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Reference interval of biochemical parameters of lactating sheep in the tropics

Intervalo de referência de parâmetros bioquímicos de ovelhas lactantes nos trópicos

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Highlights

The biochemical parameters of lactating ewes in the tropics are different from literature.
The first reference intervals for HDL and VLDL for lactating ewes.
The biochemical parameters of ewes change during lactation.

Abstract

The objective was to estimate the reference intervals of biochemical parameters of lactating ewes in the tropics. Information on up to 290 lactating ewes was compiled from 2006 to 2017. All animals were healthy and were not subjected to forced malnutrition. Animals were raised in different management systems (pasture, total confinement, partial confinement, collective and/or individual confinement). The biochemical profile consisted of information on cholesterol, triglycerides, fructosamine, HDL (high density lipoprotein), VLDL (very low-density lipoprotein), total protein (TP), uric acid, urea, albumin, creatinine, calcium, phosphorus and magnesium, AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase) and alkaline phosphatase (ALP). Reference values were estimated using the Dixon test, when parametric, and by boostrap, when non-parametric, with a 95% confidence interval. Estimated intervals were 36.3 - 94; 262.22 - 450.66; 7 - 43; 18.6 - 69.4; 1.4 - 8.4; 0.61 - 1.66; 5.4 - 11; 0 - 1.4; 8.4 - 61.5; 1.9 - 3.57 for cholesterol, fructosamine, triglycerides, HDL, VLDL, creatinine, TP, uric acid, urea and albumin, respectively. For enzymes and mineral profile, 59 - 160; 37.9 - 127.8; 30 - 190; 8.23 - 12.45; 3.7 - 8.5; 1.7 - 3.8 for AST, GGT, ALP, Ca, P,

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and Mg, respectively. It can be concluded that the biochemical parameters of lactating ewes in the tropics are different from those determined in the literature.
Key words: Energetic. Metabolism. Sheep. Protein.

Resumo

Objetivou-se estimar os intervalos de referência dos parâmetros bioquímicos de ovelhas lactantes nos trópicos. Foram compiladas informações de até 290 ovelhas lactantes no período de 2006 a 2017. Todos os animais eram saudáveis e não passaram por condições de desnutrição forçada. Os animais foram criados em diferentes sistemas de manejo (pasto, confinamento total, semiconfinamento, confinamento coletivo e/ou individual). O perfil bioquímico constituiu informações de colesterol, triglicerídeos, frutosamina, HDL (lipoproteína de alta densidade), VLDL (lipoproteína de muito baixa densidade), proteínas totais, ácido úrico, ureia, albumina, creatinina, cálcio, fósforo e magnésio, AST (aspartato aminotransferase), GGT (gama glutamil transferase) e fosfatase alcalina (FA). Os valores de referência foram estimados através do teste de Dixon, quando paramétrico e por boostrap quando não paramétrico, sendo o intervalo de confiança a 95% de probabilidade. Os intervalos estimados foram 36,3 - 94; 262,22 - 450,66; 7 - 43; 18,6 - 69,4; 1,4 - 8,4; 0,61 - 1,66; 5,4 - 11; 0 - 1,4; 8,4 - 61,5; 1,9 - 3,57 para colesterol, frutosanima, trigligeríceos, HDL, VLDL, creatinina, PT, ácido úrico, ureia e albumina, respectivamente. Para as enzimas e o perfil mineral foram 59 - 160; 37,9 - 127,8; 30 - 190; 8,23 - 12,45; 3,7 - 8,5; 1,7 - 3,8 para AST, GGT, FA, Ca, P, e Mg, respectivamente. Conclui-se que os parâmetros bioquímicos das ovelhas lactantes nos trópicos são discrepantes aos determinados na literatura
Palavras-chave: Energético. Metabolismo. Ovinos. Proteico.

Introduction

The biochemical profile of production animals is a tool for nutritional, metabolic, diagnostic assessment and for disease treatment. For an adequate biochemical interpretation, it is necessary to consider factors, such as species, breed, climate and physiological state (Onasanya et al., 2015).

In this context, for sheep, Varanis et al. (2021a) and Souza et al. (2020) evaluating pregnant ewes and lambs, respectively, demonstrated the importance of physiological state and environment in establishing reference intervals and interpreting biochemical parameters. In this way, magnitude and variation of parameters can be used to identify metabolic disorders, for example beta-hydroxybutyrate in pregnancy toxemia (Brondani et al., 2016). In addition, assist in monitoring the growth and development of lambs (Varanis et al., 2021b).

Although the importance of categorization has been demonstrated, there are gaps in knowledge about the biochemical profile of lactating ewes raised in tropical climate. Lactation is a challenging period for ewes, mainly due to the recovery of consumption capacity throughout lactation and the increase in nutritional requirements for milk production (Antunović et al., 2017). Consequently, metabolic disturbances can occur, as ketosis showed by Fiore et al. (2021) reducing milk production, lower lamb weight gain and even death of the lactating female.

