Abstract
Late pregnancy is accompanied by dramatic changes in the metabolism of sheep, in which the nutrient requirements increase and the metabolic capacity of ewes is under severe stress due to the rapid foetal growth and development of the mammary gland. The blood metabolic profile can be used to monitor these alterations, which can lead to metabolic disorders such as pregnancy toxaemia. However, data available on serum parameters in sheep do not consider physiological state. Therefore, the present study aimed to determine the biochemical reference ranges for pregnant ewes, including serum energy, protein and enzyme-related metabolites. Data from a variety of metabolites were obtained from experiments performed in several institutions and commercial farms using Santa Inês, Dorper, Lacaune, Morada Nova, Bergamacia and Suffolk ewes reared under different conditions (grazing, feedlot, semi-feedlot, collective and/or individual pens, and metabolic cages) from 2006 to 2017. All animals were healthy and without feed restriction. Data from ewes with any clinical manifestations were removed. The metabolic energy profile included data of glucose, cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein and very-low-density lipoprotein levels; the metabolic protein profile included the metabolites total protein, uric acid, albumin, and creatinine; and the metabolic enzymatic profile included the enzymes aspartate aminotransferase, gamma-glutamyl transferase and alkaline phosphatase. The reference ranges were estimated using the software RefVal 4.11. Dixon’s test was used to identify and remove outliers. The confidence intervals and percentiles were estimated using the nonparametric method of bootstrap when data were not normally distributed. A 95% confidence level was used. The serum biochemical reference ranges for pregnant sheep determined in our study were strongly divergent from those established by one of the most cited books on the topic, especially considering the high serum urea and cholesterol concentrations and low levels of blood glucose observed. Therefore, it is essential to consider physiological status when evaluating the blood metabolic profile of pregnant ewes in order to maintain an adequate nutritional management and to prevent health disorders that may lead to productive and reproductive losses.

Keywords: Ewes. Metabolic Energy Profile. Metabolites. Ovis aries.
1. Introduction

Pregnancy is a physiological state accompanied by dramatic changes in the metabolism of sheep to ensure the successful maintenance of gestation and fetal growth and development (Kandiel, El-Khaiat and Mahmoud 2016). As gestation progresses, the nutrient requirements increase and the metabolic capacity of ewes is under severe stress due to the rapid fetal growth and the development of the mammary gland (Gurgoze et al. 2009), since approximately 80% of the development of the conceptus takes place in the last six weeks of pregnancy (Rook 2000).

It is known that the energy needs of the fetal-placental unit are primarily met by maternal glucose and lactate (Kaneko, Harvey and Bruss 2008), whereas maternal tissues tend to use lipid sources for energy metabolism aiming at facilitating glucose availability to the fetus through increased gluconeogenesis (Bell 1995). The combination of increased energy demands, inadequate energy intake and metabolic alterations related to high lipid mobilization during late pregnancy results in a condition of negative energy balance in sheep, which could progress into a severe metabolic disorder named pregnancy toxemia (Van Saun 2000; Dzadzowski et al. 2015). Therefore, with the advancement of pregnancy in ruminants, those changes in maternal metabolism to meet fetal nutrient demands might also have an influence on serum biochemistry values (Mohammadi, Anassori and Jafari 2016), which can be used to assess the nutritional status and individual health disorders in livestock (Van Saun 2000). In addition to the physiological status, many aspects, such as breed, sex, age, underfeeding and health might influence the blood metabolic profile in sheep (Yokus et al. 2006).

The analysis of serum biochemistry parameters is a low-invasive and inexpensive alternative for the diagnosis, treatment, and prediction of diseases such as pregnancy toxemia (Bobé, Young and Beitz 2004). Consequently, the identification of changes in the metabolism of sheep through the evaluation of the blood metabolic profile during pregnancy is a useful tool for the management and nutritional decision-making in sheep (Balikci, Yildiz and Gürdoğan 2007; Swetha et al. 2018).

In the literature, the blood metabolic profile does not take into account this physiological status in sheep, which can lead to errors in the diagnosis of diseases and the nutritional management of the flock. So, the hypothesis would be that the reference values found for the pregnant category in animals raised in Brazil would be different from those found in the international literature. Therefore, the present study aimed to determine and compare the serum biochemical reference ranges for pregnant sheep in Brazil.

