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HEMATOLOGICAL AND BIOCHEMICAL PROFILE OF SHEEP SUPPLEMENTED WITH SALINOMYCIN AND SUBMITTED TO EXPERIMENTAL LACTIC RUMINAL ACIDOSIS

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ABSTRACT

We distributed twelve sheep into two groups, control (CG) and salinomycin (SG), to study clinical, hematological and biochemical alterations in sheep supplemented with the ionophore, and to evaluate its effect in preventing experimental lactic ruminal acidosis, which was induced with sucrose. Variables were analyzed at intervals of 4h, 8h, 12h, 16h, 24h, 32h and 48h post-induction (PI). The enzymes AST, GGT, ALP and CK were determined, and seric total protein (TP), albumin, urea, creatinine, blood count, total plasmatic protein (TPP), fibrinogen (PF), glucose and L-lactate were quantified. Clinical manifestations of lactic acidosis and lower ruminal pH values were observed 8h PI, with ($P < 0.05$) in SG compared to the basal moment. Neutrophils showed higher scores ($P < 0.05$) in CG compared to SG 4h PI. The PF reached significant values ($P < 0.05$) in CG 48h PI compared to SG. Urea decreased ($P < 0.05$) in both groups 12h PI. Glucose increased ($P < 0.05$) when compared to CG at basal moment. There was a decrease ($P < 0.05$) at urinary pH 12h up to 48h PI, compared to 0h time in CG, while the SG only decreased ($P < 0.05$) at 12h and 16h PI. Salinomycin did not prevent acidosis; however, it favored the reestablishment of the animals that received it.

KEYWORDS: clinical biochemistry; fermentation disturbance; ionophores; small ruminants.

PERFIL HEMATOLÓGICO E BIOQUÍMICO DE OVINOS SUPLEMENTADOS COM SALINOMICINA SUBMETIDOS À ACIDOSE LÁCTICA RUMINAL

RESUMO

Utilizaram-se 12 ovinos distribuídos nos grupos controle (GC) e no salinomicina (GS) com objetivo de estudar alterações clínicas, hematológicas e bioquímicas nos ovinos suplementados com o ionóforo e avaliar seu efeito na prevenção da acidose ruminal experimental. Induziu-se acidose ruminal com sacarose e as variáveis foram analisadas 4h, 8h, 12h, 16h, 24h, 32h e 48h pós-indução (PI). Determinaram-se as enzimas AST, GGT, FA, CK, as proteínas totais séricas (PT), albumina, ureia, creatinina, hemograma, proteína plasmática total (PPT), fibrinogênio (FP), glicose e L-lactato. Manifestações clínicas de acidose láctica ruminal e os menores valores de pH foram observadas 8h PI, com ($P<0,05$) no GS comparado ao momento 0h. Os neutrófilos apresentaram maiores contagens ($P<0,05$) no GC 4h PI comparado ao GS. O FP alcançou maiores valores ($P<0,05$) no GC 48h PI comparado ao GS. A uréia diminuiu ($P<0,05$) em ambos os grupos 12h PI. A glicose aumentou ($P<0,05$) no GC comparado ao momento 0h. Houve queda ($P<0,05$) do pH urinário no momento 12h até 48h PI, em relação ao momento 0h no GC, enquanto no GS apenas os momentos 12h e 16h PI apresentaram diminuição ($P<0,05$). A salinomicina não preveniu a acidose; no entanto, favoreceu o restabelecimento dos animais tratados.

PALAVRAS-CHAVE: Bioquímica clínica; distúrbio fermentativo; ionóforos; pequenos ruminantes.

INTRODUCTION

The acute rumen lactic acidosis is a metabolic disease characterized by fermentation disorder that occurs after sudden and / or unadapt ingestion of easily digestible carbohydrates, triggering changes in the microbial flora which compromise rumen dynamics, and often reflect on systemic acidosis and multiple secondary processes that are potentially harmful to animal production (HUNGATE et al., 1952; DUNLOP, 1972; BRAUN et al., 1992; ORTOLANI, 1995; OWENS et al., 1998).

Clinical signs vary with the severity of disease. Appetite and rumen movements are reduced or absent; diarrhea, dehydration, abdominal distension, tachycardia and tachypnea may occur, and laminitis can also be observed, pointing out that in most acute cases, the animals remain recumbent and may die due to severe circulatory failure (HUBER, 1971; CAKALA et al., 1974; DOUGHERTY et al., 1975; MARUTA & ORTOLANI, 2002; MIRANDA NETO et al., 2005).

The observation of clinical signs and analysis of rumen fluid and urine, when combined with information about blood alterations, allow better evaluation of the clinical case and directs to appropriate therapy (PATRA et al., 1993; NIKOLOV, 2003; CÂMARA, 2008). Nevertheless, due to the financial damage it causes, the disease should be prevented (VIEIRA et al., 2006).

Some preventive measures of lactic acidosis in ruminants are employed, such as the gradual supply of carbohydrates in the feed, and the use of buffer and some antibiotics in the diet. Among the practices that have shown satisfactory results, there is the use of ionophores, such as lasalocid and monensin, generating good prospects for controlling this fermentation disorder, by inhibiting the growth of Gram-positive bacteria, *Streptococcus bovis and Lactobacillus* sp, the greatest producers of lactic acid in the rumen (BEEDE & FARLIN, 1977; KEZAR & CHURCH, 1979b; MUIR et al., 1980b; BERGEN & BATES, 1984; AFONSO et al., 2000).

Another compound of this category, salinomycin, is being investigated for the control of some digestive disorders in ruminants (NAGARAJA et al., 1985; USAGAWA, 1992); however, little information is available regarding their use in sheep as a preventive of ruminal lactic acidosis.

Therefore, this study aimed to describe the hematological and biochemical profile of sheep supplemented with salinomycin and submitted to rumen lactic acidosis.

MATERIAL AND METHODS

This work was performed in the experimental goat pen of the Bovine Clinic of the Universidade Federal Rural de Pernambuco with 12 clinically healthy Santa Ines crossbred adult sheep, both male and female, weighing on average 30 kg. A permanent rumen cannula was implanted in each animal, according to DEHGHANI & GHADRDANI (1995). The period of recovery from surgery and adaptation to feeding regime lasted four weeks prior to the induction of ruminal acidosis. During the preparation time and the acidosis induction phase, the animals were fed soybean meal (150g) twice a day at 8:00 a.m. and 4:00 p.m, besides elephant grass *(Pennisetum purpureum)* and tifton *(Cynodon dactylon)*, as well as mineral salt and water *ad libitum.*

The animals were divided into two groups of six animals each, the control group (CG) and the group that received Salinomycin (Salocin 120 - Intervet.) (GS), administered directly into the rumen via the fistula, at a daily dose of 30 mg / kg of diet per animal during 42 days and during the induction phase (MERCHEN e BERGER, 1985).

