Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v39i3.35101

Inclusion of propylene glycol in the diet of sheep and its effect on their lambs' protein and mineral metabolites

Rogério Pereira dos Santos[1], Gilberto de Lima Macedo Junior[2], Simone Pedro da Silva[2*], Luciano Fernandes de Sousa[1] and Marina Elizabeth Barbosa Andrade[3]

*[1]Centro de Ciência Animal, Universidade Federal do Tocantins, Araguaína, Tocantins, Brazil. [2]Faculdade de Medicina Veterinária, Universidade Federal de Umuarama, Av. Pará, 1720, 38400-902, Campus Umuarama, Bloco 2T, Uberlândia, Minas Gerais, Brazil. [3]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Julio de Mesquita Filho" , Jaboticabal, São Paulo, Brazil. *Author for correspondence. E-mail: simone.silva@ufu.br*

ABSTRACT. The aim of this study was to assess the effect of propylene glycol (PG) added to the water sheep drank during lactation on protein and mineral metabolites of their suckling lambs. We assessed 24 lambs born to sheep fed levels of propylene glycol in the water (0; 1.5; 3.0 and 4.5%). The sheep were subdivided according to a completely randomized design, in a split-plot scheme. There was no interaction effect between lamb age and amount of PG provided to the sheep on the concentration of protein and mineral metabolites of the lambs. The concentration of total protein, globulin, and albumin: globulin ratio, uric acid and creatinine in the lambs was not affected by the inclusion of PG ($p > 0.05$). The concentration of urea and magnesium in the lambs presented a quadratic response as a function of the addition of PG; there was linear response for albumin concentration. The addition of PG to the sheep's diet and the lambs' age affect the concentration of protein and mineral metabolites in the lambs. However, using PG to feed lactating sheep does not have negative effects on the lambs' blood parameters, as evidenced by the absence of nutritional deficiency (protein and minerals) in the suckling lambs.

Keywords: albumin, globulin, sheep, protein, urea, water.

Inclusão de propilenoglicol na dieta de ovelhas e efeito nos metabólitos proteicos e minerais de suas crias

RESUMO. Objetivou-se avaliar o efeito da inclusão do propilenoglicol na água de ovelhas durante a lactação sobre os metabólitos proteicos e minerais de suas crias. Foram avaliados 24 cordeiros (as), filhos (as) de ovelhas que receberam níveis de propilenoglicol (0; 1,5; 3,0 e 4,5%) na água. As ovelhas foram distribuídas em delineamento inteiramente casualizado em esquema de parcela subdividida. Não houve efeito de interação entre a idade dos cordeiros (a)s e a quantidade(s) de propilenoglicol oferecido para as mães na concentração dos metabólitos proteicos e minerais dos cordeiros. A concentração de proteínas totais, globulinas, relação albumina:globulinas, ácido úrico e creatinina nos cordeiros não foi afetada pela inclusão de propilenoglicol na água das matrizes. A concentração de ureia e magnésio nos cordeiros apresentou resposta quadrática em função da inclusão de propilenoglicol na dieta das ovelhas, sendo que para a concentração de albumina houve resposta linear. A inclusão de propilenoglicol na dieta das ovelhas e a idade dos cordeiros alteram a concentração dos metabólitos proteicos e minerais nas crias. Contudo, a utilização do propilenoglicol na alimentação de ovelhas lactantes não proporciona efeitos negativos nos parâmetros sanguíneos dos cordeiros, o que é evidenciado pela ausência de déficit nutricional (proteína e minerais) nos cordeiros lactentes.

Palavras-chave: água, albumina, globulina, ovinos, proteína, ureia.

Introduction

The adequate growth and slaughter of lambs at early age require, in addition to genetic factors, proper feeding. Considering that good performance by these animals in the growth phase is directly related to milk production by their mothers, it is fundamental to adopt strategies aiming at increasing milk production and, consequently, a greater performance by lambs. One of the strategies that can be adopted in this sense is the supply of glycogen products in the postpartum period, such as the use of propylene glycol, which can be mixed in the water given to the animals (Santos et al., 2015).