2. Material and Methods

Data from a variety of metabolites were obtained from experiments performed in several institutions (Federal University of Uberlândia, Federal University of Minas Gerais, Federal Rural University of Rio de Janeiro, Federal University of Tocantins) and commercial farms using Santa Inês, Dorper, Lacaune, Morada Nova, Bergamacia and Suffolk ewes reared under different conditions (grazing, feedlot, semi-feedlot, collective and/or individual pens, and metabolic cages) from 2006 to 2017. Table 1 shows the different types of food used in feeding the animals whose data were used in this study.

| Energy concentrate         | Protein concentrate | Forages and silages                              | Additive                  |
|----------------------------|--------------------|--------------------------------------------------|---------------------------|
| Corn bran                  | Soybean meal       | *Braquiaria brizantha*                          | Monensin                  |
| Citrus pulp                | Urea               | *Braquiaria decumbens*                          | Mineral salt (sheep)      |
| Babassu starch flour       | Soy cone           | *Cynodon spp. (hay)*                            | Exogenous enzymes         |
| Soy molasses               | Babassu flour      | *Panicum maximum cv. Tanzânia*                  | Yeasts                    |
| Corn grain                 | Cottonseed         | *Panicum maximum cv. Aruana*                    | Virginamycin              |
| Propylene glycol           | Soybean grain      | *Panicum maximum cv. Massai*                    |                           |
| Glycerol                   | Protected urea     | Corn silage                                     |                           |
| Cookie flour               | Dairy substitute   | Sorghum silage                                  |                           |
|                            |                    | Tanzânia grass silage                           |                           |
All animals were healthy and without feed restriction. Data from ewes with any clinical manifestations were removed. Data were obtained considering the physiological status of pregnancy regardless of parity, birth type or days of gestation. The metabolic energy profile included data from glucose, cholesterol, triglycerides, HDL (high-density lipoprotein), LDL (low-density lipoprotein) and VLDL (very-low-density lipoprotein); the metabolic protein profile encompassed the metabolites total protein, uric acid, urea, albumin, and creatinine; the metabolic enzymatic profile included the enzymes AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase) and ALP (alkaline phosphatase). The analyses were performed on Bioplus 2000 and PKL-125 devices (MH-Lab) using different brands of test kits (Labtest Diagnóstica S.A., GT Group© and Biotécnica©). The VLDL and LDL values were obtained using the equations proposed by Friedewald et al. (1972) based on the total serum cholesterol, HDL, and triglycerides:

\[ \text{VLDL} = \frac{\text{TG}}{5} \]
\[ \text{LDL} = \text{TC} - \text{HDL} - \text{VLDL} \]

Where VLDL = very-low-density lipoprotein; TG = triglycerides; LDL = low-density lipoprotein; TC = total cholesterol; HDL = high-density lipoprotein.

Reference intervals were estimated using the software RefVal 4.11 (Solberg 2006). Dixon’s test was used to identify and remove outliers. The confidence intervals and percentiles were estimated using the nonparametric bootstrap method when data were not normally distributed. A 95% confidence level was used.

The physiological reference ranges determined in the present study were compared with established reference intervals for serum biochemical parameters presented by Kaneko, Harvey and Bruss (2008), which is one of the most cited books with about 2800 citations.

3. Results

Data relative to the physiological reference ranges for energy, protein and enzyme-related metabolites in pregnant sheep, the sample size and the reference ranges for comparison are shown in table 2.

