

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

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Abstract

Metabolite levels can vary according to the farming region, age, physiological state and feeding of the animal, therefore, the objective was to estimate reference values for the energy, hepatic and mineral profiles of sheep (above 12 months) in the tropics. For this, data from healthy adult sheep, raised in different management systems in Brazilian institutions from 2006 to 2017 were used. To determine the 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 the 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, data on AST (aspartate aminotransferase), GGT (gamma glutamyl transferase), alkaline phosphatase. The estimation and determination of reference values was performed in the RefVal 4.11 software, where outliers were removed, using the Dixon test, and the percentiles, as well as their confidence intervals, estimated by the non-parametric bootstrap method, when data did not show normal distribution. The defined confidence interval was 95%. Most metabolites presented intervals extrapolating both the lower and upper limits recommended in the international literature, especially cholesterol, fructosamine, urea and phosphorus. As for the enzymatic profile, the AST and alkaline phosphatase intervals assumed, as an upper limit, values below those recommended by the international literature. In conclusion, the intervals defined from the national data present differences in relation to the international ones, since only adult animals were considered, while those from international data do not make a distinction regarding the animal category.

Introduction

For interpretation of the metabolic profile of sheep, factors capable of modifying should be taken into account, for example, breed, sex, rearing system, climate and physiological state (Onasanya et al., 2015), in addition to diseases and nutritional imbalances. Since the values found in the literature are based on sheep raised in temperate climates, the objective was to estimate reference intervals of metabolites in adult sheep raised in the tropics, which will allow more accurate assessments of their metabolic profile.

Material And Methods

Experiments were carried out 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, Lew and Fredrickson (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.

Results And Discussion

The ccc found for Brazilian animals were wider, so that the lower limit was lower and the upper limit was higher than those defined in the international literature (Table 1). Among the metabolites that exceeded most the limits, fructosamine, cholesterol, urea and phosphorus stood out. The reference interval defined for fructosamine values was 163 times wider than that defined in the literature (Table 1). Gouveia et al. (2015) report that fructosamine levels may be elevated in cases of hyperglycemia, as it is a stable ketoamine, formed when glucose reacts non-enzymatically with amine groups in proteins. However, none of the animals presented a case of hyperglycemia, reinforcing the difference in the protein profile for animals in a tropical environment.

The amplitude observed in the levels of cholesterol, triglycerides, VLDL, HDL, LDL, urea, total protein and magnesium is explained by the possible correlation with the animal diet. Araújo et al. (2012) stated that nutritional factors alter metabolism and can modify blood composition. Gressler et al. (2015) concluded that total cholesterol levels are directly related to the animal diet. This also occurs for triglyceride levels (González, 2006). The behavior of VLDL is similar to that of triglycerides, since this lipoprotein is responsible for the transport of this component (Santos et al., 2015). Diets with high levels of crude and/or rumen-degradable protein 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 available highly lignified forages, 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 metabolic profile estimates compared to international literature.

For the enzymatic profile, AST and alkaline phosphatase also exceeded the limits defined in the literature (Table 1). Liver enzymes indicate good liver function, so high values are indicative of liver disease.

However, no animal showed liver disorders, which highlights the need to adapt the interpretation of these parameters for sheep raised in tropical environments.

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

Metabolite	Unit	N ¹	Observed	Kaneko et al. (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

Conclusion

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

Statements & Declarations

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Ethics approval: Approval of animal care and use committee was not needed because it used existing datasets historically collected by the universities and partners.

Consent to participate: Not applicable

Consent to publish: Not applicable

Availability of data and material: The datasets analyzed during the current study are not publicly available due to being part of several researches but are available from the corresponding author on reasonable request.

Code availability: Not applicable

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Érica Beatriz Schultz, Aline Rabello Conceição, Marco Tulio Santos Siqueira, Karla Alves Oliveira, Luciano Fernandes Souza and Gilberto de Lima Macedo Júnior. The first draft of the manuscript was written by Érica Beatriz Schultz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Da Cruz, C. H., Santos, S. A., de Carvalho, G. G. P., Azevedo, J. A. G., Detmann, E., de Campos Valadares Filho, S., ... & Alba, H. D. R. (2021). Estimating digestible nutrients in diets for small ruminants fed with tropical forages. *Livestock Science*, 249, 104532.
2. De Araújo, P. B., Andrade, R., Ferreira, M. D. A., Batista, Â., Carvalho, C. D., & Soares, P. C. (2012). Effect of replacement tifton hay (*Cynodon spp.*) for castor beans hulls (*Ricinus communis*) based diets of spineless cactus (*Nopalea cochenilifera* Salm Dick) metabolites on the profile of mineral and energy-protein in sheep. *Revista Brasileira de Medicina Veterinária*, 34(4), 327–335.
3. De Gouveia, L. N., Maciel, M. V., Soares, P. C., FS Neto, I., Goncalves, D. N., Batista, Â., & de Carvalho, F. F. (2015). Substitution of Tifton 85 hay by hay or silage maniçoba in diets based on spineless cactus on metabolic profile of sheep. *Pesquisa Veterinária Brasileira*, 35, 5–9.

4. Dos Santos, R. P., Sousa, L. F., de Sousa, J. T. L., Andrade, M. E. B., Júnior, G. D. L. M., & da Silva, S. P. (2015). Parâmetros sanguíneos de cordeiros em crescimento filhos de ovelhas suplementadas com níveis crescentes de propilenoglicol. *Revista Brasileira de Ciências Agrárias*, 10(3), 473–478.
5. González, F. H. (2009). Diagnostic tools and monitoring of metabolic diseases. *Ciência Animal Brasileira*.
6. González, F. H. D., & Silva, S. (2006). *Introdução À Bioquímica Clínica Veterinária*. 2aed. Porto Alegre: UFRGS, 360p.
7. Gressler, m. A. L., souza, m. I. L., souza, a. S. D., filiú, w. F. D. O., aguena, s. M., & franco, g. L. (2015). Biochemical profiles of ewes submitted to short-term nutritional supplementation in subtropical region. *Revista Brasileira de Saúde e Produção Animal*, 16(1), 210-222p.
8. Kakeno, J. J.; Harvey, J. W. and Bruss, M. L. *Clinical Biochemistry of Domestic Animals*. 6th ed. Academic Press, San Diego. 916p. 2008.
9. Onasanya, G. O.; Oke, F. O.; Sanni, T. M. and Muhammad, A. I. 2015. Parameters influencing hematological, serum and bio-chemical references in livestock animals under different management systems. *Open Journal of Veterinary Medicine* 5(08):181.
10. Solberg, H. E. (2006). *RefVal 4.11 software user's guide*. RefVal, Rykkinn, Norway.