# EFFECT OF GLYCERIN ON PHYSICAL AND CHEMICAL PARAMETERS OF RUMEN FLUID AND URINE OF BEEF CATTLE

## EFEITO DA GLICERINA SOBRE PARÂMETROS FÍSICOS E QUÍMICOS DO LÍQUIDO RUMINAL E DA URINA DE BOVINOS DE CORTE

**D. A. V. SILVA[1*], J. M. B. EZEQUIEL[2], E. H. C. B. VAN CLEEF[3]**

**SUMMARY**

The aim of this study was to evaluate physical and chemical parameters of urine and rumen fluid of cattle fed diet with glycerin added. Rumen fluid and urine samples were collected from five castrated Nellore bulls, cannulated in the rumen, aged approximately 24 months and weighing 400 kg. The animals were kept in individual stalls and fed five experimental diets containing 0, 7.5, 15, 22.5 and 30% of glycerin, based on dry matter and 30:70 forage:concentrate ratio. Corn silage was used as forage, and the concentrate was made of corn grain, soybean hulls, sunflower meal, glycerin and mineral supplement. The experiment lasted 105 days, divided into five sub-periods of 21 days each. The rumen fluid and urine samples were taken before and two hours after the meal on the 20th day of each experimental subperiod. The experimental design was the 5 × 5 Latin square with split plots. None of the parameters studied in rumen fluid was influenced by either the diet (P>0.05) or the times of harvest (P>0.05), except ruminal pH (P<0.0001). The analysis of urine, showed no significant effect of treatment on their physical characteristics, but there was a linear effect of treatments on pH (P<0.0001) and protein (P = 0.004) and increasing linear effect of treatments on the density (P = 0.001). The glycerin, regardless of the concentration used, increased density (P = 0.009) and decreased pH (P<0.0001). The timing of the urine sampling did not influence any of the parameters studied. Diets for beef cattle with 30% glycerin promote changes in the chemical parameters of urine, indicating changes in renal metabolism.

**KEY-WORDS:** Biodiesel. Metabolism. Ruminants.

**RESUMO**

Objetivou-se com este estudo avaliar parâmetros físicos e químicos da urina e do líquido ruminal de bovinos alimentados com glicerina. Cinco bovinos Nelore, machos, castrados, canulados no rúmen, com aproximadamente 24 meses e 400 kg de peso corporal foram utilizados para colheitas de líquido ruminal e urina. Os animais foram mantidos em baias individuais recebendo cinco dietas experimentais contendo 0; 7,5; 15; 22,5 e 30% de glicerina, com base na matéria seca, formuladas na proporção volumoso:concentrado de 30:70. A silagem de milho foi usada como volumoso e o concentrado foi composto por milho em grão, casca de soja, farelo de girassol, glicerina e suplemento mineral. O período experimental foi de 105 dias, dividido em cinco subperíodos de 21 dias cada. As colheitas de líquido ruminal e urina foram realizadas antes e duas horas após a alimentação dos animais no dia 20 de cada subperíodo experimental. O delineamento utilizado foi o quadrado latino 5×5 com parcela subdividida no tempo. Nenhum dos parâmetros estudados no líquido ruminal foi influenciado pelas dietas (P>0,05), nem pelos momentos de colheita (P>0,05), exceto o pH (P<0,0001). Quanto às análises da urina, não houve efeito significativo dos tratamentos sobre suas características físicas, porém houve efeito linear decrescente dos tratamentos sobre o pH (P<0,0001) e proteína (P = 0,004) e efeito linear crescente dos tratamentos sobre a densidade (P = 0,001). A glicerina, independentemente da concentração utilizada, proporcionou aumento da densidade (P = 0,009) e diminuição do pH (P<0,0001). O momento de colheita da urina não influenciou nenhum dos parâmetros estudados. Dietas para bovinos de corte com até 30% de glicerina, promovem alterações nos parâmetros químicos da urina, indicando modificações no metabolismo renal.