Table 2. Serum biochemical reference ranges for pregnant ewes, including energy, protein and enzyme-related metabolites.

| Metabolites                | Unit | SS¹ | Reference ranges (our study) | Reference ranges (Kaneko, Harvey & Bruss 2008) |
|----------------------------|------|-----|------------------------------|-----------------------------------------------|
| **Energy-related metabolites** |      |     |                              |                                                |
| Glucose                    | mg/dL| 93  | 29–59*                       | 50–80                                         |
| Cholesterol                | mg/dL| 273 | 13–117                       | 52–76                                         |
| Triglycerides              | mg/dL| 305 | 9–47                         | 9–30                                          |
| HDL²                       | mg/dL| 224 | 7–42*                        | ND⁵                                           |
| LDL³                       | mg/dL| 309 | 4.3–95.5*                    | ND⁵                                           |
| VLDL⁴                      | mg/dL| 358 | 1.6–9.8                      | ND⁵                                           |
| **Protein-related metabolites** |      |     |                              |                                                |
| Total protein              | g/dL | 308 | 2.26–7.18                    | 6–7.9                                         |
| Uric acid                  | mg/dL| 309 | 0.1–0.9                      | 0–1.9                                         |
| Urea                       | mg/dL| 300 | 7–55.8                       | 51–128                                        |
| Albumin                    | g/dL | 307 | 1.56–5.01*                   | 2.4–3.0                                       |
| Creatinine                 | mg/dL| 299 | 0.2–1.5                      | 1.2–1.9                                       |
| **Enzyme-related metabolites** |      |     |                              |                                                |
| AST⁶                       | U/L  | 223 | 45–251*                      | 60–280                                        |
| GGT⁷                       | U/L  | 220 | 28–104*                      | 20–52                                         |
| ALP⁸                       | U/L  | 233 | 33–331                       | 68–387                                        |

¹SS – Sample size; ²HDL – high-density lipoprotein; ³LDL – low-density lipoprotein; ⁴VLDL – very-low-density lipoprotein; ⁵Not determined, ⁶AST – aspartate aminotransferase; ⁷GGT – gamma-glutamyl transferase; ⁸ALP – Alkaline phosphatase. *Parametric data.
The reference interval for serum glucose (29–59 mg/dL) was lower than that established by Kaneko, Harvey and Bruss (2008), even though the sample size was lower compared to those of the other variables. Reference intervals for serum cholesterol (13–117 mg/dL) were wider than those established by Kaneko, Harvey and Bruss (2008).

The reference interval for serum triglycerides (9–47 mg/dL) was similar to that established by Kaneko, Harvey and Bruss (2008). Reference intervals for serum HDL (7–42 mg/dL), LDL (4.3–95.5 mg/dL) and VLDL (1.6–9.8 mg/dL) were established in this study for pregnant sheep but were not determined by Kaneko, Harvey and Bruss (2008).

The interval for serum total protein (2.26–7.18 g/dL) was more extensive than that established by Kaneko, Harvey and Bruss (2008), with a lower reference limit 37.6% lower than that reported by them. On the other hand, the reference interval for serum uric acid (0.1–0.9 mg/dL) was narrow, since Kaneko, Harvey and Bruss (2008) established an upper reference limit 47.3% higher than that observed in the present study.

The reference interval for serum urea (7–55.8 mg/dL) was slightly lower than the reference interval established by Kaneko, Harvey and Bruss (2008). The intervals for serum albumin (1.56–5.01 g/dL) were similar to the reference intervals established by Kaneko, Harvey and Bruss (2008), the minimum reference value being 35% lower and the maximum reference value for pregnant sheep 67% higher. Reference intervals for serum creatinine (0.2–1.5 mg/dL) included as the lower limit a minimum value 83% lower than that established by Kaneko, Harvey and Bruss (2008).

Intervals for serum AST (45–251 U/L) included as the minimum reference a value 25% lower than that established by Kaneko, Harvey and Bruss (2008), whereas reference intervals for GGT (28–104 U/L) included as the maximum reference a value 100% higher than the upper limit defined by Kaneko, Harvey and Bruss (2008). The reference interval for serum ALP (33–331 U/L) included as the lower limit a minimum value 38% lower than that established by Kaneko, Harvey and Bruss (2008).