Propylene glycol, also called 1,2-propanediol, is a product with applications in the food, beverage, cosmetic and pharmaceutical industries. It is obtained through the hydrogenolysis of glycerol in the presence of a metallic and hydrogenic catalyst (Chun, Beltramini, Fan, & Lu, 2007). After ingestion by the animal, propylene glycol escapes from ruminal fermentation, being absorbed and turned into glucose in the liver.

Propylene glycol has been used to feed ruminants as an alternative to lessen the negative energy balance generated by an increase in the milk production capacity of matrices. Some studies have reported a successful use of propylene glycol, such as reduced plasma concentrations of beta-hydroxybutyrate, free fatty acids, urea, and increased plasma concentrations of glucose, insulin, cholesterol and IGF-I (Lien, Chang, Horng & Wu, 2010; Rukkwamsuk & Panneum, 2010).

With the knowledge of some specific biochemical parameters it is possible to obtain the clinical diagnosis of some metabolic diseases (Peixoto & Osório, 2007). Thus, the biochemical profile represents an important parameter that allows for improvements in production rates through the control of the feed supplied. Caldeira, Belo, Santos, Vazques, and Portugal (2007) verified that glucose, non-esterified fatty acids, and insulin enabled a better understanding of the energy nutritional status in sheep at different life stages.

Plasma concentration of total proteins, urea and albumin expresses the nutritional status of ruminants. Likewise, concentration of minerals in fluids, such as in ruminants' blood and urine, is important to diagnose deficiencies of macro and microelements because, based on these analyses, it is possible to know the metabolic balance of a certain mineral (Schweigel, Voigt, & Mohr, 2009).

A study conducted by Chiofalo et al. (2005) assessed the supply of propylene glycol to lactating sheep and identified an increase in milk production and an increment of 26 g day^{-1} in the weight gain of lambs born to sheep fed with high levels of propylene glycol. From this perspective, it is possible that the offspring of sheep fed with this product have their protein and mineral metabolite profile affected. In fact, Santos et al. (2015) found that the use of propylene glycol changed the concentration of energy metabolites and liver enzymes of lambs born to sheep fed with this product.

In this sense, the objective was to assess how the addition of propylene glycol to the water of lactating sheep affects the concentration of protein and mineral metabolites of their offspring.

Material and methods

The experiment was carried out in the sheep-goat farming sector of Capim Branco Farm [*Fazenda Capim Branco*], Federal University of Uberlândia [*Universidade Federal de Uberlândia*] (UFU), from April to June 2012. The present study was approved by the Ethics Committee on Animal Use [*Comissão de Ética na utilização de Animais*] (CEUA) of the Federal University of Uberlândia.

Twenty-four lambs were assessed, being 12 males and 12 females, with initial average body weight of 3.95±0.63 kg, from birth to approximately 90 days of age, in which weaning was performed. The animals were born to 24 Santa Inês sheep, by simple delivery. The sheep were confined in collective stalls, distributed in a completely randomized way into four treatments. One was the control, without addition of propylene glycol, and the others differentiated as to the amount of addition of the product to the water (1.5, 3.0 and 4. 5% of propylene glycol in the water), for instance, 1.5% of propylene glycol was included by adding 15 mL of the product diluted in 985 ml of water.

Water intake per stall was measured during all the days of the experiment, being obtained through the difference between amount offered and residual amount in a 24-hour period. The water was cleaned by filtration for removal of remaining food. After filtration, graduated beakers (1L) were used to determine water intake. After measurement of the remaining volume, the water was again returned to the drinking fountains, and propylene glycol was diluted in the water, according to the respective treatment.

The feed provided to the mothers was balanced to meet their needs, in compliance with National Research Council recommendations (NRC, 2007). The ingredients used in the feed were corn silage, ground corn, citrus pulp, soybean meal and mineral salt (Table 1). The filler concentrate ratio adopted for the sheep was 68:32.