**PALAVRAS-CHAVE:** Biodiesel. Metabolismo. Ruminantes.

[1] Veterinary Medicine under graduate student of Unesp - Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal. Amador Joly Street, 87, Chácara São João, Mogi Guaçu-SP. davidattuy@hotmail.com.
[2] Professor of Animal Science Department of Unesp - Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal.
[3] Post-doctoral student of Animal Science Department of Kansas State University - Manhattan Campus, EUA.

Submitted: 17/10/2011 Accepted: 16/05/2012

## INTRODUCTION

The surplus of glycerin resulting from biodiesel production should be the focus of detailed analysis, since there are few studies about its use and economic viability. An alternative is to use glycerin in animal feeding (DONKIN, 2007, PARSONS et al., 2009, MACH et al., 2009); however, the residues of methanol and other contaminants may pose a problem, generating metabolic changes that can harm animals and producers.

The evaluation of alternative ingredients to feed ruminants should include studies about rumen fermentation pattern that is associated with the potential to promote animal performance. Rumen fluid analysis is valuable to diagnose diseases of the digestive tract of ruminants, because the rumen microbiota is highly sensitive to internal and external changes to which animals are routinely subjected (BORGES et al., 2002).

Urine is one of the main fluids to excrete substances that are harmful to the body. The greater or lesser rate of filtration, absorption and excretion of certain nutrients by the kidneys and catabolites allow urine to be used as a diagnosis tool of certain metabolic disorders (ORTOLANI, 2003).

The initial changes caused by most metabolic disorders can be detected in the ruminal fluid and urine first, since the changes of their reference values are significantly more evident than in the blood. During the subclinical disease, the deviations of standard values in the blood are very small due to homeostatic mechanisms. Therefore, the diagnosis by simple laboratory tests in the rumen fluid and urine that may be performed in the field is very important (BOUDA et al., 2000).

There are few studies in the literature about the use of crude glycerin in diets for beef cattle and it is believed that this byproduct of the biodiesel production can be used as alternative energy source in diets of beef cattle to replace conventional foods such as corn. Therefore, considering the importance of the cattle industry and biodiesel production in Brazil, the objective of this study was to investigate ruminal and renal changes in cattle that were fed up to 30% of crude glycerin in the diet.

## MATERIAL AND METHODS

The experiment was conducted at the Unidade Animal de Estudos Digestivos e Metabólicos in the Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Julio de Mesquita Filho", Jaboticabal (Unesp/ FCAV).

The following diets were tested: G0 – control diet, without glycerin; G7.5 – 7.5% of glycerin in the diet dry matter; G15 – 15% of glycerin in the diet dry matter; G22.5 – 22.5% glycerin in the diet dry matter; and G30 – 30% of glycerin in the diet dry matter. The feed was supplied twice daily, at 8 a.m. and 5 p.m. The concentrate and forage were supplied freely and weighed separately in equal amounts every time. The experimental diets were prepared to meet the minimum nutrient requirements, according to NRC (1996). The five isonitrogenous (12.2% CP DM) and isocaloric (2.5 Mcal EM/ kg DM) diets contained 30:70 forage:concentrate ratio. The diet was prepared with corn silage forage plus the concentrate made of corn hulls, soybean husks, sunflower meal and mineral supplement, and glycerin was added, except in the control diet.

Five castrated Nelore bulls aged approximately 24 months and weighing 400 kg, fitted with permanent ruminal cannulas, were used to evaluate the physical characteristics (color, smell and viscosity) of urine, and some chemical parameters (pH, density, presence of ketone bodies, blood, proteins, urobilinogen, bilirubin and leukocytes) of urine using dry chemistry Combur Test® strips, and the physical (color, smell, viscosity and floating time) and chemical (pH and reductive bacterial activity) characteristics of the ruminal fluid.