4. Discussion

Glucose is the primary metabolic fuel used by the sheep fetus (Haffaf and Benallou 2016), even though it is not a sensitive parameter of energy status since glucose homeostasis is subject to tight control (Kaneko, Harvey and Bruss 2008). The greater fetal demand in pregnant ewes is not accompanied by increased endogenous glucose production, resulting in lower glucose levels during pregnancy (Raoofi et al. 2013). In addition to the increased maternal glucose diffusion and use to supply the fetal demand (Deghnouche et al. 2013), the lower plasma glucose levels in pregnant sheep are also attributed to impaired gluconeogenesis by the liver from glucogenic precursors such as propionate, which is derived from rumen fermentation (Dzadzowski et al. 2015). In an assay with 110-day pregnant Border Leicester x Romney ewes carrying multiple pregnancies, Hu et al. (1990) reported values of blood glucose reaching 29.9 mg/dL. According to Safsaf et al. (2014), blood glucose levels in multiparous Ouled Djellal ewes reached 34.6 mg/dL after 15 weeks of pregnancy, whereas Castillo et al. (1999) observed mean blood glucose levels of 34.52 mg/dL in Assaf ewes under an intensive management system at the 90th day of pregnancy. A study conducted by Haffaf and Benallou (2016) using pregnant ewes reported blood glucose levels of 46, 41 and 39 mg/dL for the periods of 61–90, 91–120 and 121–145 days of pregnancy, respectively. Therefore, with advancing pregnancy, blood glucose levels tend to decrease since the fetus demands glucose as the primary energy source, consuming up to 70% of the maternal production (Kaneko, Harvey & Bruss 2008). Low glucose levels at the end of pregnancy should be monitored because hypoglycemic sheep may develop pregnancy toxemia, which can lead to death. However, based on our data and on the reports above, it is possible to observe that pregnant ewes raised under tropical conditions tend to have low levels of blood glucose without showing any sign of pregnancy toxemia. This endorses the importance of determining a suitable reference interval that includes data from animals raised under similar conditions of management, nutrition and location. In addition, it is known that stress factors such as management changes can lead to a severe negative energy balance, triggering ketosis in sheep. The studies mentioned above corroborate our reports that the lower range for blood glucose level in pregnant sheep is lower than that reported by Kaneko, Harvey and Bruss (2008) of 50 mg/dL, reaching 29 mg/dL.

It is known that serum cholesterol metabolisms in pregnant and non-pregnant sheep are markedly distinct (Zywicki et al. 2018). That is because, during ovine gestation, cholesterol is a substrate for steroids;
first the corpus luteum and then the placenta, and the ovine placenta relies solely on cholesterol for progesterone production. Decreased cholesterol levels may occur in sheep fed low-energy diets, in cases of hyperthyroidism and during prepartum (Santos et al. 2014) or when there is a decrease in ruminal acetate, which is the primary precursor for cholesterol synthesis (Marai et al. 2008). In contrast, Balicci, Yildiz and Gürgoan (2007) reported that there is a gradual increase in cholesterol levels in sheep at the end of pregnancy due to its reduced responsiveness to insulin, which directly affects the metabolism of adipose tissue during late pregnancy, leading to an increase in the cholesterol and lipoprotein concentrations (Schlumbohm and Harmeyer 1997). The mean serum cholesterol is 22.3 mg/dL in primiparous Ouled Djellal ewes during early pregnancy (Safaf et al. 2014) and 26.48 mg/dL in Iranian fat-tailed Baloochi ewes one day before lambing (Taghipour et al. 2010). On the other hand, Khatun et al. (2011) reported a mean serum cholesterol level of 107.6 mg/dL in early-pregnant ewes. According to Kandiel, El-Khaiat and Mahmoud (2016), the blood cholesterol levels in Barki ewes at early (60 d), mid (90 d) and late pregnancy (135 d) were 36.7, 36.4 and 34.4 mg/dL, respectively. Such results are within the range defined in the present study (13–117 mg/dL), but do not agree with the physiological values proposed by Kaneko, Harvey and Bruss (2008). In sheep with pregnancy toxemia, Van Saun (2000) reported a mean serum cholesterol level of 59 mg/dL. It is known that there is a trend towards reduction in serum cholesterol levels in animals diagnosed with ketosis, suggesting that the ability of the liver to transport this metabolite in the blood as VLDL may be compromised. Under this circumstance, the liver starts to accumulate fat, contributing to the onset of pregnancy toxemia if associated with body condition scores above 3 (on a 1–5 scale) (Santos et al. 2011).

