monitored from birth to 120 days of age, were used. The evaluation period extended from October to March (Spring/Summer).

As prophylactic measures, we certified that the colostration was adequate, and the postpartum umbilical disinfection was performed as well as disinfection of the premises; vaccinations against clostridiosis (Sintoxan Polivalente T®, Merial) and ecthyma contagiosum (Ectima Vac®, CEVA) were performed at 41 days of age, on average; we also performed weighing [body weight (BW) at birth and at 18-day intervals] and calculations of average daily weight gain (ADG) and body condition score (BCS) according to Russel et al. (1969), and parasitological monitoring [by observation of clinical signs such as diarrhea and the application of the Famacha method according to Molento et al. (2004)]. Because of the initial small size of the animals, there was difficulty in obtaining the required amount of feces for the parasitological exams, and, therefore, the count of eggs per gram of feces (EPG) was not carried out. No complication was observed after these interventions. The animals showed normal complete blood count and no lesions or apparent pathological signs throughout the experiment.

The lambs presented growth according to NRC (2007). The mean and standard error (M±SE) for birth weight was 4.6±0.2 kg, and the values of BW and ADG recorded during the experiment are in Table 1. Also, the means for Famacha grade and BCS were 1.6±0.2 and 2.8±0.1, respectively, indicating that the lambs presented adequate sanitary status.

The lambs remained housed with their mothers in collective pens from birth to weaning, which occurred between 70 and 80 days of age. From 14 days of age, they had access to feed in creep feeding composed of corn silage and protein-energy concentrate (Table 2), provided as total mixed ration (TMR), twice a day (at 08.00 and 16.00 h). The diet of the ewes was composed of the same silage provided to the lambs plus energy concentrate (Table 2), adjusted for the category of lactating ewes according to NRC (2007). Lambs were fed corn silage *ad libitum* from 61 to 90 days of age (Table 3), allowing leftovers of up to 200 g $kg^{-1}$ of the fresh matter offered to not limit the feed intake.

After weaning, the lambs remained housed in collective pens until 90 days of age. From this age, they were allocated to limpograss pastures [*Hemarthria altissima*; whose chemical composition was determined from two samples collected in the last evaluation period by the grazing simulation method,

according to Burns et al. (1989); Table 2] during the daytime (from 08.00 h), being gathered in the fold in the evening (after 16.00 h) when they received supplementation in the trough (of the same composition of TMR provided in the creep feeding during pre-weaning; Table 2), and remained overnight.

The diets were formulated to meet the nutritional requirements recommended for growing lambs, with maturity of 0.3 at four months of age and expressing 200 g day$^{-1}$ of mean daily gain, according to NRC (2007). The requirements of dry matter intake, crude protein, and metabolizable energy were adjusted to the BW of lambs at the ages of 14, 30, 60, 75 (considered as the weaning day), 90, and 120 days, and milk intake (from 14 days of age until weaning) was estimated. The diets were provided

**Table 1 -** Performance of ½ White Dorper × ½ Suffolk lambs in the first four months of age

| Performance trait | Age (days) | Mean ± standard deviation |
|---|---|---|
| Body weight (kg) | 14 | 8.54±1.81 |
| | 30 | 13.07±3.03 |
| | 60 | 18.94±3.77 |
| | 90 | 27.63±4.96 |
| | 120 | 31.27±4.72 |
| Average daily gain (g day$^{-1}$) | 14-30 | 283.1±80.7 |
| | 31-60 | 195.6±59.3 |
| | 61-90 | 289.7±74.6 |
| | 91-120 | 166.8±62.3 |

**Table 2 -** Ingredients of the energy and protein-energy concentrates and chemical composition of energy and protein-energy concentrates, corn silage, and limpograss samples collected by simulated grazing during the experiment

| Components | Energy concentrate | Protein-energy concentrate | Corn silage | Limpograss |
|---|---|---|---|---|
| Ingredients (g kg$^{-1}$) | | | | |
| Ground corn | 504.0 | 574.0 | | |
| Soybean hulls | 0.0 | 134.0 | | |
| Soybean meal | 287.0 | 261.0 | | |
| Wheat bran | 160.0 | 0.0 | | |
| Limestone | 24.0 | 13.0 | | |
| Mineral supplement[1] | 25.0 | 18.0 | | |
| Chemical composition[2] | | | | |
| DM (g kg$^{-1}$) | 905.3 | 888.3 | 326.3 | 267.0 |
| CP (g kg$^{-1}$ DM) | 189.7 | 214.1 | 71.8 | 128.8 |
| EE (g kg$^{-1}$ DM) | 32.1 | 28.6 | 25.0 | 18.5 |
| NDF (g kg$^{-1}$ DM) | 139.0 | 188.4 | 594.5 | 625.4 |
| ADF (g kg$^{-1}$ DM) | 42.9 | 47.4 | 336.1 | 296.1 |
| Lignin (g kg$^{-1}$ DM) | 16.7 | 16.1 | 46.6 | 71.1 |
| NFC (g kg$^{-1}$ DM) | 581.5 | 519.4 | 266.5 | 177.2 |
| Ash (g kg$^{-1}$ DM) | 57.6 | 49.5 | 42.2 | 50.1 |
| TDN (g kg$^{-1}$ DM) | 707.8 | 701.9 | 621.7 | 554.0 |
| DE (Mcal kg$^{-1}$ DM) | 3.12 | 3.11 | 2.62 | 2.39 |
| ME (Mcal kg$^{-1}$ DM) | 2.56 | 2.55 | 2.15 | 1.96 |