Urine physical characteristics were scored for: viscosity, 1 (aqueous) and 2 (mucous); color, from 1 to 5 (aqueous colorless, yellow, golden yellow, red brownish and dark red, respectively); and smell, from 1 to 3 ( aromatic, sweet and ammoniacal, respectively). The chemical parameters, urobilinogen, nitrite and leukocytes were attribute the following scores, 1 (present) or 2 (absent), while protein, blood, presence of ketone bodies and bilirubin, were scored 1, 2 and 3, according to increasing concentrations (BOUDA et al., 2000).

The physical characteristics of the ruminal fluid were also scored. Score 1 was given for the olive green fluid with aromatic non-repulsive smell and aqueous consistency; score 2 for greyish fluid with acrid smell and slightly viscous consistency; and score 3 for dark green fluid with putrid smell. The pH was determined using a digital Digimed pHmeter, model DM20 and bacterial reductive activity was evidenced by methylene blue redox proof (BOUDA et al., 2000).

The experiment lasted 105 days, divided into five sub-periods of 21 days each. Urine and ruminal fluid samples were collected two hours before and after animal first feeding, at the end of each sub-period. The urine was collected by the spontaneous urination method.

The experimental design was 5 x 5 Latin square with split plot in time. The analysis of contrasts for linear, quadratic, cubic and control treatment compare to glycerin added treatments effects was performed using the software MIXED of Statistical Analysis System (SAS) 9.1. The significance level was set at 5%.

## RESULTS AND DISCUSSION

The parameters of the ruminal fluid were affected by neither the diets ($P>0.05$) nor the sampling times ($P>005$) or the interaction between diets and sampling times ($P>0.05$), except for the ruminal pH ($P<0.0001$)

that displayed higher mean value when measured before feeding (6.64) compared to the value two hours after the first feeding (5.88). This result was expected due to ruminal fermentation and consequent acidification of the medium, the mean pH between treatments 6.26, with respective mean standard deviation error (SDE) 0.3273 and coefficient of variation (CV) 5.21% are presented in Figure 1.

Vargas et al (2001), while working with cattle fed different levels of concentrate in the diet (0, 25, 50 and 75%), also reported significant decreasing linear effect over time (0, 3 and 6 hours after feeding), pH values varied from 6.8 to 6.4. The authors did not report significant effect of different concentrate levels, thus evidencing the action of compensatory mechanisms to the production and absorption of fatty acids, such as the presence of buffers in the rumen from the saliva and the release of buffers or bases from the food.

The ruminal fluid on macroscopic examination was colored olive green, unlike the milky color reported by Zilio et al. (2008) for high concentrate diets, but the animals were not fed only concentrate in the diet. Therefore, the corn forage, despite having grains in its composition may have affected favorably the color of the rumen fluid and maintained its olive green color.

Barbosa et al (2003) evaluated six animals, 3 cattle and 3 buffaloes, and reported that ruminal fluid color changed from olive green to straw yellow, results consistent with the elephant grass diet they were fed. Thus, animals fed diets containing high levels of grain together with forage may have the ruminal fluid color close to olive green, as it was observed in this study.

Ruminal fluid has a slightly sticky viscosity and an aromatic odor, which although strong is not repulsive (BOUDA et al., 2000), similar to what was found in this study. Changes in color and/or viscosity of rumen fluid suggest ruminal conditions, such as foamy bloat (VAN CLEEF, 2009), simple indigestion or ruminal alkalosis (QUIROZ-ROCHA & BOUDA, 2000).

The floating time, which measures the fermentation capability through gas production by the rumen bacteria, was on average 14 minutes with SDE 5.2561 and CV 37.02%. This result was above the standard time ranging from 4 to 8 min suggested by Bouda et al. (2000) for cattle. In most cases, animals that are fed diets with higher forage:concentrate ratio display ruminal floating time of approximately 4 minutes (BARBOSA et al., 2003, TABELEÃO et al., 2007). Therefore, animals that are fed lower ratio, display longer floatation time as it evidenced in this study, since the forage:concentrate ratio was 30:70.