The maternal lipid metabolism is extensively modified during pregnancy to ensure the maintenance of nutrient supply to the growing fetus (Kandiel, El-Khaiat and Mahmoud 2016), especially when we consider the physiological adaptations of sheep to meet their increased energy demands for gestation. Under this condition, the rate of adipose tissue metabolism is changed, resulting in increased triglyceride metabolism aimed at obtaining energy and producing steroid hormones such as progesterone (Castillo et al. 1999). Therefore, high serum triglyceride levels may be a result of energy mobilization of adipose tissue, particularly in animals under energy deficit (Oliveira et al. 2014). On the other hand, the number of total insulin receptors reduces with the advancement of pregnancy, which could result in decreased triglyceride concentration because of an inefficient stimulation of lipogenesis by insulin (Yokus et al. 2006). Sheep diagnosed with hyperketonemia also have low serum triglyceride concentration due to the reduced appetite (Santos et al. 2011). In an assay with Makouei sheep seven days before the expected lambing time, Mohammadi, Anassori and Jafari (2016) reported a mean serum triglyceride concentration of 46.54 mg/dL, similar to that found by Castillo et al. (1999) in twin-bearing Assaf ewes 10 days before parturition (45.4 mg/dL). Serum triglyceride concentrations were also above the upper range stated by Kaneko, Harvey and Bruss (2008) in Akkaraman ewes at 150 days of pregnancy (Balicci, Yildiz and Gürgoan 2007) and Ouled Djellal sheep (Deghnouche et al. 2013) of 32.7 and 34 mg/dL, respectively. These authors observed a mean concentration of 81.24 mg/dL in Santa Inês sheep at the end of pregnancy. In contrast, Kandiel, El-Khaiat and Mahmoud (2016) reported a mean serum triglyceride concentration of 16.2 mg/dL in Barki ewes at mid-pregnancy.

High- and low-density lipoproteins are the primary endogenous sources of cholesterol for steriodogenesis by luteal cells, which is critical for the establishment and maintenance of pregnancy, and they carry most of the circulating cholesterol in the serum of adult animals. Although Kaneko, Harvy and Bruss (2008) did not report HDL and LDL cholesterol concentrations in their study, several research papers corroborate the reference ranges found in the present study for pregnant sheep. The mean HDL and LDL serum concentrations were 18.01 and 13.15 mg/dL for Tsigai ewes (Antunović et al. 2011) and 12.15 and 32.81 mg/dL for Santa Inês sheep seven days before lambing (Nasciutti et al. 2012). With the advancement of pregnancy in Barki ewes, the mean HDL concentrations at early, mid and late gestation were 11, 9.7 and 11.2 mg/dL, respectively, whereas the corresponding serum LDL concentrations were 16.9 19.5 and 16.7 mg/dL (Kandiel, El-Khaiat & Mahmoud 2016). According to the authors, high HDL concentration in ewes may be evidence of low steroidogenesis during late pregnancy. The diet can also influence the concentrations of HDL and LDL in sheep, since ewes fed low-fat diets tend to have reduced serum levels of both lipoproteins.

Fatty acids are esterified to triglycerides, incorporated into the hepatic tissue and exported from the liver during the synthesis and secretion of hepatic VLDL (Berchielli, Pires and Oliveira 2006). Pregnant sheep...
have a deficiency in the production of VLDL and, under situations of compromised lipid metabolism, triglycerides cannot be removed from hepatocytes, leading to hepatic insufficiency (Dzadzowski et al. 2015). Concurrent low cholesterol levels indicate that the liver’s capacity to export fat as VLDL is impaired, triggering the process of fat accumulation in the liver (Grummer 1993). In an assay with Sakiz-Awassi crossbreed sheep, the VLDL concentrations during early and late pregnancy were 4.75 and 3.13 mg/dL, respectively (Yokus et al. 2006), whereas in pregnant Santa Inês hair sheep the concentration was 6.92 mg/dL. With the advancement of pregnancy in Barki ewes, the mean VLDL concentrations at early, mid and late gestation were 7.0, 7.4 and 6.5 mg/dL, respectively (Kandiel, El-Khiaiat and Mahmoud 2016). All the reports above corroborate our range for VLDL in pregnant sheep.