The physiological values (0h) for the studied variables were established during the two days prior to induction by clinical evaluation, which consisted of physical examination and laboratory blood count, serum chemistry and ruminal and urinary pH, as recommended by JAIN (1993) and RADOSTITS et al. (2007). On clinical examination of the animals, the following characteristics were observed: attitude, behavior, appetite, mucous membrane color, heart and respiratory rates, reticulo-ruminal motility (frequency and amplitude), rectal temperature and feces aspect.

After the initial acclimation period, the antibiotic administration was maintained and acidosis was induced, supplying as substrate 10g sucrose / kg of body weight through the ruminal fistula, at 8:00 a.m., before the morning feeding (DELAK & ADAMIC, 1959).

Clinical observations during the experiment and the sampling of rumen fluid, blood and urine were carried out every 4h, 8h, 12h, 16h, 24h, 32h and 48h post-induction (PI), in order to observe the appearance of probable clinical and laboratory alterations indicative of lactic acidosis, as recommended by KEZAR & CHURCH (1979a).

The rumen fluid was obtained by the tube coupled to the suction pump introduced through the cannula, and pH was determined in a digital pH meter (pH meter: Corning 30) (DIRKSEN, 1993). For hematological analysis, three blood samples were obtained in vacuum tubes by jugular vein puncture; one tube contained 10% EDTA for complete blood count; another tube, sodium fluoride for plasma glucose and lactate determination; and the third tube was siliconized to obtain serum for the assessment of serum total

protein (TP), albumin, urea, creatinine, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and creatine kinase (CK). Urine pH was analyzed as recommended by ORTOLANI (2002).

The values were statistically analyzed over eight experimental moments, comparing each time with the initial moment (0h) within the group and between control and salinomycin groups. The variables were submitted to analysis of variance, using F statistics, being significant when $p < 0.05$, making the contrast between the means by Turkey method and by calculating the minimum significant difference (msd) for alpha equal to 0.05. For the variables ruminal pH, eosinophils, basophils, plasma fibrinogen (PF), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT), we obtained the median as a measure of central tendency, employing the Friedman test for dependent samples and Mann Whitney method for the comparison between groups using the $\chi 2$ and calculating the msd for alpha equal to 0.05 (CURI, 1997).

RESULTS

Acidosis induction in the animals caused a mild clinical case of ruminal acidosis, characterized by moderate tachycardia, appetite loss, apathy and consequent anorexia. Most animals in both groups presented decreased motility of the rumen (frequency and amplitude) over the period of ruminal acidosis (8h to 16h PI), and soft feces excretion. Variation in hydration of some animals was observed, with more intense dehydration in CG compared to SG, with no clinical significance related to other changes. These findings were evident from 8h PI on, returning to normal from 32h PI on.

The ruminal pH decreased progressively from the initial values, reaching the lowest levels at 8h PI, showing 6.06 in CG and 6.07 in SG, with statistically significant differences ($P < 0.05$) in the group receiving the ionophore when compared to the initial moment. Even so, animals from SG presented more expressive recovery (Figure 1) compared to animals of CG, despite any significant statistical difference ($P > 0.05$).

In erythrogram, red blood cells, hematocrit and hemoglobin showed the highest values (Table 1) between 12h and 16h PI in CG, while showing an increase in later hours, between 16h and 32PI, in SG. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) varied little compared to the primary indices. There was no significant difference ($P > 0.05$) for the variables described or between groups over the observation moments within each group.

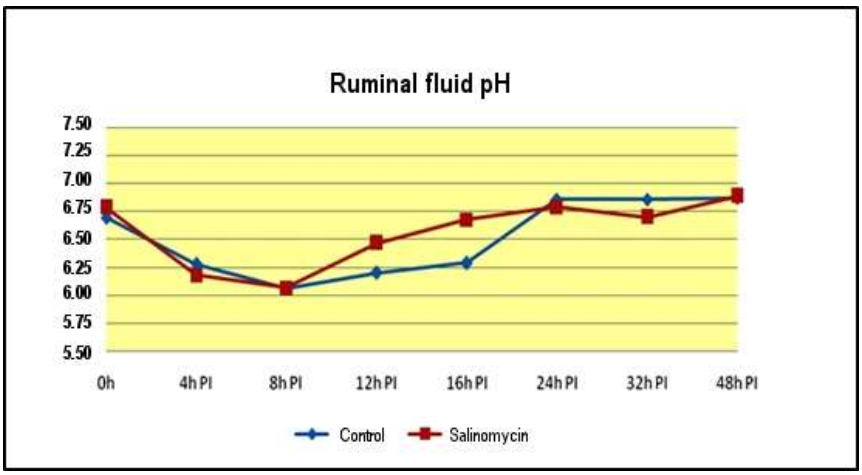

FIGURE1. Median values of ruminal pH of sheep in CG and SG, with experimental ruminal lactic acidosis in basal time (0h) and after induction of rumen acidosis with sucrose (10g/kg body weight)

TABLE 1. Hematological and biochemical profiles of sheep in control group (CG) and salinomycin group (SG) at basal time (0h) and after induction of rumen acidosis with sucrose (10g/kg body weight)