This longer time suggests ruminal acidosis; however, this single parameter is not enough to diagnose this disease and can also be explained by the high level of concentrate in the supplied diet, which contributed to the rumen fermentation pattern displayed. Furthermore, it is not possible to conclude that the ruminal pattern displayed was due to the addition of glycerin in the diet, since the longest floatation times were observed in the control and the treatment with 15% of glycerin added in the diet.

Urine physical characteristics were not significantly affected by the treatments (Figure 2). There was, however, a decreasing linear effect of treatments on pH ($P<0.0001$) and protein ($P = 0.004$) and an increasing linear effect of treatments on the density ($P = 0.001$). The mean scores and concentrations observed were 8.06 for pH with SDE = 0.5117 and CV = 6.34%, 2.53 for protein with SDE = 0.5026 and CV = 19.86% and 1012 for density with SDE = 3.5494 and CV = 29.98%. Glycerin, independent of the concentration, increased urine density ($P = 0.009$) and decreased pH ($P<0.0001$). Urine parameters did not change over time (Figure 3). There was no significant interaction ($P>0.05$) between treatments and sampling time.

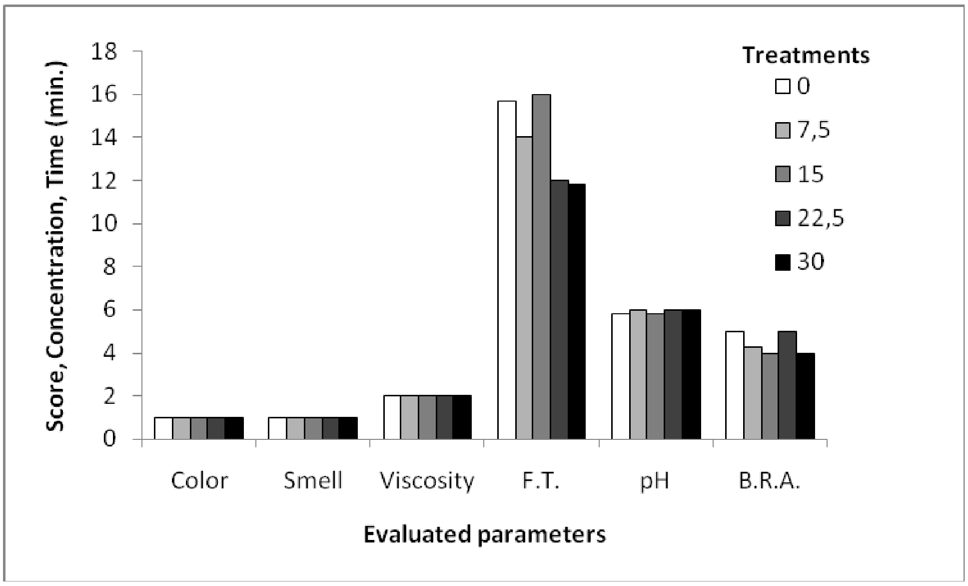


**Figure 1 -** Physico-chemical parameters of the ruminal fluid of cattle fed diets containing different levels of crude glycerin .
T.F. = floating time; A.R.B.= bacterial reductive activity.