DM - dry matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; NFC - non-fibrous carbohydrates; TDN - total digestible nutrients; DE - digestible energy; ME - metabolizable energy.
[1] Guaranteed analysis of macrominerals: 110-135 g kg$^{-1}$ calcium, 87 g kg$^{-1}$ phosphorus, 147 g kg$^{-1}$ sodium, 18 g kg$^{-1}$ sulphur; trace minerals: 15 mg kg$^{-1}$ cobalt, 590 mg kg$^{-1}$ copper, 20 mg kg$^{-1}$ chrome, 870 mg kg$^{-1}$ fluorine, 50 mg kg$^{-1}$ iodine, 2000 mg kg$^{-1}$ manganese, 300 mg kg$^{-1}$ molybdenum, 20 mg kg$^{-1}$ selenium, 3800 mg kg$^{-1}$ zinc.
[2] The contents of DM, CP, EE, lignin, and ash were analyzed following the methods of AOAC (1990); the contents of NDF and ADF were determined as proposed by Van Soest et al. (1991); the content of NFC was calculated according to Hall et al. (1999); and the contents of TDN, DE, and ME were calculated using the equations described by Weiss et al. (1992).

to complement the milk intake until weaning and to allow the lambs to achieve the pre-stablished performance (Table 3).

The biochemical tests for each of the 34 female lambs were performed at 30, 60, 90, and 120 days of age, totaling 134 samples. Blood samples (4 mL) were collected by jugular venipuncture in vacuum tubes without anticoagulant, after a 10-h fasting (without water deprivation). All collections were performed in the period between 06.00 and 09.00 h. The samples were rested until clot formation and then centrifuged at 2,400 × *g* for 10 min. The obtained serum was transferred and stored in Eppendorf tubes at −20 °C until analysis.

The serum samples were thawed at room temperature and then processed in an automated biochemical analyzer (BS-200, Myndray®), previously calibrated with commercial control serum (Control Lab®). The evaluated parameters and the respective methods applied were as follows: the concentration of total proteins (biuret method, Katal®), albumin (bromocresol green method, Katal®), total globulins (by the difference between the total proteins and albumin), urea (UV enzymatic method of urease-GLDH, Kovalent®), creatinine (Jaffé method, Kovalent®), total and conjugated bilirubins (Jendrassik Grof method, Dialab®), unconjugated bilirubin (by the difference between the total and conjugated bilirubins), total cholesterol (CHOD-PAP enzymatic method, Kovalent®), triglycerides (GPO-PAP enzymatic

**Table 3 -** Nutritional requirements, estimated diet intake, and nutritional balance for ½ White Dorper × ½ Suffolk lambs in the first four months of age

| Nutritional parameter | Age (days) | | | | |
|---|---|---|---|---|---|
| | 14-30 | 31-60 | 61-75 | 76-90 | 91-120 |
| Nutritional requirements[1] | | | | | |
| DMI (g day$^{-1}$) | 371.7 | 550.6 | 726.4 | 817.8 | 889.4 |
| CPI (g day$^{-1}$) | 59.7 | 88.4 | 116.7 | 129.3 | 138.5 |
| MEI (Mcal day$^{-1}$) | 0.79 | 1.17 | 1.54 | 1.73 | 1.88 |
| Diet | | | | | |
| Milk[2] | | | | | |
| FMI (g day$^{-1}$) | 1555.4 | 1378.6 | 995.0 | 0.0 | 0.0 |
| DMI (g day$^{-1}$) | 237.2 | 210.2 | 151.7 | 0.0 | 0.0 |
| CPI (g day$^{-1}$) | 56.9 | 50.5 | 36.4 | 0.0 | 0.0 |
| MEI (Mcal day$^{-1}$) | 1.58 | 1.40 | 1.01 | 0.00 | 0.00 |
| Total mixed ration | | | | | |
| F:C ratio | 10:90 | 10:90 | 20:80 | 30:70 | 70:30 |
| DMI (g day$^{-1}$) | 134.5 | 340.3 | 250.0 | 300.0 | 300.0 |
| CPI (g day$^{-1}$) | 26.9 | 68.0 | 46.4 | 51.4 | 34.3 |
| MEI (Mcal day$^{-1}$) | 0.34 | 0.85 | 0.62 | 0.73 | 0.68 |
| Corn silage *ad libitum* | | | | | |
| DMI (g day$^{-1}$) | 0.0 | 0.0 | 324.6 | 517.8 | 0.0 |
| CPI (g day$^{-1}$) | 0.0 | 0.0 | 23.3 | 37.2 | 0.0 |
| MEI (Mcal day$^{-1}$) | 0.00 | 0.00 | 0.70 | 1.11 | 0.00 |
| Grazing | | | | | |
| DMI (g day$^{-1}$) | 0.0 | 0.0 | 0.0 | 0.0 | 589.4 |
| CPI (g day$^{-1}$) | 0.0 | 0.0 | 0.0 | 0.0 | 75.9 |
| MEI (Mcal day$^{-1}$) | 0.00 | 0.00 | 0.00 | 0.00 | 1.16 |
| Balance | | | | | |
| DMI (g day$^{-1}$) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CPI (g day$^{-1}$) | +24.10 | +30.03 | −10.55 | −40.73 | −28.25 |
| MEI (Mcal day$^{-1}$) | +1.13 | +1.08 | +0.78 | +0.11 | −0.05 |