Variabel	Control Group (CG) Post-induction hours							
	0	4	8	12	16	24	32	48
RBC ($x10^6$/µL)	12.71± 1.49	13.00± 1.93	12.74± 1.92	13.88± 2.40	13.53± 1.99	12.99± 2.32	13.48± 1.93	12.60± 1.69
Hematocrit (%)	33.21± 3.09	33.14± 4.53	33.57± 4.20	36.14± 4.53	35.29± 4.31	34.14± 4.45	35.29± 4.03	34.57± 3.91
Hemoglobin (g/dL)	11.21± 1.11	11.37± 1.50	11.21± 1.46	11.94± 1.46	12.21± 1.42	11.35± 1.16	11.83± 1.53	11.64± 1.20
TPP (g/dL)	7.60± 0.31	7.34± 0.32	7.43± 0.37	7.73± 0.36	7.80± 0.35	7.66± 0.22	7.76± 0.37	7.66± 0.30
TP (g/dL)	8.30± 0.38	7.71± 0.52	7.84± 0.81	8.27± 0.64	8.16± 0.56	7.94± 0.57	7.99± 0.39	7.92± 0.69
Albumin (g/dL)	2.58± 0.18	2.53± 0.26	2.61± 0.24	2.66± 0.34	2.67± 0.27	2.59± 0.27	2.64± 0.26	2.62± 0.26
Creatinine (mg/dL)	0.67± 0.09	0.65± 0.11	0.63± 0.07	0.68± 0.12	0.68± 0.11	0.66± 0.12	0.74± 0.17	0.69± 0.16
AST (U/L)	96.90	96.93	99.53	102.16	104.78	107.39	104.74	94.29
GGT (U/L)	49.73	45.90	45.90	53.55	53.55	49.73	49.73	45.90
FA (U/L)	132.67± 51.38	129.91± 57.37	124.38± 49.18	123.00± 55.60	117.46± 50.13	127.14± 47.38	116.10± 41.63	109.18± 42.09
CK (U/L)	186.20± 46.52	161.90± 25.10	165.95± 32.30	178.10± 36.58	174.05± 28.41	165.95± 18.29	174.05± 28.41	186.20± 33.20
	Salinomycin Group (SG) Post-induction hours							
	0	4	8	12	16	24	32	48
Hematocrit (%)	12.28± 1.14	12.23± 2.86	12.32± 2.00	12.52± 2.02	12.52± 2.11	12.67± 2.48	13.59± 3.27	12.13± 3.10
Hemoglobin (g/dL)	30.43± 2.96	31.71± 4.50	32.14± 6.15	31.71± 4.54	33.29± 6.78	33.14± 9.03	33.86± 8.75	31.43± 8.08
TPP (g/dL)	10.48± 0.95	10.83± 1.28	10.50± 1.79	10.61± 1.22	10.67± 1.35	10.71± 2.21	11.06± 2.47	10.43± 2.12
TP (g/dL)	7.36± 0.44	7.44± 0.71	7.43± 0.62	7.53± 0.63	7.57± 0.81	7.56± 1.07	7.61± 0.77	7.33± 0.77
Albumin (g/dL)	7.74± 0.87	7.81± 0.91	7.66± 0.65	7.67± 0.85	7.88± 0.70	7.66± 0.47	7.67± 0.44	7.46± 0.73
Creatinine (mg/dL)	2.57± 0.30	2.55± 0.46	2.58± 0.29	2.67± 0.33	2.67± 0.32	2.62± 0.29	2.62± 0.34	2.48± 0.27
AST (U/L)	0.76± 0.14	0.77± 0.22	0.79± 0.27	0.80± 0.25	0.83± 0.25	0.81± 0.29	0.83± 0.30	0.76± 0.25
GGT (U/L)	85.12	83.81	78.57	94.29	91.67	91.67	91.67	81.19
FA (U/L)	49.73	53.55	53.55	57.38	53.55	57.38	53.55	53.55
CK (U/L)	149.25± 53.93	161.70± 78.26	157.52± 65.06	153.39± 63.32	157.54± 68.16	156.16± 71.01	158.94± 78.55	163.07± 72.78
Hematocrit (%)	176.07± 58.88	226.66± 135.36	202.37± 101.50	190.22± 83.30	190.22± 91.40	174.04± 77.44	174.03± 60.30	149.74± 62.23

The inflammatory response observed in the leukocyte count was moderate and presented similar pattern in both experimental groups from 8 a.m. PI on. In both CG and SG, the highest values were observed at the moments 12h and 16h PI, without significant differences (P> 0.05) within the groups or between them. For SG, the leukocyte count decreased gradually; however, the values remained slightly above the initial levels. Segmented neutrophils were the main defense cells responsible for this distribution pattern (Figure 2), with significant difference (P <0.05) at the time 4hPI in CG (4774.29/µL) compared to SG (3430/µL).

The values of total plasma protein (TPP) evolved gradually, reaching the highest levels at 16h PI (7.8g/dL ± 0.35) and 32h PI (7.61g/dL ± 0.77) for CG and SG, respectively. No statistical significance (P> 0.05) was observed when the initial levels and each time between the groups were compared. In CG, plasma fibrinogen (PF) had the largest value at 48h PI (400mg/dL), which was statistically significant (P <0.05) when compared to SG (200mg/dL). In animals of SG, PF rates showed no variation at most of the experimental moments. No statistically significant difference (P> 0.05) was verified for both groups in the

comparison between post-induction moments and time 0h (Figura 3).

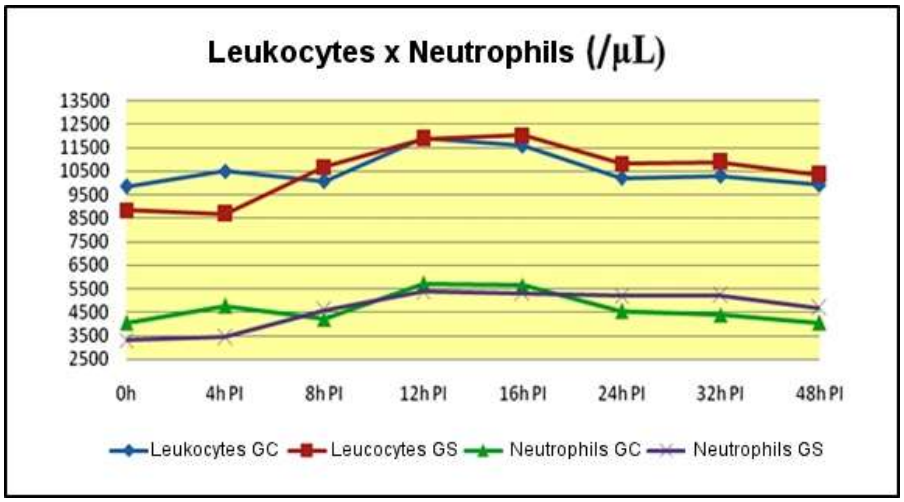

FIGURE 2. Mean values of leukocytes and segmented neutrophils (/µL) of sheep in CG and SG, with experimental ruminal lactic acidosis at the basal time (0h) and after induction with sucrose (10g/kg body weight).

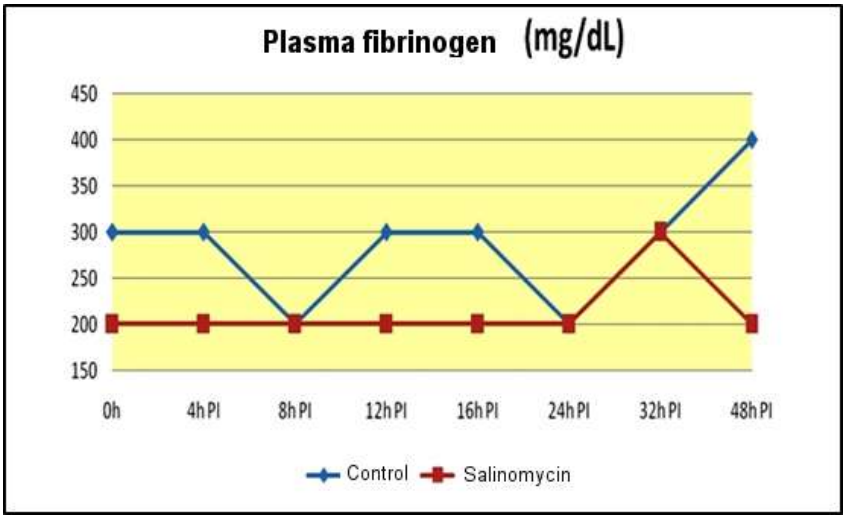

FIGURE 3. Median values of plasma fibrinogen concentration (mg/dL) of sheep in CG and SG, with experimental ruminal lactic acidosis at the basal time (0h) and after induction with sucrose (10g/kg body weight).