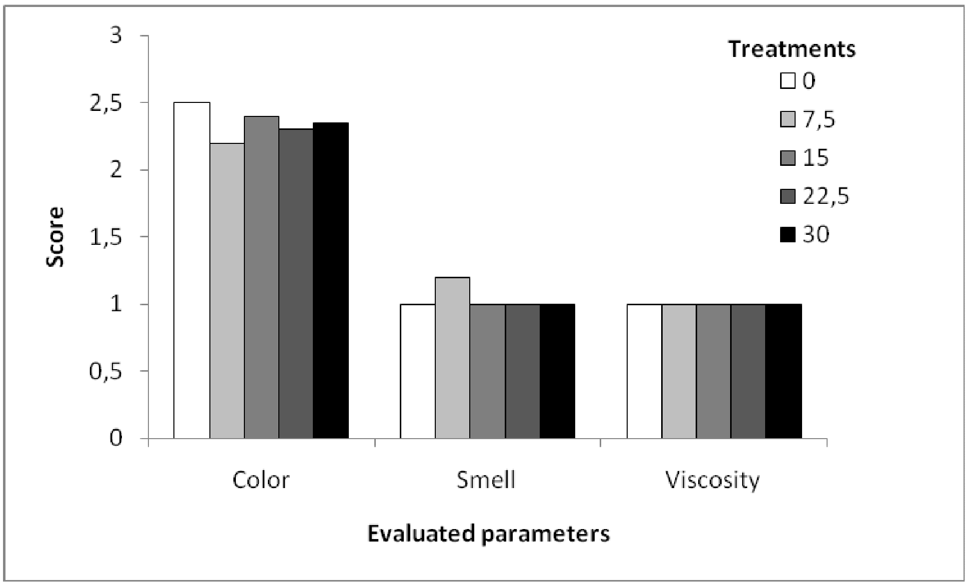


**Figure 2 -** Physical parameters of urine of cattle fed diets containing different levels of crude glycerin.

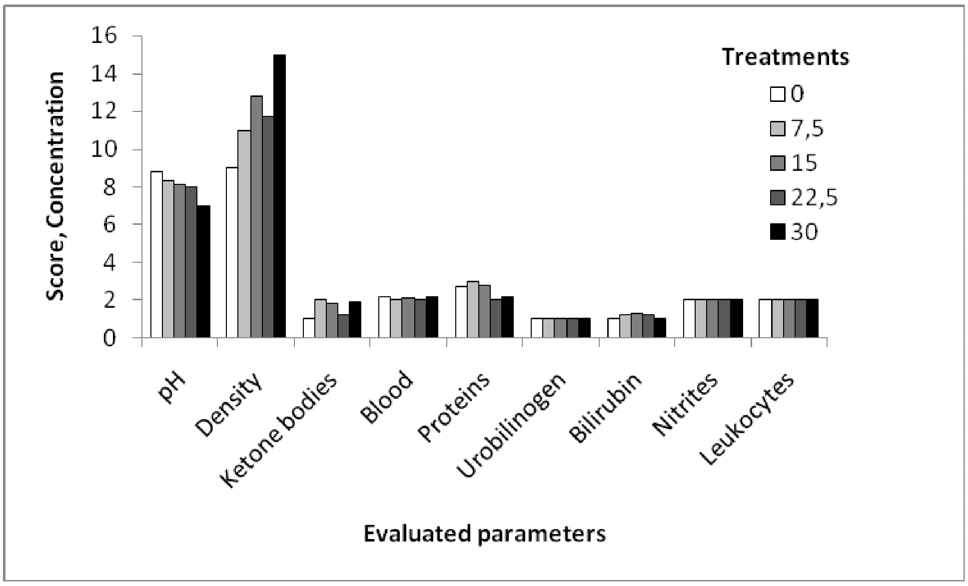


**Figure 3 -** Chemical parameters of urine of cattle fed diets containing different levels of crude glycerin.

The colorless, watery urine indicates increased excretion (polyuria) or water intake, ketonemia or severe renal impairment (BOUDA et al., 2000). Golden yellow urine indicates decreased urine as in febrile or major general disorders, while brownish to dark red shows the presence of blood or hemoglobin (BOUDA et al., 2000). The sweet odor is frequent in ketonemia, while the ammoniacal smell may indicate bacterial infection (BOUDA et al., 2000). Urine viscosity may increase to mucous consistency due to the presence of pus or mucous resulting from pyelonefritis processes (BOUDA et al., 2000). These changes in urine color, smell and viscosity were not observed in this study.