DMI - dry matter intake; CPI - crude protein intake; MEI - metabolizable energy intake; FMI - fresh milk intake; F:C ratio - forage to concentrate ratio.
[1] Estimated based on NRC (2007).
[2] Milk intake was estimated based on the data reported by Degen and Benjamin (2003, 2005); milk composition was estimated from the data reported by Hentz et al. (2012) for the same ewes used in the current study; and milk energy content was calculated according to Šebek and Everts (1993) and Walker and Norton (1971).

method, Kovalent®), glucose (glucose enzymatic method, Katal®), gamma-glutamyltransferase (GGT; kinetic method of GGT, Katal®), aspartate aminotransferase (AST; UV kinetic method of AST, Katal®), and creatine kinase (CK; UV kinetic method of CK, Katal®).

The data were analyzed in a mixed model with repeated measures on time (PROC MIXED), in which the time represented the age of lambs and was the fixed effect, and the animal was the random effect. The analyses were performed using the model:

$$\hat{Y}_{ij} = \mu + A_i + lamb_j + \varepsilon_{ij},$$

in which $\hat{Y}_{ij}$ = value of dependent variable for the j-th lamb at the i-th age; $\mu$ = mean value of dependent variable (constant); $A_i$ = effect of the i-th age; $lamb_j$ = random effect of j-th lamb; and $\varepsilon_{ij}$ = random error. The best covariance structure for each dependent variable was selected based on the corrected Akaike (AICC) and Bayesian (BIC) information criteria. The means were fitted to the statistical model and compared between ages by Tukey's test (PROC LSMEANS).

A regression analysis (PROC REG) in which the age of lambs was considered the independent variable was performed up to the second order (quadratic), according to:

$$\hat{Y}_{ij} = \beta_0 + \beta_1 A_{i1} + \beta_2 A_{i2} + \gamma_{ij} + \varepsilon_{ij},$$

in which $\hat{Y}_{ij}$ = value of the dependent variable for the j-th lamb at the i-th age; $\beta_0$ = regression intercept; $A_i$ = independent variable (age); $\beta_1$ = linear regression coefficient for the dependent variable; $\beta_2$ = quadratic coefficient of regression for the dependent variable; $\gamma_{ij}$ = regression deviations; and $\varepsilon_{ij}$ = random error.

Differences between ages in the mixed models and the adjustment of regression equations were considered at a 0.05 significance level.

To verify the differences between lambs and adult sheep, the intervals for the blood biochemical parameters of the lambs between 30 and 120 days of age were determined, with the lower and upper limits of the 0.95 confidence interval, the median, and the mean and standard deviation (M±SD) through descriptive analyses (PROC MEANS). The values of serum triglycerides were compared with those found for ewes by Caldeira et al. (2007), and the values of other parameters were compared to the reference interval for adult sheep established by Kaneko et al. (2008).

All statistical analyses were carried out by SAS software (Statistical Analysis System, version 9.0).

## 3. Results

There was an effect of age on all protein parameters (Tables 4 and 5). The means for total protein showed a linear response, increasing between 30 and 60 days and stabilization until 120 days. The means for total globulin showed a linear increase with advancing age. A quadratic effect was observed for albumin concentrations, which showed an increase between 30 and 90 days, although with its inflection point at 78 days, so that a decrease was observed at 120 days.

There was an effect of age on the kidney function parameters (Tables 4 and 5). The urea concentration showed an increasing linear response with an increase between 60 and 90 days, peaking at 54.29 mg $dL^{-1}$ at 120 days of age. The mean creatinine concentrations remained constant between 30 and 90 days and showed a decrease at 120 days, in a quadratic response with the inflection point at 65 days.

The total bilirubin concentration showed a peak of 0.39 mg $dL^{-1}$ at 30 days, which was followed by a gradual decrease until 90 days and a subtle increase between 90 and 120 days, in a quadratic response with its inflection point at 115 days (Tables 4 and 5). The conjugated bilirubin concentration showed a linear behavior, although oscillating, with an increase between 30 and 60 days, decrease at 90 days, and a further increase at 120 days, when it reached its peak of 0.26 mg $dL^{-1}$. On the opposite way, through a quadratic effect, the peak of the unconjugated bilirubin concentration occurred at 30 days (0.20 mg $dL^{-1}$), then decreased gradually until reaching 0.05 mg $dL^{-1}$ at 120 days of age.