The total serum protein (TSP) did not show significant variations over the observation periods (P> 0.05), returning to slightly lower levels at the end of the experiment (Table 1). The fraction represented by albumin presented an increase at 16h PI for both groups, remaining slightly above the initial value for CG and slightly below for SG, but there was no significant expression of this behavior (P> 0.05).

After lactic acidosis induction, urea rates decreased significantly (P <0.05) compared to the initial moment (0h), and more significantly at 12h PI for both groups (Figure 4), reaching the values of 45.03mg/dL (± 12.68) and 54.38mg/dL (± 11.69) for CG and SG, respectively. No statistically significant difference (P> 0.05) was verified between the groups.

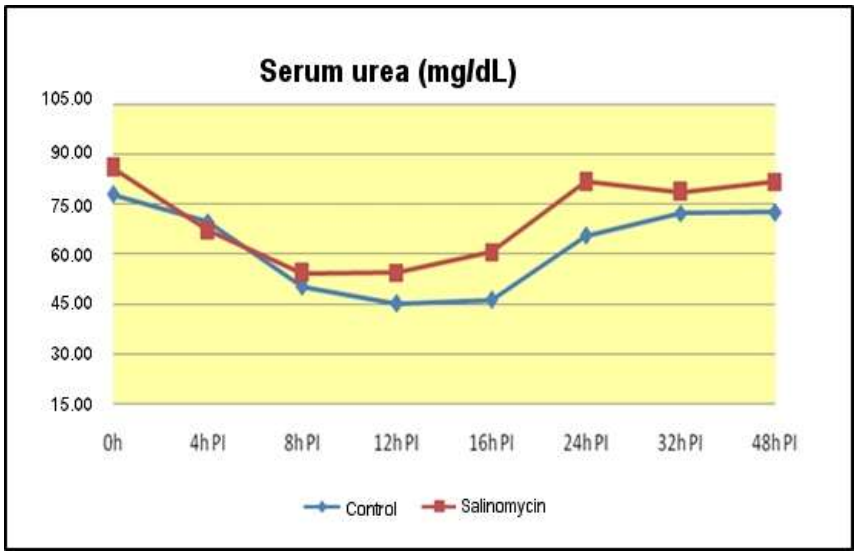

FIGURE 4. Mean values of serum urea (mg/dL) of sheep in CG and SG, with experimental ruminal lactic acidosis at basal time (0h) and after induction with sucrose (10g/kg body weight).

Creatinine showed homogeneous behavior (Table 1), with linear distribution in both groups, being the highest values observed at 32h PI, but there was no statistically significant difference (P> 0.05) between the experimental moments and basal time. There was no statistically significant difference (P> 0.05) between CG and SG.

Regarding the enzymes AST, GGT and ALP, no significant variation (P> 0.05) was observed throughout the experimental period (Table 1) when compared to the initial time and between the CG and SG.

After rumen acidosis induction, plasma glucose increased in both groups, which was significant (P <0.05) in CG at 4h PI (76.95 mg / dL), compared to the initial time (59.63mg/dL). In GS, despite the increase, the highest mean value (88.05 mg/dL) was observed at 16h PI, but without statistical significance (P> 0.05). By analyzing the behavior of this variable between groups, no significant difference was observed (P> 0.05) over the experimental moments (Figure 5).

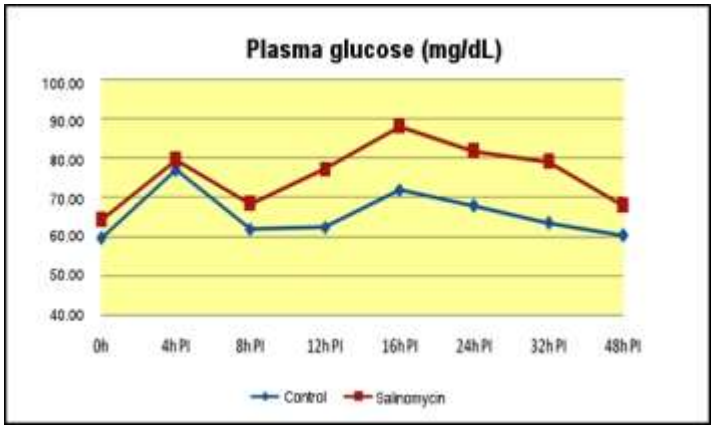

FIGURE 5. Mean values of plasma glucose (mg/dL) of sheep in CG and SG, with experimental ruminal lactic acidosis at basal time (0h) and after induction with sucrose (10g/kg body weight).

Values of L-lactate increased, being the highest average observed at 4h PI, reaching 16.50 mg/dL in CG and 30.72 mg/dL in the SG, with no statistical significance (P> 0.05) when compared with findings of the initial moment. Comparing the groups, no significant difference was observed (P> 0.05): animals in CG returned to initial levels and the ones in SG showed slightly lower values (Figure 6).

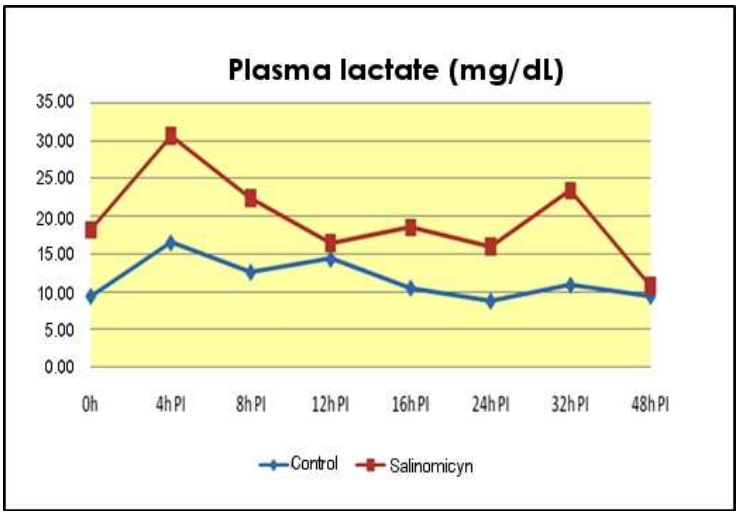

FIGURE 6. Mean values of plasma lactate (mg/dL) of sheep in CG and SG, with experimental ruminal lactic acidosis at basal time (0h) and after induction with sucrose (10g/kg body weight).