The lambs stayed with their mothers, without access to the food of the latter, from birth until 30 days. After this period, controlled feeding was managed until the lambs reached adequate weight for weaning, which was done at 90 days of age. For controlled feeding management, the lambs were separated from their mothers in the morning and housed in two collective stalls (nursery) and, in the late afternoon, were taken to the stalls where their mothers were. In the nursery, the lambs had free food containing corn silage, and concentrate containing the same ingredients of the feed provided to the sheep, but with 20% of crude protein and 70% of total digestible nutrients, in addition to mineral salt and water; the mineral salt was mixed with the concentrate. The filler concentrate ratio used for the lambs was 30:70.

Table 1. Chemical composition (g kg^{-1}) of the diet, feed provided to the sheep and propylene glycol.

Ingredients	DM	CP	NDF	ADF	HCEL	CEL	Ash	EE
Corn silage	278.1	66.6	600.3	352.5	247.8	159.0	43.5	27.4
Concentrate*	907.2	179.3	210.4	97.2	113.2	143.8	45.0	18.4
Diet chemical composition (g.kg^{-1})								
Total Protein					92.2			
Total Digestible Nutrients					690.0			
Neutral Detergent Fiber					338.8			

Propylene Glycol: Tests performed by Vetec company		
Tests	Limits	Results
Content (CG)	Min. 99.5%	99.95%
Color (Alpha)	Max. 10	< 10
Residue on ignition	Max. 0.005%	0.002%
Titratable acids	Max. 0.0005 meq.g^{-1}	<0.0005 meq.g^{-1}
Chloride (Cl)	Max. 1ppm	< 1 ppm
Water (K.F.)	Max. 0.2%	0.03%
Density 20ºC/20ºC	1.035-1.040	1.039

DM: Dry matter, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, HCEL: Hemicellulose, CEL: Cellulose, Ash: Mineral Matter, EE: Ethereal Extract, *Concentrate composition: ground corn (45%), soybean meal (29.7%), citrus pulp (22.2%) and mineral salt (3%).

Blood samples were collected by jugular venipuncture with the aid of *vacuntainner*, in the following periods: 9; 18, 27, 36; 45; 60; 75 and 90 days of age. All blood samples were collected in the morning, before the first feed was given. The blood samples collected were centrifuged at 5,000 rotations per minute (RPM) for 10 minutes; the serum was separated into aliquots, stored in eppendorf tubes and kept in a freezer at -5ºC for further laboratory analysis.

For protein metabolism determination, the concentration of total protein, albumin, globulins, albumin/globulins ratio, creatinine, uric acid and urea was analyzed. The concentration of phosphorus, calcium and magnesium was also determined in the blood samples as well. All samples were processed in an automated biochemical analyzer, using a Lab Test commercial kit.

The experimental data were subjected to normality, homoscedasticity and sphericity tests. The experimental design used was the completely randomized one, in an experimental split-plot scheme in time. In the plots, there were levels of propylene glycol in the water (0, 1.5, 3.0 and 4.5%) and, in the subplots, the lambs' age in days (9, 18, 27, 36, 45, 60 75 and 90 days). After obtaining the individual variances of the plots and subplots, regression analysis was performed. Statistical analyses were carried out on the Sistema para Análises Estatísticas program, version 9.1 (SAEG, 2007).

Results and discussion

There was no interaction between lamb age and propylene glycol (PG) levels in the sheep's food for concentration of total protein, globulin and albumin: globulin ratio. The concentration of these metabolites in the offspring was not affected by the addition of PG to the water the mothers drank ($p \geq 0.05$), remaining within the range considered normal for species. The concentration of the previously mentioned metabolites, considered as reference for sheep, is shown in Table 2.