Despite the variation of urine pH, the values remained within the standard range suggested by Kaneko et al. (2008), from 7.4 to 8.4. The cases where pH values were below this range may suggest acidosis and when above, it suggests alkalosis, pyelonefritis and cystitis. Typically, cattle raised under extensive management have very alkaline urine pH, since the grasses contain more cations than anions, whereas animals fed diets rich in soluble carbohydrates have a more acid urine pH, mainly due to increased ruminal formation of short chain fatty acids (ORTOLANI, 2003).

Del Claro et al (2002) worked with basal and anionic diets for cattle and found that anionic diets lowered urine pH (5.56), while basal diets maintained a mean pH of 8.17, thus the authors conclude that the kidneys are important to maintain the acid-base balance, because when the blood pH tends to become acidic, as in the case of metabolic acidosis induced by diets with high concentrate, the kidneys respond by excreting ammonium ions ($NH_4^+$), lowering the urine pH, which corroborates the present study.

Due to the significant decrease of pH values, it is possible to suggest that higher glycerin concentrations in the diets associated with high levels of concentrate in the diet and longer time of contact with such ingredients could significantly harm animal health by lowering further the pH, which could cause health hazards and consequent decrease of animal performance.

Proteins should not be present in the urine, but sometimes they may appear at very low concentrations. When present at high levels, they can be associated with an inflammatory process or nephrosis (BOUDA et al., 2000).

Saglam et al. (2003), Rossetti et al. (2004) and Haanwinckel et al. (2004) mentioned that changes such as degeneration and necrosis of tubular epithelial cells occur in the proximal and distal tubules in infectious diseases that manifest by abnormal urinalysis, often showing proteinuria and hyaline casts in the tubular lumen. In the present study, there was no evidence of large quantity of protein in the urine, therefore, we ruled out possible changes of inflammatory, degenerative or infectious origin.

Urine specific gravity test is used clinically because the low ability to concentrate urine is an early sign of renal tubular disease (BOUDA et al., 2000). The treatment with 30% glycerin resulted in the highest urine specific gravity. Therefore, it is possible to conclude that the addition of glycerin in the diet did not favor the appearance of tubular diseases, since the control treatment had the lowest urine density.

## CONCLUSION

The addition of up to 30% of crude glycerin in the diet, did not affect the ruminal fluid of the studied animal; but, it does change somewhat the chemical parameters of the urine. However, these changes are not enough to cause significant harm to animal health and consequent impairment of animal performance.

## REFERENCES

BARBOSA, J. D.; ÁVILA, S. C.; DIAS, R. V. C.; PFEIFER, I. B.; OLIVEIRA, C. M. C. **Estudo comparativo de algumas provas funcionais do fluido ruminal e de metabólitos sangüíneos de bovinos e bubalinos.** Pesquisa Veterinária Brasileira**.** v.23, n.1, 2003.

BORGES, N. C.; SILVA, L. A. F.; FIORAVANTI, M. C. S.; CUNHA, P. H. J.; MORAES, R. R.; GUIMARÃES, P. R.; MARTINS, M. E. P. Avaliação do suco ruminal de bovinos "a fresco" e após 12 horas de conservação. **Ciência Animal Brasileira**. v.3, n.2, p.57-63, 2002.

BOUDA, J.; QUIROZ-ROCHA, G.; GONZÁLEZ, F. H. D. Importância da coleta e análise do líquido ruminal e urina. In: GONZÁLEZ, F. H. D.; BORGES, J. B.; CECIM, M. (Eds.). **Uso de provas de campo e de laboratório clínico em doenças metabólicas e ruminais dos bovinos.** Porto Alegre, Brasil, Gráfica da Universidade Federal do Rio Grande do Sul, p.13-17, 2000.

DEL CLARO, G. R.; ZANETTI, M. A.; SALLES, M. S. V. **Influência da dieta aniônica no balanço macromineral em novilhos holandeses.** Arquivo Brasileiro de Medicina Veterinária e Zootecnia**.** v.54, n.3, 2002.

DONKIN, S. S. Performance of dairy cows fed glycerol as a primary feed ingredient. **Journal of Animal Science**. v.85, n.1, p.350, 2007.