There was a significant decrease (P <0.05) in mean urinary pH values (Figure 7) after ruminal acidosis induction, at the moment 12h PI (6.43) and at the following moments, until the last experimental time trial (5.5), compared with time 0h (7.86) in CG. For SG, there was significant decrease of pH (P <0.05) only at 12h PI (5.5) and 16h PI (5.64) compared with the initial moment (8.18). The comparison between groups showed significant differences (P <0.05), and the lowest mean value in SG (5.5) compared to CG (6.43) at 12h PI. However, at the end of the experiment (48hPI), pH of CG remained low (5.5), with a significant difference (P <0.05), compared to the mean pH values of SG (7.6). Over the experimental moments, SG stabilized the pH, returning to basal-like levels.

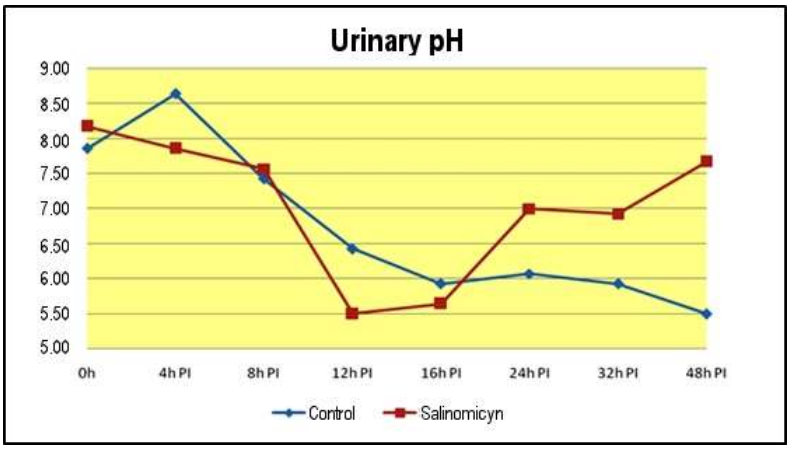

FIGURE7. Mean values of urinary pH of sheep in CG and SG, with experimental ruminal lactic acidosis at basal time (0h) and after induction with sucrose (10g/kg body weight).

DISCUSSION

We observed the clinical signs in sheep after acidosis induction are similar to those reported by other authors that have either caused or described the digestive disorder by using different substrates, both in small and large ruminants. Neverhteless, in this study, the clinical state of the animals was milder compared to other reports, in which the animals presented rumen stasis, diarrhea, severe dehydration, neurological disorders and even death in severe cases (CAKALA et al., 1974; CAO et al., 1987; BRAUN et al., 1992; NAGARAJA & LECHTENBERG, 2007; COMMUN et al., 2009; MIRANDA NETO et al., 2011). These differences probably occurred due to the smaller quantity and the type of substrate used to induce acidosis in animals in this study. These findings differ from trials with monensin, in which the ionophore was effective in preventing acidosis in ruminants, probably by a more effective mechanism for optimizing the energy status and improving the microbiota that produces propionic acid, at the expense of those that produce lactic acid, when compared to the use of salinomycin (NAGARAJA et al., 1985; MOUSSA, 1994; AFONSO et al., 2002b).

The decrease in rumen pH values, although less intense, was similar to the descriptions in the literature, returning to close to the initial levels, and doing it in a more prominently way in the SG, showing the favorable aspect of using this ionophore. This change is justified in cases of lactic acidosis, when pH falls to critical levels 4-8 hours after the ingestion of the substrate that triggered the condition, being lower when the production of volatile fatty acids (VFAs) by Gram-negative bacteria is higher and the production of lactic acid by Gram-positive bacteria, which predominate due to low pH, is continued (NOCEK, 1997; OWENS et al., 1998). These findings reflect the type and amount of the substrate used in inducing acidosis as well as the salinomycin interaction with the microbial population, demonstrating low power of controlling the fermentation disorder when compared to studies on other selective antibiotics such as monensin, capreomycin and laidlomycin, in which the ionophore enables a good pH control in fermentation disorders, making the healing of ill animals apparently faster (MUIR et al., 1980a; MUIR et al., 1980b; AHUJA et al., 1990; BAUER et al., 1995; MBANZAMIHIGO et al., 1995; AFONSO et al., 2002a).

In erythrogram, although there was no statistical difference between groups for any variable, we verified that the increase in values was higher for the animals in CG. This behavior was probably due to the greater intensity of the fermentative disorder undergone by the control animals, which is reflected in the degree of dehydration observed. The findings reflect better performance of animals in SG then control animals, regarding the pathological changes of osmotic balance and the stress caused by experimental acidosis, since in cases of lactic acidosis, the efflux of liquid from intra- and extracellular compartments to the rumen in order to maintain intra rumen balance results in an hematocrit increase (TELLE & PRISTON, 1971). On the other hand, the stress produced by acidosis causes splenic contraction due to the action of epinephrine; hemoconcentration may occur because of the amount of red blood cells released into the peripheral bloodstream and subsequent increase in hematocrit (JAIN, 1993). However, this last case is unusual in ruminants and is not accompanied by increases in the values of plasma protein as observed in this study.

PATRA et al. (1997) observed similar although more intense behavior for hematocrit when they induced ruminal acidosis in sheep. The highest increase in Hb in CG probably reflects the dehydration that occurred and was demonstrated by a higher hematocrit value after induction in these animals compared to animals in SG, which kept a more stable plasma volume compared to control. There are reports of an increase of hemoglobin rates in ruminants with acute indigestion, due to decreased plasma volume and the splenic contraction. However, HUBER (1971) induced acidosis in sheep and verified that the decrease in plasma volume was greater than the increase in hematocrit, indicating that the latter underestimates the degree of plasma dehydration. The rates observed for TPP are considered normal for the species, with the highest values related to the intensity of dehydration occurred in animals of each group (ANGELOV et al., 1996; ALMEIDA et al., 2008).

The leukogram findings are similar to those reported by CAO et al. (1987), UNDERWOOD (1992), MOHAMED NOUR et al. (1998) and NIKOLOV (2000), for goats, cattle and buffalo with acidosis. The authors described the mobilization of neutrophils is related to the inflammation of the rumen mucosa caused by the high concentration of lactic acid in the ruminal fluid, which being irritant to epithelium triggers the entire process of ruminitis. The recovery of leukocyte count at the following moments was due to the improvement of the clinical condition of the animals, since the evolution of induced lactic acidosis was mild, as verified by GOZHO et al. (2007) and DANSCHER et al. (2010) in cows with under acute ruminal acidosis.

The increase in acute protein phase and plasma fibrinogen (PF) was more intense in the animals of CG than in those of SG, although both groups remained within the limits considered normal for sheep (GARRY, 2002). This finding in animals of SG indicates better control of the adverse aspects caused by acidosis, such as lysis of Gram-negative bacteria, which are directly related to the increase of the inflammatory response as suggested by ECKERSALL (2000), GOZHO et al. (2007), BRAUN et al. (2010) and DANSCHER et al. (2010). In this study, the inflammatory stimulus was low, and no significant response occurred, as described by those authors, probably due to the smaller amount and the type of substrate used, also to the action of salinomycin preventing bacterial lysis, because there was almost no increase in concentrations of fibrinogen in animals treated with the ionophore compared to the CG animals.