A quadratic effect of age on the energy profile of the lambs was noted (Tables 4 and 5). The total cholesterol and triglycerides concentrations presented a similar dynamics, marked by peaks at 30 days, which were followed by a gradual decrease until 90 days (with their inflection points at 90 and 86 days, respectively) and a further increase at 120 days, although showing lower values than the initial concentrations. The glucose concentration showed a decrease between 30 and 60 days, with an inflection point at 71 days, from which there was a gradual increase of the values until 120 days.

Regarding the enzymatic profile, there was also an effect of age on the serum concentrations of GGT, AST, and CK (Tables 4 and 5). The GGT concentration linearly decreased between 30 and 120 days of age. On the other hand, the AST concentration linearly increased throughout the study. The CK means

**Table 4 -** Means and standard errors (M±SE) for blood biochemical parameters in ½ White Dorper × ½ Suffolk lambs until four months of age

| Parameter | Age (days) | | | | P-value |
|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | |
| Total proteins (g $L^{-1}$) | 53.3±0.7b | 56.5±0.7a | 57.2±0.7a | 58.0±0.7a | <0.0001 |
| Total globulins (g $L^{-1}$) | 19.8±0.5c | 22.0±0.6b | 22.4±0.5b | 24.3±0.5a | <0.0001 |
| Albumin (g $L^{-1}$) | 33.5±0.3c | 34.5±0.3ab | 34.8±0.3a | 33.7±0.3bc | 0.0002 |
| Urea (mg $dL^{-1}$) | 39.67±1.57b | 38.01±1.62b | 50.28±1.57a | 54.29±1.57a | <0.0001 |
| Creatinine (mg $dL^{-1}$) | 0.69±0.03a | 0.71±0.03a | 0.71±0.03a | 0.59±0.03b | 0.0018 |
| Total bilirubin (mg $dL^{-1}$) | 0.39±0.02a | 0.35±0.02ab | 0.27±0.02c | 0.31±0.02bc | <0.0001 |
| Conjugated bilirubin (mg $dL^{-1}$) | 0.19±0.01c | 0.24±0.01ab | 0.21±0.01bc | 0.26±0.01a | 0.0007 |
| Unconjugated bilirubin (mg $dL^{-1}$) | 0.20±0.01a | 0.11±0.01b | 0.06±0.01c | 0.05±0.01c | <0.0001 |
| Total cholesterol (mg $dL^{-1}$) | 83.88±3.60a | 74.93±3.71ab | 48.82±3.60c | 68.36±3.60b | <0.0001 |
| Triglycerides (mg $dL^{-1}$) | 35.85±1.26a | 23.14±1.30bc | 20.87±1.26c | 25.71±1.26b | <0.0001 |
| Glucose (mg $dL^{-1}$) | 83.04±1.74a | 75.55±1.79b | 80.28±1.74ab | 83.48±1.76a | 0.0029 |
| GGT (U $L^{-1}$) | 75.7±2.0a | 67.1±2.0b | 62.3±2.0b | 51.9±2.0c | <0.0001 |
| AST (U $L^{-1}$) | 68.4±2.4c | 74.3±2.5bc | 81.2±2.4b | 92.6±2.4a | <0.0001 |
| CK (U $L^{-1}$) | 223.8±8.1a | 153.6±8.3b | 181.9±8.0b | 166.6±8.0b | <0.0001 |

GGT - gamma-glutamyltransferase; AST - aspartate aminotransferase; CK - creatine kinase.
Lowercase letters in the row compare means by Tukey's test (P<0.05).

**Table 5 -** Regression equations for blood biochemical parameters in relation to age of ½ White Dorper × ½ Suffolk lambs

| Parameter | Equation | $R^2$ | Inflection point[1] | | P-value | |
|---|---|---|---|---|---|---|
| | | | Age | Value | Linear | Quadratic |
| Total proteins (g $L^{-1}$) | TP = 52.5291 + 0.0493Age | 0.14 | | | <0.0001 | 0.0877 |
| Total globulins (g $L^{-1}$) | TGlob = 18.6596 + 0.0461Age | 0.20 | | | <0.0001 | 0.7875 |
| Albumin (g $L^{-1}$) | Alb = 31.2301 + 0.0916Age – 0.00059$Age^2$ | 0.12 | 78 | 34.8 | 0.4258 | <0.0001 |
| Urea (mg $dL^{-1}$) | Urea = 31.6374 + 0.1864Age | 0.31 | | | <0.0001 | 0.0936 |
| Creatinine (mg $dL^{-1}$) | Creat = 0.5684 + 0.0052Age – 0.00004$Age^2$ | 0.10 | 65 | 0.74 | 0.0098 | 0.0058 |
| Total bilirubin (mg $dL^{-1}$) | TB = 0.5199 – 0.0046Age + 0.00002$Age^2$ | 0.16 | 115 | 0.26 | <0.0001 | 0.0165 |
| Conjugated bilirubin (mg $dL^{-1}$) | CB = 0.1788 + 0.0006Age | 0.08 | | | 0.0017 | 0.9615 |
| Unconjugated bilirubin (mg $dL^{-1}$)[2] | UB = 0.3394 – 0.0052Age + 0.00002$Age^2$ | 0.60 | | | <0.0001 | <0.0001 |
| Total cholesterol (mg $dL^{-1}$) | TChol = 123.2387 – 1.4527Age + 0.00808$Age^2$ | 0.20 | 90 | 57.94 | <0.0001 | 0.0002 |
| Triglycerides (mg $dL^{-1}$) | TG = 56.4510 – 0.8376Age + 0.00486$Age^2$ | 0.39 | 86 | 20.36 | <0.0001 | <0.0001 |
| Glucose (mg $dL^{-1}$) | Gluc = 92.4546 – 0.4255Age + 0.00298$Age^2$ | 0.07 | 71 | 77.27 | 0.4723 | 0.0030 |
| GGT (U $L^{-1}$) | GGT = 83.3086 – 0.2545Age | 0.36 | | | <0.0001 | 0.6662 |
| AST (U $L^{-1}$) | AST = 59.0415 + 0.2665Age | 0.30 | | | <0.0001 | 0.2682 |
| CK (U $L^{-1}$) | CK = 283.3406 – 2.6648Age + 0.01454$Age^2$ | 0.16 | 92 | 161.2 | 0.0003 | 0.0027 |