Serum total protein is composed of albumin and globulins and varies according to the physiological status and age of the livestock. Overall, decreases in total protein concentration are observed with the advancement of pregnancy for several reasons: a normal pregnancy-induced haemodilution effect as a result of the increased blood volume and body water content (Zywicki et al. 2018; Swetha et al. 2018), decreases in globulin and immunoglobulin levels when colostrum is being produced in the udder (Balıkcı, Yıldız and Gürdoğan 2007; Kaneko, Harvey and Bruss 2008) and increased needs for amino acids for fetal muscle development (Castillo et al. 1999; Jainudeen & Hafez 1994). However, low total protein levels are also associated with an impairment of the usual hepatic functions in sheep diagnosed with pregnancy toxemia (Santos et al. 2011). The serum total protein concentration was 4.22 g/dL in Iraqi ewes during the second month of pregnancy (Jodan and Al-Hamedawi 2017). According to Gurgoze et al. (2009) analysing Awassi ewes at the 120th day of pregnancy and Nasciutti et al. (2012) studying Santa Inês hair sheep seven days before lambing, the total protein concentrations were 5.96 and 4.79 g/dL, respectively. In Karakul sheep at late pregnancy, Baumgartner and Pernthaner (1994) reported a mean value of 6.13 g/dL. The cited studies agree with our findings that the serum biochemical references for total protein in pregnant sheep are wider than that established by Kaneko, Harvey and Bruss (2008).

Albumin is considered the most sensitive indicator of the protein nutritional status in the long term, since low concentrations generally indicate inadequate protein intake (Cardoso et al. 2010; Oliveira et al. 2014). Albumin plays a role in carrying non-esterified fatty acids to be used by peripheral tissues as an energy
source (González and Silva 2006), besides being a significant source of amino acids to supply the demands of the fetus (Jainudeen and Hafez 1994). Even in animals diagnosed with pregnancy toxemia, serum albumin levels remain within the physiological limits for the species, as observed for Santa Inês, Dorper and crossbred sheep, with a mean value of 3.1 g/dL (Santos et al. 2011). According to these authors, serum albumin concentrations in animals diagnosed with pregnancy toxemia do not differ from those of healthy sheep at the same period of gestation, and alterations in serum albumin levels are associated with chronic problems or acute changes in the animals’ hydration. Contrary to the narrow range determined for serum albumin in the study by Kaneko, Harvey and Bruss (2008), the values from our report are broader, varying from 1.56 to 5.01 g/dL in pregnant sheep. The serum albumin concentration of 1.79 g/dL in Sakiz-Awassi crossbreed sheep (Yokus et al. 2006) was within the range for the proposed physiological values although approximately 25% lower than the lower limit determined by Kaneko, Harvey and Bruss (2008). In NARI Suwarna Ewes in India, the serum albumin concentration was 3.67 g/dL at the second month of pregnancy (Swetha et al. 2018), whereas it was 3.64 g/dL in Awassi ewes at the 145th day of pregnancy (Gurgoze et al. 2009) and 4.91 g/dL in Makouei sheep seven days before the expected lambing time (Mohammadi, Anassori and Jafari 2016).

Creatine is produced mainly in the kidneys and liver and then transported to the muscle and brain in the blood, where it is converted to phosphocreatine (Marai et al. 2008). In muscle, approximately 1–2% of free creatine is converted to creatinine daily (Burtis and Ashwood 1996). Creatinine excretion is primarily modified by the urinary flow velocity, and levels above the physiological values have been observed as the glomerular filtration rate decreases (Ognik et al. 2015). High levels of creatinine may indicate renal failure (Nascimento et al. 2015). For serum creatinine, the values found in the literature are lower than that reported by Kaneko, Harvey and Bruss (2008). Morsy et al. (2016) reported a mean serum creatinine of 0.49 mg/dL in late-pregnant Santa Inês ewes in Brazil, whereas it was 0.50 mg/dL in Awassi ewes of 21 days of pregnancy (Gurgoze et al. 2009). In an assay with Sakiz-Awassi crossbreed sheep in early and late pregnancy, the serum creatinine levels were 0.84 and 0.65 mg/dL, respectively (Yokus et al. 2006). The values were higher in Santa Inês hair sheep at late gestation (Nascimento et al. 2015) reaching 0.95 mg/dL, but still within limits determined by our study. However, for sheep diagnosed with pregnancy toxemia, Santos et al. (2011) reported a mean serum creatinine concentration of 2.01 mg/dL.