The results obtained for TP were similar to those reported by VIHAN et al. (1982) and METKARI et al. (2001), who observed no changes for this variable in animals with the disorder, which is justified by the lower intensity of the process occurred in the animals analyzed in this study. On the contrary, ALMEIDA et al. (2008) explained this finding by hemoconcentration due to dehydration during illness. Similar findings were described for albumin by VIHAN et al. (1982) in goats with acidosis, and by AUSTIN & WILDE (1985) in pregnant ewes with induced acidosis, supplemented or not with the ionophore monensin, in which there were no remarkable changes in this protein.

The pattern of decrease in urea values and subsequent return to levels similar to those in the initial phase of the study corroborate the findings by ALMEIDA et al. (2008) on acidosis in goats. This finding is probably due to the change in intrarumen fermentation pattern by decreasing the microbial population that produces NH_3, reflecting a decline in serum urea during the most critical moments of ruminal acidosis (MOUSSA, 1994; BRAUN et al., 2010). Data from this study differ from those obtained by PATRA et al. (1996) and METKARI et al. (2001), for sheep and goats with induced acidosis, which showed an inverse distribution, with the highest values in the most critical periods of acidosis. Urea distribution in this study demonstrates the lowest severity of clinical acidosis in animals when compared to the greater severity of acidosis and dehydration described in the previously mentioned experiments.

Creatinine levels remained within the limits considered normal for the species, similar to those reported by ALMEIDA (2008) for goats with experimental ruminal lactic acidosis. The described findings disagree with those observed by NAGARAJA et al. (1985) for bovines, when these authors compared the efficiency of salinomycin to the ionophores lasalocid and monensin in the prevention of acidosis, and those verified by BROWN et al. (1999), who studied sheep with the same disorder, because both studies had higher values of creatinine in a period similar to that described in this paper, which can be explained by the lower renal blood perfusion caused by the increased intensity of the fermentative disorder in those works, as explained by GONZÁLEZ & SCHEFFER (2002).

The increase of AST levels was mild during the monitoring of acidosis signs in both experimental groups, showing no apparent impairment of liver function or tissue damage, because the animals showed a mild case of acidosis and did not remain in decubitus, which would increase AST concentration (BROWN et al., 1999; ALMEIDA et al., 2008). Different findings were reported by BRAUN et al. (1992), DAS & MISRA (1992) and PATRA et al. (1996), who verified remarkable increases in this enzyme from 24 h after the induction of disease, relating this finding to liver and muscle damages. These differences are probably related to the type of substrate employed – sucrose – and the amount administered, which was much lower in this study.

The enzyme GGT was not affected during acidosis induction, because, despite the alterations observed, they were not indicative of liver damage as they remained within normal levels for the species (KANEKO et al., 2008). BRAUN et al. (1992) and PATRA et al. (1996) observed different results for sheep and goats with acidosis. According to these authors, the values for this enzyme were elevated due to hepatobiliary injury.

The values obtained for the ALP remained within the normal levels for the species (KANEKO et al., 2008). Similar findings were reported by ALMEIDA et al. (2008), in cases of lactic acidosis induced in goats, and by NAGARAJA et al. (1985), employing salinomycin as a prevention model in cattle.

Similarly to this study, ALMEIDA et al. (2008) observed no changes for CK in goats with acidosis. However, LAL et al. (1991), BRAUN et al. (1992) and UNDERWOOD (1992) studied lactic acidosis cases in goats, and reported significant increases in CK, justified by muscle damage caused by longer time in recumbence. Acidosis induction in the animals of this study produced a case of mild lactic acidosis, in which the animals, despite decreased appetite, ruminal atony, clusters of feces, among other symptoms, did not stay prostrated or

lying down for a long time, causing no sufficient stimulus to increase the amount of this enzyme that is a sensitive marker of muscle damage (GARRY, 2002).

Elevations in glucose values, using different substrates and generating clinical manifestations of varying intensity in ruminants with lactic acidosis have been reported by ANGELOV et al. (1995), PATRA et al. (1997), MOHAMED NOUR et al. (1998) and ALMEIDA et al. (2008), justifying the temporary hyperglycemia due to increased glucose reabsorption from the surplus that was not metabolized by the rumen microbiota, but also due to an increase in the synthesis of glucose by the liver, arising from higher production and absorption of volatile fatty acids in the rumen (NAGARAJA et al., 1985). Although no difference was verified between the experimental groups, the sheep which received salinomycin showed higher levels of glucose, during the period of rumen acidosis, justified by the selective action of the ionophore to favor the growth of bacteria that synthesize propionate, the main gluconeogenic precursor (AUSTIN & WILDE, 1985; NAGARAJA et al., 1985; MOUSSA, 1994).

Considering the results for plasma L-Lactate, we could find that the animals of both groups presented a mild case of lacticemia, due to the absorption of the acid into the bloodstream because of its increase in the rumen fluid, leading to the described clinical manifestations. This change is very characteristic in the process of lactic acidosis and arises as a result of the imbalance between its synthesis and utilization by the producer and consumer bacterial flora, because, in the rumen, it is only an intermediate product of Gram-positive bacterial fermentation (ANGELOV et al., 1996; NOCEK, 1997; MOHAMED NOUR et al., 1998). The findings are similar to those reported by BAUER et al. (1995), who employed the laidlomycin in cattle, in order to minimize the occurrence of experimental sub-acute acidosis, and observed that it was not as effective. However, these findings differ from those reported by AHUJA et al. (1990) and NAGARAJA et al. (1985), who, using this and other types of ionophores in cattle and buffalos, observed favorable results in animals with induced acidosis in which the compounds were effective in controlling the disease by lowering the levels of lactic acid in the rumen and consequently the blood.

The findings of this study demonstrate the favorable aspect of the use of salinomycin in animals exposed to situations that may trigger ruminal lactic acidosis and, therefore, metabolic acidosis, because the animals treated with the ionophore returned to normal values of urine pH in a faster and more consistent way than animals of CG, which remained showing low values of urinary pH indicating more difficulty in metabolizing the excess of AGVs and other fermentation products such as the lactic acid accumulated, being within normal limits considering the sudden carbohydrates intake. This decrease in the values of urinary pH is mainly attributed to the excretion of the H+ ion, which is associated to the disposal of ammonia, as well as phosphate and lactate molecules, being a precocious and more reliable indicator in mild cases of ruminal acidosis because the kidneys secrete H+ ions before the mechanism of lactic acid reabsorption is overshot (UNDERWOOD, 1992; PATRA et al., 1993; ORTOLANI, 2002). On the other hand, BROWN et al. (1999), in a study with adult sheep, found that urinary pH was not affected in induced acidosis, contrary to the findings of this study which demonstrated a significant decrease in urinary pH of animals affected by experimental lactic acidosis, but that did not undergo treatment or preventative measure as the use of salinomycin. These authors consider that individual variations may interfere with the pH value, making limited use as an indicator of acidosis.