GGT - gamma-glutamyltransferase; AST - aspartate aminotransferase; CK - creatine kinase.
[1] Only for the parameters adjusted to quadratic regression.
[2] An inflection point for the quadratic regression was not found up to 120 days of age.

decreased between 30 and 60 days and then remained stable until 120 days, in a quadratic effect with its inflection point at 92 days.

With respect to the comparison of biochemical parameters of lambs with those for adult sheep (Table 6), the mean values for total protein in lambs were lower than the reference interval for adults, such that even when the mean was added to the standard deviation (60.6 g $L^{-1}$), it remained close to the lower limit of the reference. Similarly, the mean for total globulins, even when added to its standard deviation (25.6 g $L^{-1}$), remained well below the lower limit of the reference interval. Conversely, the value of the lower limit for the albumin concentration of the lambs exceeded the upper value of the reference interval for adults.

The urea and creatinine concentration values of the lambs were also not within those referenced for adults. However, the mean for urea when subtracted from its standard deviation (34.25 mg $dL^{-1}$) was within of the reference, whereas the mean for creatinine added to its standard deviation (0.83 mg $dL^{-1}$) was much lower than the lower limit of the reference.

The means for total, conjugated, and unconjugated bilirubins were included within the reference interval for adults, although both the mean values for conjugated and unconjugated bilirubin when added to their standard deviations (0.31 and 0.18 mg $dL^{-1}$, respectively) were higher than the values of the upper limits of the reference interval for these metabolites in older animals.

The mean for total cholesterol of lambs was also inserted in the interval for adults, although its value when added to its standard deviation (93.41 mg $dL^{-1}$) exceeded the upper value of the reference interval. The mean for triglycerides, even when subtracted from its standard deviation (17.18 mg $dL^{-1}$), remained higher than the mean referenced for adults. The mean glucose level was a little over the upper limit of the reference, reaching a value of 91.15 mg $dL^{-1}$ when added to its standard deviation.

The GGT values of the lambs were also higher than those of the reference, and even when standard deviation was subtracted from the mean (49.9 U $L^{-1}$), its value remained very close to the upper limit of the interval established for adults. The AST values remained within the reference interval for adults, although when the standard deviation was subtracted from the mean (62.9 U $L^{-1}$), the value was very close to the lower limit of the reference. The mean for CK was at least 14 times higher than the upper value of the interval determined for adults.

**Table 6 -** Descriptive analysis of biochemical parameters in ½ White Dorper × ½ Suffolk lambs in the first four months of age and reference intervals reported for adult sheep

| Parameter | Interval | | Median | M±SD | Reference for adult sheep[1] |
|---|---|---|---|---|---|
| | Lower limit | Upper limit | | | |
| Total proteins (g $L^{-1}$) | 55.5 | 57.0 | 56.0 | 56.2±4.4 | 60.0–79.0 |
| Total globulins (g $L^{-1}$) | 21.5 | 22.7 | 22.0 | 22.1±3.5 | 35.0–57.0 |
| Albumin (g $L^{-1}$) | 33.8 | 34.4 | 34.0 | 34.1±1.6 | 24.0–30.0 |
| Urea (mg $dL^{-1}$) | 43.71 | 47.61 | 45.60 | 45.66±11.41 | 17.18–42.88 |
| Creatinine (mg $dL^{-1}$) | 0.65 | 0.70 | 0.70 | 0.67±0.16 | 1.2–1.9 |
| Total bilirubin (mg $dL^{-1}$) | 0.31 | 0.35 | 0.32 | 0.33±0.11 | 0.1–0.5 |
| Conjugated bilirubin (mg $dL^{-1}$) | 0.21 | 0.24 | 0.23 | 0.23±0.08 | 0–0.27 |
| Unconjugated bilirubin (mg $dL^{-1}$) | 0.09 | 0.12 | 0.08 | 0.10±0.08 | 0–0.12 |
| Total cholesterol (mg $dL^{-1}$) | 64.71 | 73.09 | 62.10 | 68.90±24.51 | 52–76 |
| Triglycerides (mg $dL^{-1}$) | 24.87 | 28.04 | 25.10 | 26.45±9.27 | 12.22[2] |
| Glucose (mg $dL^{-1}$) | 78.84 | 82.45 | 81.40 | 80.65±10.50 | 50–80 |
| GGT (U $L^{-1}$) | 61.7 | 66.6 | 64.1 | 64.2±14.3 | 20–52 |
| AST (U $L^{-1}$) | 76.4 | 82.0 | 81.6 | 79.2±16.3 | 60–280 |
| CK (U $L^{-1}$) | 172.4 | 190.7 | 176.0 | 181.5±52.3 | 8.1–12.9 |