On the other hand, there was interaction between lamb age and propylene glycol levels for albumin, which reduced linearly with the addition of PG (Table 2), which may be related to the reduction in milk production by the sheep, which consumed more PG and had intoxication, causing lower food consumption and lower milk production. Similarly, a study conducted by Cruz et al. (2014) also observed signs of mild intoxication in sheep that received 4.5 and 6% of PG in water, with a reduction in dry matter intake of approximately 14% when compared to the dry matter intake established by the National Research Council (NRC, 2007), for these animals. Therefore, it is probable that the lower consumption of milk by the lambs may have resulted in lower protein consumption by these animals, which culminated in a reduction of serum albumin concentration in the lambs.

As the lambs aged, their serum levels of total protein decreased linearly, probably due to increased protein demand for muscle tissue deposition, which, during the lamb's initial life phase, is on the rise (Resende, Silva, Lima, & Teixeira, 2008; Regadas Filho et al., 2011).

Globulin concentrations decreased as the lambs aged and there was an increase in concentrations near weaning age. In this case, at 60 days of age, the animals showed lower globulin concentration (3.85 mg dL^{-1}) (Table 2). Because immunoglobulins are related to body immunological conditions the highest globulin concentrations, at 9 days of age, are due to the intake of colostrum, which is rich in immunoglobulins. An increase in the concentration of this metabolite near 60 days of age occurred because the animal in this period already has its immune system formed.

There was no interaction between lamb age and propylene glycol levels in the sheep's food as to uric acid, creatinine and urea concentrations.

Table 2. Average concentration of total protein (TP), albumin (ALB), globulins and albumin:globulins ratio (A/G) as a function of propylene glycol addition levels (mg.dL^{-1}) and the lambs' age, and reference values for sheep (mg dL^{-1}).

Treatment	TP	ALB[A]	Globulins	A/G
0.0%	7.10	2.75	4.35	0.65
1.5%	6.83	2.75	4.07	0.69
3.0%	6.78	2.69	4.08	0.68
4.5%	6.53	2.68	3.95	0.67
Lambs' age (days)				
Days	TP	ALB[C]	Globulins[D]	A/G[E]
9	7.58	2.39	5.19	0.47
18	6.78	2.89	3.88	0.75
27	6.44	2.82	3.62	0.80
36	6.89	2.78	4.11	0.68
45	6.90	2.72	4.18	0.66
60	6.85	2.67	4.17	0.64
75	6.60	2.77	3.81	0.74
90	6.55	2.48	4.06	0.61
OM	6.82	2.63	4.13	0.67
CV (%)	6.97	9.01	12.12	17.34
Reference values★	6.0-7.9	2.4-3.0	3.5-5.7	-

[A]$\hat{Y}=2.78-0.037x$, $R^2=82.36\%$; [B]$\hat{Y}=7.14-0.007x$, $R^2=31.30\%$; [C]$\hat{Y}=2.438+0.016x-0.0002x^2$, $R^2=43.57\%$; [D]$\hat{Y}=4.93-0.036x+0.0003x^2$, $R^2=31.41\%$; [E]$\hat{Y}=0.53+0.008x-0.00008x^2$, $R^2=24.06\%$; OM = Overall Mean; CV = Coefficient of Variation; ★Reference values in mg dL^{-1} according to Kaneko, Harvery, and Bruss (2008).

Uric acid and creatinine concentrations in the lambs were not influenced by the inclusion of propylene glycol in the food provided to the sheep. Creatinine concentrations remained within the normal values for species, which indicates that the animals in this study did not have hepatic alteration. The concentration of the above-mentioned metabolite considered as reference for sheep is shown in Table 3.

On the other hand, there was lamb age effect on uric acid and creatinine concentrations ($p \geq 0.05$). Creatinine values increased linearly as the offspring aged (Table 3). Plasma creatinine comes from the catabolism of creatine, which originates from the metabolism of amino acids in the muscle tissue. Possibly, the greater musculature deposition in the organism promoted an increase in creatinine concentration throughout the animal's growth.