CONCLUSIONS

Given the proposed objectives, we concluded that acidosis induction caused characteristic clinical manifestations of the disease in a mild form in both experimental groups. Due to the low intensity clinical evaluation, the hematological and biochemical alterations were not remarkable. The use of salinomycin did not prevent the emergence of fermentative disorder, however, the animals that used it showed earlier clinical recovery of hematological and biochemical parameters.

REFERENCES

AFONSO, J. A. B.; MENDONÇA, C. L.; FIORAVANTE, M. C. S.; KUCHEMBUCK, M. R. G. Características e indicações clínicas dos ionóforos para ruminantes. **Revista do Conselho Federal de Medicina Veterinária**, v. 6, n. 20, p. 29-36, 2000.

AFONSO, J. A. B.; KUCHEMBUCK, M. R. G.; FELTRIN, L. P. Z.; LAPOSY, C. B.; KOHAYAGAWA, A.; MENDONÇA, C. L.; TAKAHIRA, R. K. Efeito da monensina sódica sobre as características do suco rumenal na acidose láctica experimental em ovinos. **Revista Brasileira de Medicina Veterinária**, v. 24, n.5, p. 203-210, 2002a.

AFONSO, J. A. B.; CIARLINI, P. C.; KUCHEMBUCK, M. R. G.; KOHAYAGAWA, A.; FELTRIN, L. P. Z.;

CIARLINI, D. R. P.; LAPOSY, C. B.; MENDONÇA, C. L.; TAKAHIRA, R. K. Metabolismo oxidativo dos neutrófilos de ovinos tratados com monensina sódica e experimentalmente submetidos à acidose ruminal. **Pesquisa Veterinária Brasileira**, v.22, n.4, p. 129-134, 2002b.

AHUJA, A. K.; RANDHAWA, S. S.; RATHOR, S. S. Effect of monensin in ameliorating subacute lactic acidosis in buffalo calves. **Acta Veterinaria Brno**, v. 59, p. 171-178, 1990.

ALMEIDA, M. Z. P. R. B.; MENDONÇA, C. L.; AFONSO, J. A. B.; MIRANDA NETO, E. G. Estudo clínico, hematológico e bioquímico em caprinos submetidos à acidose láctica ruminal induzida experimentalmente. **Veterinária e Zootecnia**, v. 15, n. 1, p. 100-113, 2008.

ANGELOV, G.; NIKOLOV, Y; ANGELOV, A.Changes in acid-base variables and some biochemical parameters in caprine acute rumen acidosis. **Veterinarski Arhiv**, v. 65, n. 2, p. 43-48, 1995.

ANGELOV, G.; NIKOLOV, Y; ANGELOV, A. Changes in acid-base parameters, blood sugar and blood lactate in experimental acute rumen acidosis in sheep. **Indian Veterinary Journal**, v. 73, p. 309-314, 1996.

AUSTIN, A. R.; WILDE, R. M. The effect of sodium monensin on pregnant ewes. **British Veterinary Journal**, v. 141, n. 6, p. 628-634, 1985.

BAUER, M. L.; HEROLD, D. W.; BRITTON, R. A.; STOCK, R. A.; KLOPFENSTEIN, T. J.; YATES, D. A. Efficacy of laidlomycin proprionate to reduce ruminal acidosis in cattle. **Journal of Animal Science**, London, v. 73, p. 3445-3454, 1995.

BEEDE, D. K.; FARLIN, S. D. Effects of capreomycin disulfate and oxamycin on ruminal pH, lactate and volatile fatty acid concentrations in sheep experiencing induced acidosis. **Journal of Animal Science**, v. 45, n. 2, p. 393-401, 1977.

BERGEN, W. J.; BATES, D. B. Ionophores: Their effect on production efficiency and mode of action. **Journal of Animal Science**, v. 58, p. 1465-1483, 1984.

BRAUN, U.; RIHS, T.; SCHEFER, U. Ruminal lactic acidosis in sheep and goats. **Veterinary Record**, v. 130, p. 343-349, 1992.

BRAUN, J. P.; TRUMEL, C.; BÉZILLE, P. Clinical biochemistry in sheep: A selected review. **Small Ruminant Research**, v. 92, p. 10-18, 2010.

BROWN, M. S.; HALFORD, D. M.; GALYEAN, M. L.; KREHBIEL, C. R.; DUFF, G. Effect of ruminal glucose infusion on dry matter intake, urinary nitrogen composition, and serum metabolite and hormone profile in ewes. **Journal of Animal Science**, v. 77, p. 3068-3076, 1999.

CAKALA, S.; BORKOWSKI, T.; ALBRYCHT, A. Rumen acidosis in sheep induced with different doses of saccharose. **Polskie Archiwun Weterynaryjne**, v.17, p. 117-130, 1974.

CÂMARA, A. **Efeito da salinomicina na prevenção da acidose láctica ruminal experimental em ovinos**. Mossoró: UFERSA, 2008, 76p. Dissertação (Mestrado em Ciência Animal) – Programa de Pós-Graduação em Ciência Animal, Universidade Federal Rural do Semi-Árido, Mossoró, 2008. http://www2.ufersa.edu.br/portal/view/uploads/setores/80/Disserta%C3%A7%C3%A3o_Adaucides%C2%A0C%C3%A2mera.pdf

CAO, G. R.; ENGLISH, P. B.; FILIPPICH, L. J.; ONGLIS, S. Experimentally induced lactic acidosis in the goat. **Australian Veterinary Journal**, v. 64, n.12, p. 367-370, 1987.

COMMUN, L.; MIALON, M. M.; MARTIN, C.; BAUMONT, R.; VEISSIER, I. Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. **Journal of Animal Science**, v 87, p. 3372-3379, 2009.

CURI, P.R. **Metodologia e análise da pesquisa em ciências biológicas.** Botucatu: Tipomic, 1997. 263 p.

DANSCHER, A. M.; THOEFNER, M. B.; HEEGAARD, P. M. H.; EKSTRØM, C. T.; JACOBSEN, S. Acute phase protein response during acute ruminal acidosis in cattle. **Science**, 2010. Doi:10.1016/j.livsci2010.06.009.

DAS, S. K.; MISRA, S. K. Liver function in experimental acidosis in goat. **Indian Journal of Animal Sciences**, v. 62, n. 3, p. 243-244, 1992.

DEHGHANI, S. N.; GHADRDANI, A. M. Bovine rumenotomy: Comparision of four surgical techniques. **Canadian Veterinary Journal**, v. 36, n.11, 693-697 1995.

DELAK, M., ADAMIC, S. Contribution to the knowledge of saccharose intoxication in sheep. **Veterinary Archives,** v. 29, p. 214-222, 1959.