GGT - gamma-glutamyltransferase; AST - aspartate aminotransferase; CK - creatine kinase; M±SD - mean and standard deviation.
[1] According to Kaneko et al. (2008).
[2] According to Caldeira et al. (2007).

## 4. Discussion

The total protein concentrations are directly influenced by the dynamics of total globulins (Souza et al., 2014). Therefore, the lower values for total proteins and globulins verified at 30 days probably represent the transition period in the immunoglobulin content in the bloodstream, which is characterized by the end of the degradation process of immunoglobulins passively received via colostrum and by the initial phase of active production of immunoglobulins by the animal itself (Mohri et al., 2007; Silva et al., 2010). In subsequent months, the increase in values results mainly from the increase in gamma globulin concentrations resulting from the antigenic stimulation of lambs (exposure to antigens in the extrauterine environment and by vaccination) and from the maturation of the immune system (Silva et al., 2010; Delfino et al., 2014; Santos et al., 2017).

The albumin concentrations also affect the total protein levels. Albumin is the main plasma protein synthesized by the liver and corresponds to approximately 35 to 50% of total serum proteins and is responsible for 80% of the colloid osmotic pressure (Kaneko et al., 2008). It is influenced by dietary protein intake and is considered the most sensitive indicator for the determination of protein nutritional status in the long term, since changes in its concentrations are detected only after a minimum period of one month due to its low rate of synthesis and degradation (Peixoto and Osório, 2007). According to Silva et al. (2010) and Chai et al. (2015), lambs older than 30 days already present rumination and become proficient in the use of nitrogenous compounds from the diet, which causes a gradual increase in albumin concentrations until 90 days, as observed in this study. The decrease observed at 120 days, as a delayed response from albumin, in turn, might have been caused mainly by weaning, since depriving the lambs of milk reduces the total dietary protein and energy intake (as observed at 76-90 days; Table 3) and, consequently, the use of available forage protein and albumin synthesis (Fernandes et al., 2012; Santos et al., 2017). It is noteworthy that the dynamics of albumin is not only changed by nutrition, but also by the ability of the liver to synthesize it, which, during the neonatal period, is still influenced by the development and maturation of hepatic metabolic processes (Mohri et al., 2007; Souza et al., 2014).

Regarding the parameters related to kidney function, it is known that shortly after birth, with the rupture of the umbilical cord, the kidneys assume the hydroelectrolytic control and excretory functions previously performed by the placenta, and there is a gradual increase in the efficiency of kidney function with age (Benesi et al., 2005). In sheep, it is important to remember that although urea is not considered a very accurate metabolite for renal evaluation, it is a quick and sensitive indicator of the protein nutritional status, since it is directly related to the input of degradable proteins and to the energy:protein ratio of the diet (González and Scheffer, 2002; Peixoto and Osório, 2007; Braun et al., 2010). In fact, serum urea concentration is proportional to the ammonia levels produced in the rumen (Karimizadeh et al., 2017). Most of the protein ingested is degraded by ruminal microorganisms, releasing ammonia, which is used for microbial protein synthesis (Geron et al., 2018). However, when large amounts of protein are fermented in combination with low availability of energy from carbohydrate degradation, a high rumen ammonia concentration is generated. The capacity of ammonia utilization by ruminal microorganisms is exceeded, so the excess is absorbed into the enterohepatic circulation, and in the liver is converted into urea, which, added to urea from amino acid metabolism, constitutes the major portion of plasma urea (Fernandes et al., 2012; Karimizadeh et al., 2017; Geron et al., 2018). However, it can be observed that the energy requirement for the lambs in this study was practically met (Table 3), with only a small deficit in its balance at 91-120 days, while the protein deficit was much higher (not being the protein requirement fully met). In this case, then, the increase in urea from 90 days onwards probably reflected the increase in urea recycled by the nitrogen conservation mechanism, through which part of the urea synthesized in the liver, after being released into the bloodstream, returns to the rumen through the bloodstream itself (by difusion through the ruminal wall) or via saliva (Santos and Pedroso, 2011).