Manifestations during lactation can be monitored through the metabolic profile, since this allows to evaluate the nutritional, metabolic and health status of the animal. Therefore, the objective was to establish reference intervals for protein, energy, enzymatic and mineral biochemical parameters of lactating ewes raised in tropical climate.

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 University of Lavras, Federal Rural University of Rio de Janeiro, Federal University of Tocantins) and commercial farms using Santa Inês (39%), Dorper (28%), Lacaune (11%), Morada Nova (17%), Bergamacia (2%) and Suffolk (3%) ewes reared under different conditions (grazing, feedlot, semi-feedlot, collective and/or individual pens, and metabolic cages) from 2006 to 2017. Table 1 lists the different types of food used for animal feeding, whose data were used in this study.

The age of the ewes ranged from 1.5 to 3.8 years, the average lactation period was 90 days, for the calving order, an average of 92% multiparous and 8% primiparous. All animals were healthy, were not subjected to forced malnutrition, and data from ewes showing any clinical manifestations were discarded.

Table 1
Main feeds used in production systems

Energy concentrate	Protein concentrate	Forage and silage	Additive
Corn meal	Soybean meal	*Braquiaria brizantha*	Monensin
Citrus pulp	Urea	*Braquiaria decumbens*	Mineral salt (sheep)
Babassu starch meal	Soybean husk	*Cynodon spp.*(hay)	Exogenous enzymes
Soybean molasses	Babassu meal	*Panicum maximum* cv. Tanzania	Yeast
Corn grain	Cottonseed meal	*Panicum maximum* cv. Aruana	Virginiamycin
Propylene glycol	Soybean grain	*Panicum maximum* cv. Massai	
Glycerol	Protected urea	Corn silage	
Biscuit meal		Sorghum silage	
		Tanzania grass silage	

To determine the metabolic energy profile, data on cholesterol, triglycerides, fructosamine, HDL (high-density lipoprotein) and VLDL (very low-density lipoprotein) were obtained; for protein profile, total protein, uric acid, urea, albumin and creatinine data; for the mineral profile, values of calcium, phosphorus and magnesium; and for the enzymatic profile, AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase) and alkaline phosphatase (ALP) data. Laboratory analyses were performed on Bioplus 2000 and PKL-125 (MH-Lab) devices, using kits of different brands (Labtest, Biotecnica, GT Group). Values of VLDL were obtained as proposed by Friedewald et al. (1972).

Reference values were estimated using the RefVal 4.11 software. Outliers were removed using the Dixon's test, and the percentiles, as well as their confidence intervals, were estimated by the non-parametric boostrap method, when the data did not meet normal distribution assumption. The defined confidence interval was 95%. The comparison of the calculated reference intervals was performed with the values proposed by Kaneko et al. (2008), as it is one of the most cited books with around 6,520 citations.

Results and Discussion

The mean of the energy biochemical parameters was 65.15, 356.14, 25, 44 and 4.9 for cholesterol, fructosamine, triglycerides, HDL and VLDL, respectively. There was a greater range in reference intervals of estimates of cholesterol, fructosamine and triglycerides compared to literature (Table 2).

The lower threshold of cholesterol was 30% below the reference, while the upper limit was 23% above. Within the interval estimated in Table 2 and recommended by Kaneko et al. (2008), Lima et al. (2016) observed an average of 64.73 mg/dL for Santa Inês ewes 30 days post-partum (DPP). J. S. Silva et al. (2013), for sheep with 60 DPP, observed a mean concentration of 52.25 mg/dL. Contrary to the recommendation by Kaneko et al. (2008) and within the estimated interval, for Lacaune ewes, Brito et al. (2006) found a mean value of 89.71 mg/dL at 60 DPP, and F. M. S. C. Santos et al. (2014), a mean of 44.08 mg/dL for Morada Nova ewes at 15 DPP.

In addition, during lactation, there is a gradual increase in cholesterol levels (Brito et al., 2006). This increase may be related to an increase in plasma lipoproteins, weight loss, and an increase in steroid hormone precursors, as reproductive activity is reestablished (J. S. Silva et al., 2013).