The highest concentrations of uric acid, at 27, 36 and 60 days, occurred due to increased ruminal activity, since the lambs decreased their consumption of liquid food (sheep milk) and began to eat more solid foods (forage and concentrated feed). After 30 days of age, the offspring was subjected to controlled feeding management because, before that, ingestion of solids was limited due to lack of access to their mothers. They were able to eat only the leftovers from the sheep's trough as they fell on the ground. The increase in uric acid concentration is related to the increase in microbial protein synthesis by ruminal microorganisms. In recent years, researches have confirmed the correlation between microbial protein production and urinary excretion of purine derivatives (Aguiar et al., 2015, Pessoa, Leão, Ferreira, Valadares Filho, Valadares, & Queiroz, 2009, Rennó et al., 2008).

The concentration of urea showed a quadratic response as a function of the inclusion of PG in the sheep's diet; the addition of 2.0% of PG resulted in higher concentration of blood urea (34.42 mg dL^{-1}) (Table 3). The concentration of urea in the plasma is directly related to the protein level of the feed, as well as to the energy/protein ratio of the diet. Studies conducted with lactating cows (Andersen, Larsen, Nielsen, & Ingvartsen, 2002; Nielsen & Ingvartsen, 2004) found that the use of PG increased the energy density of the diet, since this product is an additive with a high concentration of energy, around 23.7 MJ of gross energy kg^{-1} DM (Miyoshi, Pate, & Palmquist, 2001). It led to an increase in milk production and changes in milk fat and protein yielding (Chiofalo et al., 2005). In this sense, it is possible that the lambs born to sheep that received PG around 2% ingested more protein coming from the greater yielding of milk protein, which may have resulted in increased consumption of degradable protein and higher serum concentration of urea in the animals

There was no interaction between lamb age and inclusion of propylene glycol in the sheep's diet for blood concentration of calcium, phosphorus and magnesium. Calcium and phosphorus levels in the lambs were not influenced by the inclusion of propylene glycol in their mothers' diet ($p > 0.05$). However, the inclusion of PG in the sheep's diet affected magnesium levels, which presented a quadratic behavior. The addition of 2.5% of PG promoted the highest blood concentration of magnesium (2.94 mg dL^{-1}) (Table 4). There is no homeostatic control for magnesium; therefore, its blood concentration directly reflects the level of the diet. In fact, a study conducted by Moallem, Katz, Arieli, & Lehrer (2007) found that dairy cows supplemented with PG at a dosage of 500g day^{-1} animal^{-1} had an increase of 4.5% in milk production compared to animals that did not receive supplementation. Thus, it is possible that sheep supplemented with 2.5% PG had a greater milk production and this may have caused a higher milk consumption by the lambs and, consequently, higher consumption of magnesium from milk.

Table 3. Average concentrations of uric acid (UA), creatinine and urea metabolites (mg dL^{-1}) as a function of propylene glycol levels and the lamb age.

Treatments	UA*	Creatinine	Urea
0.0%	0.165	0.85	31.19
1.5%	0.149	0.77	32.26
3.0%	0.159	0.86	35.16
4.5%	0.160	0.83	27.56
Age (Days)	UA	Creatinine^C	Urea^D
9	0.083	0.69	34.33
18	0.169	0.72	30.39
27	0.245	0.64	30.00
36	0.184	1.06	29.68
45	0.101	0.79	32.92
60	0.193	1.04	20.08
75	0.180	0.66	38.17
90	0.121	1.04	33.87
OM	0.159	0.83	31.18
CV (%)	62.86	26.30	26.37
Reference values★	-	1.2-1.9	17.12 – 42.80

*Log(+1); $^A\hat{Y}=30.58+3.80x-0.97x^2$, $R^2=74.28\%$; $^B\hat{Y}=0.092+0.004x-0.00004x^2$, $R^2=22.22\%$; $^C\hat{Y}=0.70+0.00378x$, $R^2=20.60\%$; $^D\hat{Y}=36.88-0.36x+0.004x^2$, $R^2=22.96\%$.OM=Overall Mean; CV = Coefficient of Variation; Reference values according to Kaneko et al. (2008) expressed as mg dL^{-1}.