HAANWINCKEL, M. C. S.; MEGID, J.; SOUZA, L. C. Avaliação da prova de imunoperoxidase como recurso diagnóstico na leptospirose animal. **Arquivos do Instituto Biológico.** v.71, p.293-01, 2004.

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. C. **Clinical biochemistry of domestic animals.** 6.ed. San Diego: Academic Press, 2008. 928p.

MACH, N.; BACH, A.; DEVANT, M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. **Journal of Animal Science**, v.87, p.632-638, 2009.

NRC. **Nutrient Requirements of Beef Cattle**. Washington, DC: National Academic Press, 7.ed., 1996.

ORTOLANI, E. L. Diagnóstico de doenças nutricionais e metabólicas por meio de exame de urina em ruminantes. In: I SIMPÓSIO DE PATOLOGIA CLÍNICA VETERINÁRIA DA REGIÃO SUL DO BRASIL, 2003, Porto Alegre. **Anais...** Porto Alegre: UFRGS, 2003, p.91-102.

PARSONS, G. L.; SHELOR, M. K.; DROUILLARD, J. S. Performance and carcass traits of finishing heifers fed crude glycerin. **Journal of Animal Science***,* v.87, p.653-657, 2009.

QUIROZ-ROCHA, G.F.; BOUDA J. Diagnóstico de indigestão simples, alcalose ruminal e intoxicação por uréia. In: GONZÁLEZ, F.H.D.; BORGES, J.B.; CECIM, M. (Eds.). **Uso de provas de campo e de laboratório clínico em doenças metabólicas e ruminais dos bovinos.** Porto Alegre: Gráfica da Universidade Federal do Rio Grande do Sul, 2000, p.23-27.

ROSETTI, C. A.; VANASCO, B. N.; PINI, N.; CARFAGNINI, J. C. Comparison of three diagnostic techniques for the detection of leptospires in the kidneys of wild house mice (*Mus musculus*). **Pesquisa Veterinária Brasileira**. v.24, p.6-10, 2004.

SAGLAM, Y. S.; TEMUR, A.; ASLAN, A. Detection of leptospiral antigens in kidney and liver of cattle. **Deutsche Tierarztliche Wochenschrift.** v.110, p.75-7, 2003.

TABELEÃO, V. C.; DEL PINO, F. A. B.; GOULART, M. A.; WEISER, M. A.; SCHWEGLER, E.; MOURA, S. V.; SILVA, V. M.; ROOS, T. B.; GIL-TURNES, C.; GONZÁLEZ, F. H. D.; CORRÊA, M. N. Caracterização dos parâmetros ruminais e metabólicos de cordeiros mantidos em pastagem nativa. **Ciência Animal Brasileira**. v.8, n.4, p.639-646, 2007.

VAN CLEEF, E. H.; PATIÑO, R. P.; NEIVA JR, A. P.; SERAFIM, R.S.; REGO, A. C.; GONÇALVES, J. S. Distúrbios metabólicos por manejo alimentar inadequado em ruminantes: Novos conceitos. **Revista Colombiana de Ciencia Animal**. v.1, n.2, 2009.

VARGAS, L. H.; LANA, R. P.; MÂNCIO, A. B.; CAMPOS, J. M. S.; JHAM, G. M.; OLIVEIRA, M. V. M. Influência de Rumensin®, óleo de soja e níveis de concentrado sobre o consumo e os parâmetros fermentativos ruminais em bovinos. **Revista Brasileira de Zootecnia**. v.30, n.5, p.1650-1658, 2001.

ZILIO, B. S.; CRUZ, E. V., ANDRADE JÚNIOR, J. P.; MERLINI, G. P.; MARQUES, L. E.; DUQUE, P. V. T.; SACCO, S. R. Análise do líquido ruminal – Revisão de literatura. **Revista Científica Eletrônica de Medicina Veterinária**. v.6, n.11, 2008.