Table 2
Reference intervals for energy and protein biochemical parameters of lactating ewes

Item	Unit	N	Reference interval	Reference (Kaneko et al., 2008)
Cholesterol	mg/dL	275	36.3 - 94	52 - 76
Fructosamine	µmol/L	228	262.22 - 450.06*	170 - 174
Triglycerides	mg/dL	64	7 - 43	9 -30
HDL[2]	mg/dL	225	18.6 - 69.4*	SIL
VLDL[3]	mg/dL	64	1.4 - 8.4	SIL
Creatinine	mg/dL	290	0.61 - 1.66	1.2 - 1.9
Total Protein	g/dL	290	5.4 - 11.0*	6 - 7.9
Uric Acid	mg/dL	289	0 - 1.4	0 - 1.9
Urea	mg/dL	280	8.4 - 61.5	17 - 43
Albumin	g/dL	290	1.90 - 3.57	2.4 - 3.0

[1]N - sample number; [2]HDL - high density lipoprotein; [3]VLDL - very low-density lipoprotein; *parametric data.

For fructosamine, the lower threshold was 54.5% above the reference, while the upper, was 158.6% above. Fructosamine is a product of glycation of plasma proteins. Quantifying fructosamine concentration is important for energy metabolic evaluation, as it depends on the mean glucose concentration and protein half-life, without being subjected to changes due to transient hyperglycemia, as showed C. F. Silva et al. (2022) for sheep fed diets containing extra-fat whole corn germ. The estimated reference interval for fructosamine includes the one proposed by F. M. S. C. Santos et al. (2014) for Morada Nova ewes, with an average of 149.33 µmol/L at 15 DPP. For Santa Inês ewes with 30 DPP, Lima et al. (2016) reported a mean value of 189.34 µmol/L, and for sheep at 60 DPP, J. S. Silva et al. (2013) obtained a mean concentration of 169.35 µmol/L.

Triglycerides were 22% below the reference at the lower threshold and 43.3% above the upper threshold (Table 2). The concentration of triglycerides reduces at the beginning of lactation compared to pregnancy, due to the increase in energy input for milk production, milk fat synthesis, in addition to the lower reserve of available free fatty acids (Pesántez-Pacheco et al., 2019). Within the estimates (Table 2), F. M. S. C. Santos et al. (2014) observed a mean value of 15.94 mg/dL for sheep at 15 DPP; for animals at 30 DPP, Lima et al. (2016) showed a mean of 13.01 mg/dL; and for ewes at 60 DPP, J. S. Silva et al. (2013) and Brito et al. (2006) obtained, respectively, means of 20 and 41.4 mg/dL.

As with cholesterol and triglycerides, lipoproteins are key to understanding energy metabolism. There are no comparative parameters for the reference intervals for HDL and VLDL for lactating ewes, demonstrating the originality of the estimates.

High-density lipoprotein (HDL) is responsible for transporting excess cholesterol from tissues to the liver, whereas very low-density lipoprotein (VLDL) transports triglycerides from the liver to adipose tissue (R. P. Santos et al., 2015). Therefore, with physiological changes during lactation, we call attention that this is the first study establishing estimates of reference intervals for lipoproteins in the metabolic and nutritional monitoring of lactating ewes (Table 2).

The mean values of creatinine, total protein, uric acid, urea and albumin were 1.16, 8.2, 0.7, 13.98 and 2.73. The range of reference intervals of the biochemical profile was similar to the literature (Table 2).

In protein metabolism, creatinine is related to protein mobilization. According to Nascimento et al. (2015), the reduction in creatinine level is related to the mobilization of proteins for lactogenesis. Within the range proposed in Table 2, F. M. S. C. Santos et al. (2014), evaluated Morada Nova ewes, and observed, at 15 days of lactation, an average of 0.87 mg/dL, while J. S. Silva et al. (2013), observed an average of 0.73 mg/dL in Santa Inês ewes at 60 DPP.

As for total protein as an indication of total circulating proteins, for ewes with an average of 30 days of lactation, Ribeiro et al. (2004), Cardoso et al. (2010) and Lima et al. (2016) observed, respectively, averages of 7.21, 6.75 and 7.06 g/dL. As for ewes at 60 DPP, Brito et al. (2006) and J. S. Silva et al. (2013) reported, respectively, mean concentrations of 6.98 and 7.54 g/dL.

Together with creatinine and total protein, urea concentration is an important indicator of protein metabolism and balance (J. S. Silva et al., 2013). Evaluating Santa Inês ewes, Cardoso et al. (2010) and Lima et al. (2016) observed, at 30 DPP, mean values of 26.48 mg/dL and 41.82 mg/dL, respectively. For Lacaune ewes, at 60 DPP, Brito et al. (2006) found a mean of 59.45 mg/dL, and J. S. Silva et al. (2013), for Santa Inês ewes, average concentration of 35.4mg/dL.

For albumin, Brito et al. (2006) reported an average of 3.44 g/dL for Lacaune dairy ewes at 60 DPP. As for Santa Inês ewes at 30 DPP, Cardoso et al. (2010) and Lima et al. (2016) observed, respectively, average concentrations of 2.11 and 2.69 g/dL; and for sheep at 60 DPP, J. S. Silva et al. (2013) reported a mean value of 2.67 g/dL albumin. Levels of this metabolite may be reduced during lactation, since during this period there is a high demand for protein for milk synthesis (González & Silva, 2006).