Table 4. Average concentration of mineral metabolites (mg dL^{-1}) as a function of propylene glycol addition levels and lamb age.

Treatments	Calcium	Phosphorus	Magnesium
0.0%	10.62	12.80	2.25
1.5%	10.49	12.56	2.31
3.0%	10.49	11.89	2.61
4.5%	10.48	10.99	2.26
Age (Days)	Calcium	Phosphorus^C	Magnesium
9	10.28	13.84	2.12
18	11.03	11.27	2.75
27	10.32	11.25	2.89
36	11.25	14.18	2.26
45	10.70	11.68	2.15
60	11.01	13.18	2.18
75	10.04	10.82	2.10
90	9.58	10.27	2.24
OM	10.51	12.06	2.34
CV (%)	8.59	23.58	20.09
Reference values★	11.5-12.8	5.0-7.3	2.0-3.0

$^A\hat{Y}=2.21+0,=.23x-0.04x^2$, $R^2=55.43\%$; $^B\hat{Y}=10.99-0.01x$, $R^2=26.18\%$; $^C\hat{Y}=13.26-0.03x$, $R^2=26.06\%$; $^D\hat{Y}=2.55-0.005x$, $R^2=19.05\%$; OM = Overall Mean; CV = Coefficient of Variation; ★Reference values according to Kaneko et al. (2008).

Conclusion

Inclusion of propylene glycol in the sheep's diet and the lambs' age affected the concentration of protein and mineral metabolites in the offspring. However, the use of propylene glycol in the lactating sheep's food does not have negative effects on the lamb's blood parameters, as evidenced by lack of nutritional deficit (protein and minerals) in the suckling lambs.

Acknowledgements

To CNPq (National Council for Scientific and Technological Development) for assistance in the research project.

References

Andersen, J. B., Larsen, T., Nielsen, M. O., & Ingvartsen, K. L. (2002). Effect of energy density in the diet and milking frequency on hepatic long chain fatty acid oxidation in early lactating dairy cows. *Journal of Veterinary Medicine*, *49*(4), 177-183.

Aguiar, M. S. M. A., Silva, F. F., Donato, S. L. R., Schio, A. R., Souza, D. D., Meneses, M. A., & Lédo, A. A. (2015). *Semina: Ciências Agrárias*, *36*(2), 999-1012.

Caldeira, R. M., Belo, A. T, Santos, C. C, Vazques, M. I., & Portugal, A. V. (2007). The effect of body condition score on blood metabolites and hormonal profiles in ewes. *Small Ruminant Research*, *68*(3), 233-241.

Chiofalo, V., Todaro, M., Liotta, L., Margiotta, S., Manzo, T., & Leto, G. (2005). Effect of propylene glycol on pre- and postpartum performance by dairy ewes. *Small Ruminant Research*, *58*(2), 107-114.

Chun, H. Z., Beltramini, J. N., Fan, Y. X., & Lu, G. Q. (2007). Chemoselective catalytic conversion of glycerol as a biorenewable source to valuable commodity chemicals. *Chemical Society Reviews*, *37*(1), 527-549.

Cruz, W. F. G., Macedo Junior, G. L., Andrade, M. E. B., Shultz, E. B., Rodrigues, V. J. C., & Silva, S. P. (2014). Consumo, digestibilidade e parâmetros fisiológicos de ovelhas suplementadas com níveis crescentes de propilenoglicol na água. *Veterinária Notícias*, *20*(1), 19-27.

Kaneko, J. J., Harvery, J. W., & Bruss, M. L. (2008). *Clinical biochemistry of domestic animals* (6th ed.). San Diego, CA: Academic Press.