In contrast, creatinine is less influenced by external factors and, therefore, is the main marker for the glomerular filtration rate in ruminants (Russell and Roussel, 2007). Carlos et al. (2015) and Lima et al. (2015), when comparing sheep in different age groups (from less than six months to over 24

months), observed an increase in creatinine concentrations with advancing age, which would be due to the expansion of creatinine reservoirs in the organism as a consequence of the increase in muscular mass deposition with growth, or a possible reduction in serum thyroxine levels that would trigger a decrease in glomerular filtration rate. However, just as among the lambs in this study, Feitosa et al. (2007), Mohri et al. (2007), and Delfino et al. (2014) when evaluating calves, and Cruz et al. (2017) studying lambs, observed higher values of creatinine in the first days of life that would be correlated with renal immaturity and muscle wasting caused by the onset of locomotion. Also, according to these authors, the subsequent decrease during the growth phase would be attributed to the renal clearance improvement from the first week of life and the decrease in phosphocreatine demand for muscle weight gain after 61 days of age.

In cattle, the effect of age on the total bilirubin dynamics is notorious, such that neonatal calves have concentrations significantly higher than those observed in older animals (Mohri et al., 2007; Russell and Roussel, 2007). Similarly, the highest values of total and unconjugated bilirubin concentrations observed in the lambs at 30 days of age appear to represent the end of the physiological hyperbilirubinemia process of newborns, which is characterized by the widespread destruction of fetal erythrocytes in the mononuclear phagocytic system of the liver and spleen (Mohri et al., 2007). In the following ages, the dynamics of the total, conjugated, and unconjugated bilirubins probably still reflect the phase of morphofunctional adaptation of the liver to extrauterine life (Souza et al., 2014).

Concerning the energy profile, it is known that cholesterol has two sources: exogenous, derived from food, and endogenous, synthesized from acetyl-CoA in the liver, under the regulation of exogenous cholesterol intake (González and Scheffer, 2002). The lambs in this study, as verified among the animals evaluated in the studies by Santos et al. (2015) and Cruz et al. (2017), showed a decrease in total cholesterol concentrations with advancing age as a consequence of the modifications to the diet during the first months of life. The dynamics of this parameter is directly influenced by the level of milk intake and metabolizable energy in the diet (Table 3). Initially, the concentrations tend to be high due to the intake of colostrum and milk rich in fats; however, as the animals are weaned and begin to ingest a diet with a higher amount of fodder and concentrated ration with a lower energy content in the form of lipids, the cholesterolemia should decrease (Fernandes et al., 2012; Santos et al., 2015).

The dynamics of triglycerides observed in the first months of life of the lambs resembled that observed by Cruz et al. (2017), showing a drop in triglyceride concentrations after 30 days of age. As in the case of cholesterol, this variation might have been caused by the restriction in milk intake and changes in feeding management (Table 3), as well as by the improvement in hepatic maturation and the ability to metabolize lipids (Bennis et al., 1992; Delfino et al., 2014).

On the other hand, the glycemia in ruminants is not influenced much by feeding, since it is regulated by an efficient hormonal homeostatic mechanism that aims to keep its concentration constant (González and Scheffer, 2002). Despite the performance of this mechanism, in the neonatal period and during the growth phase, glycemia is greatly influenced by age (Mohri et al., 2007; Bórnez et al., 2009; Cruz et al., 2017) and is related to the intake of colostrum and milk, and also to the maturation of the liver, pancreas, and enzymatic activities, as well as to the adaptation of the organism to the extrauterine environment (Kaneto et al., 2004). Thus, it is believed that the decrease in glucose observed in the lambs at 60 days of age is still the result of the action of these factors associated with age.

Concerning the enzymatic profile, it is recognized that GGT is localized mainly in the kidneys, pancreas, and intestines and shows high activity in the liver. Therefore, it is considered hepatospecific and an important indicator for hepatobiliary diseases and cholestasis in sheep (Russell and Roussel, 2007; Kaneko et al., 2008). It is also present in the epithelium of the ducts of the mammary glands, so that ovine colostrum has high concentrations of GGT. Thus, under normal conditions, such as among the lambs in this study, its high serum concentration at 30 days of age would still result from its high absorption via colostrum in the first 24-48 h of life, using the same mechanism of absorption as for immunoglobulin G (IgG), and its decrease would be due to its deactivation and/or physiological degradation with advancing age (Britti et al., 2005; Souza et al., 2014).

Aspartate aminotransferase is present in several tissues, but is localized mainly in the liver, in skeletal and cardiac muscle tissue, erythrocytes, and kidneys (González and Scheffer, 2002). Britti et al. (2005) and Cruz et al. (2017) also observed a positive correlation between AST concentration and age of lambs. It is believed that the gradual increase in AST up to 120 days of age resulted from the combination of an increase in mass and muscle activity and an improvement in the endogenous production of this enzyme with the development of the animal (Feitosa et al., 2007; Mohri et al., 2007; Cruz et al., 2017).

