








Communication

[Comunicação]

Reference intervals for metabolic profile of adult sheep in the tropics: over 12 months

[Intervalos de referência para perfil metabólico de ovelhas adultas nos trópicos: mais de 12 meses]

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The metabolic profile is a tool for monitoring nutritional status, metabolic disorders, and physiological state. For interpretation of the metabolic profile of sheep it is important to consider breed, sex, age, rearing system, climate, and physiological state (Hernandez *et al.*, 2020). As an example, Brondani *et al.* (2016) showed the importance of evaluating in categories, highlighting the metabolic changes during pregnancy. Since the values found in the literature are based on sheep raised in temperate climates without considering the categories, the objective was to estimate reference intervals of metabolites in adult sheep raised in the tropics.

A database was built with some studies. Experiments were conducted with sheep at the Federal University of Uberlândia, Federal University of Minas Gerais, Federal University of Lavras, Federal Rural University of Rio de Janeiro and Federal University of Tocantins, from 2006 to 2017. From these, metabolite data was extracted of adult sheep, male and female, non-pregnant and non-lactating, over 12 months. The experiments were developed in systems of pasture, total confinement, semi-confinement, collective or individual confinement and in metabolic cages. The present study considered only data from healthy animals, without any clinical manifestation or submission to forced malnutrition.

To determine the metabolic energy profile, data were obtained for glucose, cholesterol, triglycerides, fructosamine, HDL (high density

lipoprotein), LDL (low density lipoprotein) and VLDL (very low-density lipoprotein); 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 data. Laboratory analyses were performed on Bioplus 2000 and PKL-125 (MH-Lab) devices, using kits from Labtest, Biotecnica and GT Group). LDL and VLDL values were calculated as proposed by Friedewald *et al.* (1972).

To estimate and determine the reference values, the RefVal 4.11 software was used (Solberg, 2006). The percentiles and their confidence intervals were estimated by the non-parametric bootstrap method, while the outliers were removed by the Dixon test. All intervals were defined with 95% confidence. The reference intervals found were compared with those presented by Kaneko *et al.* (2008), which is the main reference in studies on biochemistry of domestic animals.

The reference intervals found were wider than those defined in international literature (Tab. 1). Among the highlighted metabolites are fructosamine, cholesterol, urea, and phosphorus. The reference interval defined for fructosamine values was 163 times wider than that defined in the literature. Gouveia *et al.* (2015) report that fructosamine levels may be elevated in cases of hyperglycemia, as it is a stable ketamine, formed when glucose reacts non-enzymatically with

Reference intervals...

amine groups in proteins. However, none of the animals presented hyperglycemia, reinforcing the difference in the protein profile for animals

raised in a tropical environment which are more resilient.

Table 1. Reference intervals for serum liver enzymes and metabolites in sheep over twelve months (95% confidence)

Metabolite	Unit	N ¹	Observed	Kaneko <i>et al.</i> (2008)
Glucose	mg/dL	718	29.15 - 87.18 *	50 – 80
Cholesterol	mg/dL	1,143	10.0 - 98.6	52 – 76
Triglycerides	mg/dL	1,001	4 – 40	9 – 30
Fructosamine	µmol/L	109	136 - 788.63	170 – 174
HDL ²	mg/dL	200	15.6 - 72.5 *	SIL ⁵
LDL ³	mg/dL	187	1.78 - 48.28 *	SIL ⁵
VLDL ⁴	mg/dL	436	0.9 - 7.85 *	SIL ⁵
Creatinine	mg/dL	991	0.6 - 1.7	1.2 - 1.9
Total Protein	g/dL	1,016	3.9 - 10.6	6 - 7.9
Uric acid	mg/dL	822	0.0 - 0.86	0 - 1.9
Urea	mg/dL	902	9 – 70	17 - 43
Albumin	g/dL	1,2	1.1 - 5.1	2.4 - 3.0
Calcium	mg/dL	108	7.45 - 11.10 *	11.5 - 12.8
Phosphorus	mg/dL	109	3.08 - 11.6	5 - 7.3
Magnesium	mg/dL	109	1.53-4.8	2.2-2.8
AST ²	U/L	694	12.7-160	60-280
GGT ³	U/L	772	19-75.2	20-52
Alkaline phosphatase	U/L	573	34.1-248.4	68-387

¹ N – sample number; ² HDL - high density lipoprotein; ³ LDL – low density lipoprotein; ⁴ VLDL – very low-density lipoprotein; ⁵ no information in the literature. *Parametrically transformed data

Regarding the amplitude observed in the levels of energetic metabolites, urea, total proteins, and magnesium, Araújo *et al.* (2012) stated that nutritional factors alter metabolism and can modify blood composition. Furthermore, Gressler *et al.* (2015) concluded that total cholesterol levels are directly related to the animal's diet. This also occurs for triglyceride levels (González and Silva, 2006). The behavior of VLDL is similar to that of triglycerides, as this lipoprotein is responsible for transporting this component (Santos *et al.*, 2015). Diets with elevated levels of crude and/or degradable protein in the rumen result in higher levels of plasma urea and total protein (Gressler, 2015). The same occurs with albumin, which, being the most abundant protein in plasma, indicates the protein content of the diet (Araújo *et al.*, 2012).

Sheep raised in a tropical environment have highly lignified forage available, which leads to reduced fiber digestibility and protein availability, thus increasing the nutritional

heterogeneity of the diet (Cruz *et al.*, 2021). That is, the quality and availability of food in the tropics is related to variations in the estimates of the metabolic profile in relation to international literature.

Liver enzymes indicate good liver function, so elevated values are indicative of liver disease. However, no animal showed hepatic alterations, which highlights the need to adapt the interpretation of these parameters for sheep raised in tropical environments.

The reference intervals for metabolic profile of adult sheep raised in the tropics differ from international literature. The use of metabolite reference intervals considering regionality and category enables more accurate nutritional and pathological diagnoses.

Keywords: *biochemistry, physiology, small ruminants*

RESUMO

Os níveis de metabólitos variam de acordo com a região de criação, idade, estado fisiológico e alimentação do animal. Portanto, objetivou-se estimar valores de referência para os perfis energético, hepático e mineral de ovinos adultos nos trópicos. Para isso, foram utilizados dados de ovinos saudáveis, criados em diferentes sistemas de manejo, em instituições brasileiras, de 2006 a 2017. Glicose, colesterol, triglicerídeos, fructosamina, HDL, LDL e VLDL foram determinantes para o perfil energético; para o perfil proteico: proteína total, ácido úrico, ureia, albumina e creatinina; para o perfil mineral: valores de cálcio, fósforo e magnésio; e para o perfil enzimático: AST, GGT e fosfatase alcalina. A estimativa e a determinação dos valores de referência foram realizadas pelo teste Dixon e pelo método não paramétrico bootstrap. O intervalo de confiança definido foi de 95%. A maioria dos metabólitos apresentou intervalos que extrapolaram os limites recomendados na literatura internacional, chamando a atenção para o perfil energético o colesterol, fructosamina, ureia e fósforo e no enzimático, os intervalos de AST, GGT e fosfatase alcalina. Em conclusão, os intervalos definidos com base nos dados nacionais apresentam diferenças em relação aos internacionais, pois estes não distinguem os intervalos quanto à categoria do animal.

Palavras-chave: bioquímica, fisiologia, pequenos ruminantes

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