Lien, T. F., Chang, L. B., Horng, Y. M., & WU, C. P. (2010). Effects of propylene glycol on milk production, serum metabolites and reproductive performance during the trasnsition period of dairy cows. *Asian – Australasian Journal of Animal Sciences*, *23*(3), 372-378.

Miyoshi, S., Pate, J. L., & Palmquist, D. L. (2001). Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. *Animal Reproduction Science*, *68*, 29-43.

Moallem, U., Katz, M., Arieli, A., & Lehrer, H. (2007). Effects of peripartum propylene glycol or fats differing in fattys acid profiles on feed intake production, and plasma metabolites in dairy cows. *Journal Dairy Science*, *90*(8), 3846-3856.

National Research Council [NRC]. (2007). *Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids*. Washington, D.C.: Hardcover.

Nielsen, N. I., & Ingvartsen, K. L. (2004). Propylene glycol for dairy cows. A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis *Animal Feed Science and Technology*, *115*(3-4), 191-213.

Peixoto, L. A. O., & Osório, M. T. M. (2007). Perfil metabólico proteico e energético na avaliação do desempenho reprodutivo em ruminantes. *Revista Brasileira de Agrociência, 13*(3), 299-304,

Pessoa, R. A. S., Leão, M. I., Ferreira, M. A., Valadares Filho, S. S., Valadares, R. F. D., & Queiroz, A. C. (2009). Balanço de compostos nitrogenados e produção de proteína microbiana em novilhas leiteiras alimentadas com palma forrageira, bagaço de cana-de-açúcar e ureia associados a diferentes suplementos. *Revista Brasileira de Zootecnia*, *38*(5), 941-947.

Regadas filho, J. G. L., Pereira, E. S., Villarroel, A. B. S., Pimentel, P. G., Fontenele, R. M., Costa, M. R. G. F., Maia, I. S. G., ... Sombra, W. A. (2011). Efficiency of metabolizable energy utilization for maintenance and gain and evaluation of small ruminant nutrition system model in Santa Ines sheep. *Revista Brasileira de Zootecnia*, *40*(11), 2558-2564.

Rennó, L. N., Valadares Filho, S. C., Valadares, R. F. D., Paulino, M. F., Rennó, F. P., & Silva, P. A. (2008). Níveis de ureia na ração de novilhos de quatro grupos genéticos: estimativa da produção de proteína microbiana por meio dos derivados de purinas na urina utilizando duas metodologias de coleta. *Revista Brasileira de Zootecnia*, *37*(3), 546-555.

Resende, K. T., Silva, H. G. O., Lima, L. D., & Teixeira, I. A. M. A. (2008). Avaliação das exigências nutricionais de pequenos ruminantes pelos sistemas de alimentação recentemente publicados. *Revista Brasileira de Zootecnia, 37*(spe), 161-177.

Rukkwamsuk, T., & Panneum, S. (2010). Effect of oral administration of propylene glycol during periparturient period on blood biochemical parameters and liver triacylglicerol accumulation in postparturient dairy cows. *African Journal of Agricultural Research*, *5*(23), 3239-3245.

Sistema para Análises Estatísticas [SAEG]. (2007). *Versão 9.1.* Viçosa, MG: UFV.

Santos, R. P., Sousa, L. F., Sousa, J. T. L., Andrade, M. E. B., Macedo Júnior, G. L., & Silva, S. P. (2015). Parâmetros sanguíneos de cordeiros em crescimento filhos de ovelhas suplementadas com níveis crescentes de propilenoglicol. *Agrária - Revista Brasileira de Ciências Agrárias*, *10*(3), 473-478.

Schweigel, M., Voigt, J., & Mohr, E. (2009). Indication of intracellular magnesium deficiency in lactating dairy cows revealed by magnesium loading and renal fractional excretion. *Journal of Animal Physiology and Animal Nutrition, 93*(1), 105-112.

Received on January 30, 2017.
Accepted on April 4, 2017