Creatine kinase is a muscle-specific enzyme characterized as a very sensitive bioindicator of the degree of activity, damage and/or muscular effort (Russell and Roussel, 2007; Bórnez et al., 2009). Bórnez et al. (2009) verified that in different stress conditions, older lambs show higher levels of CK than lambs still in suckling, and Antunović et al. (2012) did not observe CK variation among lambs with 30 and 70 days of age. According to Braun et al. (2010), CK has high intramuscular activity and sensitivity and might vary quickly after minimal damage. Russell and Roussel (2007) and Lepherd et al. (2009) reported variations caused by the instability of this enzyme even in the face of common activities of routine management, such as restraint and weighing of animals, or as a result of intramuscular injections, exercise, or physical effort. Therefore, the possibility that the variations in the present study might have occurred due to subtle differences in movement and the time for animal restraint for the blood collection at different times of evaluation cannot be ruled out.

Observing the comparison of biochemical parameters between the lambs and adult sheep, it was confirmed that the biochemical values of younger animals often differ from the reference intervals for adults. Values for total globulins, creatinine, triglycerides, and CK are still far from the values established for adults. However, some other parameters are close to the adult reference interval (total proteins, albumin, urea, glucose, and GGT), but could still result in a misinterpretation of findings, and others are within the intervals of normal physiological variation for adults of the species (as in the case of total, conjugated, and unconjugated bilirubins, total cholesterol, and AST).

In the case of triglycerides, although Kaneko et al. (2008) did not present values for comparison, Caldeira et al. (2007) found a value of 12.22 mg $dL^{-1}$ in ewes with balanced metabolic status, which corresponds to less than half the value observed in the lambs. However, the values of the lambs were below the value of 40.7 mg $dL^{-1}$ observed by Carlos et al. (2015) in Morada Nova lambs aged less than six months, possibly due to the difference between the breeds and food management, and approached the value of 32.6 mg $dL^{-1}$ observed by Cruz et al. (2017) in Dorper lambs between 15 and 121 days of age, still remaining slightly lower, probably due to the greater precocity in the initial age of the evaluations by these authors.

It is also important to note that the CK values of the lambs are much higher than those of the adults, which would lead to serious misinterpretations if compared directly with the reference values of Kaneko et al. (2008), but they are close to the reference interval established by Lepherd et al. (2009) for weaned Merino lambs between nine to 16 weeks of age (ranging from 180 to 454 U $L^{-1}$).

During the first months of life, the age factor is naturally associated with several processes of changes, whether due to variations in feed management (colostrum intake, suckling, weaning, inclusion of solid feeds in the diet), or changes from intrauterine to extrauterine environment, which triggers new biological challenges and intense changes in the maturation of organs, body systems, and metabolic activity, or by evident changes in the body growth of the animal. These changes alter the blood biochemical profile, so that this variation structure cannot be considered normal in other ages (Kaneto et al., 2004; Benesi et al., 2005; Fernandes et al., 2012; Souza et al., 2014).

For example, the evaluation of the metabolic profile according to age, during suckling or fattening, can expose specific nutritional mistakes, which could otherwise be undetectable because animal uses its own body reserves (Antunović et al., 2012). Also, in this period, higher values for albumin, urea, triglycerides, glucose, and GGT, together, can be related to the morphofunctional adaptation of the liver, but in adults, they would probably indicate some kind of liver problem (Feitosa et al., 2007; Souza et al., 2014).

It is still important to remember that some diseases are more predisposed to certain ages. Thus, being able to rule out normal changes due to age in the clinical biochemical profile ensures a greater accuracy

for this evaluation, a better chance to confirm the diagnosis of a disease, and a quick and effective intervention, preventing it from becoming chronic or leading to death. In addition, it allows surgical procedures to be performed with greater safety (Mohri et al., 2007; Russell and Roussel, 2007; Kaneko et al., 2008; Delfino et al., 2014).

## 5. Conclusions

The biochemical parameters of healthy ½ White Dorper × ½ Suffolk female lambs are influenced by age until four months of life. However, the magnitude of variation in the parameters within this period is small and is often associated with the nutritional, immunological, and adaptation physiological factors. Except for the total, conjugated, and unconjugated bilirubins, total cholesterol, and AST means, the values of the biochemical parameters of female lambs differ from the reference intervals established for adult sheep. Thus, it is recommended that the interpretation of tests performed on animals in the growth phase should be made based on specific reference intervals for this age group.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: R.L. Dittrich and A.L.G. Monteiro. Formal analysis: D.F. Souza, R.L. Dittrich and A.L.G. Monteiro. Funding acquisition: A.L.G. Monteiro. Investigation: D.F. Souza, T.S.S.S. Reijers, S. Gilaverte, T.A. Cruz, F. Hentz, B.Q. Castilhos, R.L. Dittrich and A.L.G. Monteiro. Methodology: D.F. Souza, S. Gilaverte, B.Q. Castilhos, R.L. Dittrich and A.L.G. Monteiro. Project administration: D.F. Souza, R.L. Dittrich and A.L.G. Monteiro. Resources: A.L.G. Monteiro. Writing-original draft: D.F. Souza, R.L. Dittrich and A.L.G. Monteiro. Writing-review & editing: D.F. Souza, S. Gilaverte, T.A. Cruz, B.Q. Castilhos and A.L.G. Monteiro.

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