Aspartate aminotransferase (AST) is a liver enzyme involved in the process of transamination, yielding oxaloacetate as the end product of metabolism (Dzadzowski et al. 2015). This enzyme is found in hepatocytes, red blood cells and muscles (Al-Hadithy, Badawi and Mahmood 2013), and its serum levels are used to estimate liver function, since increased AST levels are associated with liver fatty infiltration (Lubojacka et al. 2005). The serum AST has a positive correlation with milk yield, physical activity and heart problems (Feijó et al. 2014; Antunović, Šperanda and Steiner 2004). Despite the physiological ranges determined in this study for serum aspartate aminotransferase in pregnant sheep were similar to those of Kaneko, Harvey and Bruss (2008), some studies reported values more like the interval found in this work. Nascimento et al. (2015) observed a serum aspartate aminotransferase level of 53.7 U/L in Santa Inês sheep three weeks before lambing, and Yokus et al. (2006) reported a mean of 46.23 U/L in Sakiz-Awassi crossbreed sheep during early pregnancy. In Merino landschaf pregnant ewes on the 20th day before lambing, the serum AST concentration was 104.87 U/L, whereas it was 97.20 U/L in Tsigai ewes (Antunović et al. 2011).

Gamma-glutamyl transferase is a liver enzyme found exclusively in hepatocytes and considered a biomarker of liver injury (Copeland 2000). The GGT and AST activity should be taken into consideration together, since both can indicate an injury to the liver tissue. The GGT levels may increase immediately if there is an acute hepatic injury due to the release of membrane fragments containing the enzyme (Thrall et al. 2007). Data found in the literature for GGT exceed the upper limit recommended by Kaneko, Harvey and Bruss (2008), which ranges from 20 to 52 U/L. This result may be related to the fact that their data do not consider the category and physiological status of the animal. The serum GGT concentration in Sakiz-Awassi crossbreed sheep during late pregnancy was 54.84 U/L (Yokus et al. 2006), a value still lower than that observed by Zywicki et al. (2018) in crossbreed Western-breed ewes during the third-trimester pregnancy, which reached up to 76 U/L. In Lacaune ewes with a healthy pregnancy, with subclinical ketosis and with clinical ketosis, the GGT activities averaged 28.4, 65.9 and 65.1 U/L, respectively (Marutsova 2015). Santos et al. (2011) reported an average GGT level of 83.4 U/L in Santa Inês, Dorper and crossbred ewes diagnosed with pregnancy toxemia after their discharge.
Alkaline phosphatase (ALP) is an intrahepatic enzyme found mainly in the biliary ducts and placenta (Al-Hadithy, Badawi and Mahmood 2013) and its serum concentrations are considered valuable parameters for the diagnosis of ketosis in sheep (Sargison et al. 1994). In an assay with Chios sheep that manifested clinical signs of pregnancy toxaemia, the ALP concentration reached 196.55 U/L (Dzadzovski et al. 2015). Studying white-faced Western-breed ewes at late gestation, Zywicki et al. (2018) reported a minimum serum ALP concentration of 36 U/L, whereas Yokus et al. (2006) observed an average value of 59 U/L in Sakiz-Awassi crossbreed sheep during early pregnancy. In Mehraban ewes 10 days before expected lambing, the values for ALP were slightly higher, reaching 166.3 U/L (Aliarabi et al. 2018). Ewes diagnosed with pregnancy toxaemia with high ALP activity recovered more slowly compared with those with lower ALP serum levels (Marutsova et al. 2015).

5. Conclusions

The serum biochemical reference ranges for pregnant sheep determined in our study are strongly divergent from those established by one of the most cited books on the topic, especially considering the high serum urea and cholesterol concentrations and low levels of blood glucose observed. Therefore, it is essential to consider the physiological status when evaluating the blood metabolic profile of pregnant ewes in order to maintain an adequate nutritional management and to prevent health disorders that may lead to productive and reproductive losses.

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