Data on Aspartate aminotransferase (AST), Gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were used for enzyme profile (Table 3). The mean of the enzymatic biochemical parameters was 109.5, 82.85 and 110 for AST, GGT and alkaline phosphatase. The range of the estimated intervals for enzymatic metabolites was different from that reported in the literature. The lower limit of the AST level was only 1.67% below the reference, however the upper limit was 42.8% below. For GGT, the lower limit was 89.5% higher than the reference, and the upper limit was 145.1% above. As for alkaline phosphatase, the lower limit was 69.3% below, and the upper limit was 50.9% below the reference.

Table 3
Reference intervals for enzymatic and mineral biochemical parameters of lactating ewes

Item	Unit	N[1]	Reference interval	Reference (Kaneko et al., 2008)
AST[2]	U/L	284	59 - 160	60 - 280
GGT[3]	U/L	278	37.9 - 127.8	20 - 52
ALP	U/L	64	30 - 190	68 - 387
Calcium	mg/dL	228	8.23 - 12.45*	11.5 - 12.8
Phosphorus	mg/dL	226	3.7 - 8.5*	5 - 7.3
Magnesium	mg/dL	228	1.7 - 3.8	2.2 - 2.8

[1]N - sample number; *parametric data; 2AST: aspartate aminotransferase; 3GGT: Gamma-glutamyl transferase.

Analysis of AST, GGT, and ALP aid in determining liver function problems (Kaneko et al., 2008). Within the expected interval (Table 2) for AST, Nascimento et al. (2015), evaluated Santa Inês ewes, and observed a mean of 64.3U/L at 30 DPP. For Morada Nova animals at 15 days of lactation, F. M. S. C. Santos et al. (2014) observed a mean value of 101.45 U/L. For GGT, F. M. S. C. Santos et al. (2014) registered a mean value of 48.77

U/L for ewes at 15 days of lactation, while Nascimento et al. (2015) reported a mean value of 57.4 U/L for ewes at 30 days of lactation. As for alkaline phosphatase, F. M. S. C. Santos et al. (2014) observed a mean value of 89.13 U/L, at 15 DPP.

The mean values of calcium, phosphorus and magnesium concentrations for lactating ewes were 10.34, 6.1 and 2.75, respectively.

The lower limit for calcium was 28.4% below the reference, however, the upper limit was similar to that reported in the literature. For the mineral biochemical profile, calcium values fluctuate during lactation due to their mobilization for parturition and lactation (Cardoso et al., 2010). Within the estimates (Table 2) for animals at 60 days of lactation, Brito et al. (2006) and J. S. Silva et al. (2013) reported mean values of 9.82 and 8.69 mg/dL, respectively.

For phosphorus and magnesium, the range of reference intervals was greater than the reference for pregnant ewes, drawing attention to the 26% reduction for the lower threhsold of phosphorus and an increase of 35.7% for the upper limit of magnesium.

Magnesium levels are influenced by the balance between intake and elimination, to meet the physiological needs of the lactation period (Cardoso et al., 2010). Evaluating Santa Inês ewes, Cardoso et al. (2010) observed, 30 DPP, a mean value of 2.74 mg/dL, and after 60 DPP, J. S. Silva et al. (2013) observed a mean level of 2.35 mg/dL. For Morada Nova ewes 15 DPP, F. M. S. C. Santos et al. (2014) found a mean concentration of 2.37 mg/dL; for Border Leicester x Texel ewes at 29 DPP, Ribeiro et al. (2004) observed a mean of 2.21 mg/dL; and for Lacaune dairy ewes at 60 DPP, mean value of 2.80 mg/dL (Brito et al., 2006).

For phosphorus, F. M. S. C. Santos et al. (2014) observed a mean value of 5.29 mg/dL for Santa Inês ewes at 15 DPP; Ribeiro et al. (2004) and Cardoso et al. (2010) reported, respectively, mean concentrations of 3.09 and 2.97 mg/dL at 30 DPP; with 60 DPP, Brito et al. (2006) observed a mean level of 5.04 mg/dL. F. M. S. C. Santos et al. (2014) observed that phosphorus levels decreased 7 days before parturition, maintaining this behavior until 15 DPP. If the deficiency of this mineral occurs, it can result in a low reproductive performance, even poor growth of the offspring (Ribeiro et al., 2004).

Conclusions

Reference intervals for energy, protein, enzymatic and mineral biochemical parameters of lactating ewes in the tropics differ from those in the literature. HDL and VDLD estimates aid in the metabolic and health assessment of lactating ewes.

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