DIRKSEN, G. Sistema digestivo. In: DIRKSEN, G, GRÜNDER, H. D., STÖBER, M. **Rosenberger exame clínico dos bovinos**. 3 ed., Rio de Janeiro: Guanabara Koogan, 1993. p. 166-228.

DOUGHERTY, R. W.; RILEY, J. L.; COOK, H. M. Changes in motility and pH in the digestive tract of experimentally overfed sheep. **American Journal of Veterinary Research**, v. 36, n. 6, p. 827-829, 1975.

DUNLOP, R. H. Pathogenesis of ruminant lactic acidosis. **Advances in Veterinary Science and Comparative Medicine,** v. 16, p.259-302, 1972.

ECKERSALL, P. D. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animal. **Revue de Médecine Vétérinaire**, v. 151, n. 7, p. 577-584, 2000.

GARRY, F. B. **Diseases of the alimentary tract**, p. 722-747. In: SMITH, B. P. (Ed.). Large Animal Internal Medicine. 3rd ed. Mosby: St. Louis, 2002.

GONZÁLEZ, F. H. D.; SCHEFFER, J. S. F. Perfil

sanguíneo: Ferramenta de análise clínica, metabólica e nutricional. In: GONZÁLEZ, F. H. D et al. Avaliação metabólico-nutricional de vacas leiteiras por meio de fluídos corporais (sangue, leite e urina). **Arquivos do 29º Congresso Nacional de Medicina Veterinária**, Gramado, RS, p. 5-17, 2002. http://www6.ufrgs.br/favet/lacvet/restrito/pdf/avalia_ao%20metabolica%20vacas.pdf#page=5

GOZHO, G. N.; KRAUSE, D. O.; PLAIZIER, J. C. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute acidosis in dairy cows. **Journal of Dairy Science**, v. 90, n. 2, p. 856-866, 2007.

HUBER, T. L. Effect of acute indigestion on compartmental water volumes and osmolality in sheep. **American Journal of Veterinary Research**, v. 32, n. 6, p. 887-890, 1971.

HUNGATE, R. E.; DOUGHERTY, R. H.; BRYANT, M. P.; CELLO, R. M. Microbiological and physiological changes associated with acute indigestion in sheep. **Cornell Veterinarian**, v. 42, p. 423-449, 1952.

JAIN, N. C. **Essentials of veterinary hematology**. 5. ed., Philadelphia: Lea & Febiger, 1993. 417p.

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. **Clinical biochemistry of domestic animals**. 6.ed. New York: Academic Press, 2008. 916p.

KEZAR, W.W., CHURCH, D.C. Ruminal changes during the onset and recovery of induced lactic acidosis in sheep. **Journal of Animal Science,** v.49, n. 5, p. 1161-1167, 1979a.

KEZAR, W.W., CHURCH, D.C. Effect of thiopeptin and sodium bicarbonate on the prevention of lactic acidosis induced in sheep. **Journal of Animal Science,** v.49, n. 5, p. 1396-1402, 1979b.

LAL, S. B. et al. Biochemical alterations in serum and cerebr-spinal fluid in experimental acidosis in goats. **Research in Veterinary Science**, v. 50, p. 208-210, 1991.

MARUTA, C. A.; ORTOLANI, E. L. Susceptibilidade de bovinos das raças jersey e gir à acidose láctica ruminal: I – Variáveis ruminais e fecais. **Ciência Rural**, v. 32, n. 1, p. 55-59, 2002a.

MBANZAMIHIGO, L.; VAN NEVEL, C. J.; DEMEYER, D. I. Adaptation of rúmen fermentation to monensin. **Reproduction Nutritional Development**, v. 35, n. 4, p. 353-365, 1995.

MERCHEN, N. R.; BERGER, L. L. Effect of salinomycin level on nutrient digestibility and ruminal characteristics of sheep and feedlot performance of cattle. **Journal of Animal Science,** v. 60, n. 5, p. 1338-1346, 1985.

METKARI, S. M. et al. Management of experimentally induced lactic acidosis in goats. **Indian Veterinary Journal**, v. 78, p.692-694, 2001.

MIRANDA NETO, E. G.; AFONSO, J. A. B.; MENDONÇA, C. L.; ALMEIDA, M. Z. P. R. B. Estudo clínico e características do suco ruminal de caprinos com acidose láctica induzida experimentalmente. **Pesquisa Veterinária Brasileira**, v. 25, n. 2, p. 73-78, 2005.

MIRANDA NETO, E. G.; AFONSO, J. A. B.; SILVA, S. T. G.; MENDONÇA, C. L. Aspectos clínicos e a bioquímica ruminal de caprinos submetidos à acidose experimental e suplementados ou não com monensina sódica. **Pesquisa Veterinária Brasileira**, v. 31, n. 5, p. 416-424, 2011.

MOHAMED NOUR, M. S.; ABUSAMRA, M. T.; HAGO, B. E. D. Experimental induced acidosis in Nubian goats: Clinical, biochemical and pathological investigations. **Small Ruminant Research**, v. 31, p. 7-17, 1998.

MOUSSA, H. M. Ruminal and blood characteristics of nubian goats dosed with the growth promoter monensin. **Acta Veterinaria Brno**, v. 63, p. 13-17, 1994.

MUIR, L. A.; RICKES, E. L.; DUQUETTE, P. F.; SMITH, G. E. Control of wheat induced lactic acidosis in sheep by thiopeptin and related antibiotics. **Journal of Animal Science,** v.50, n. 3, p. 547-553, 1980a.

MUIR, L. A.; DUQUETTE, P. F.; RICKES, E. L.; SMITH, G. E. Thiopeptin for the prevention of ovine lactic acidosis induced by diet change. **Journal of Animal Science,** v.51, n. 5, p. 1182-1188, 1980b.

NAGARAJA, T. G.; AVERY, T. B.; GALITZER, S. J.; HARMON, D. L. Effect of ionophore antibiotics on experimentally induced lactic acidosis in cattle. **American Journal of Veterinary Research,** v.46, p. 2444-52, 1985.

NAGARAJA, T. G.; LECHTENBERG, K. F. Acidosis in feedlot cattle. **Veterinary Clinics of Food Animals**, v. 23, p. 333-350, 2007.

NIKOLOV, Y. Some biochemical changes in cerebrospinal flui, blood and rumen fluid in experimental ruminal acidosis in buffalo calves. **Indian Veterinary Journal**, v. 77, p. 957-960, 2000.

NIKOLOV, Y. Biochemical alterations in rumen liquor, blood, cerebrospinal fluid and urine in experimental acute ruminal lactic acidosis in sheep. **Indian Veterinary Journal**, v. 80, p. 36-39, Jan. 2003.

NOCEK, J. E. Bovine acidosis: Implications on laminitis. **Journal of Dairy Science**, v. 80, p. 1005-1028, 1997.

ORTOLANI, E. L. Induction of lactic acidosis in cattle with sucrose: relationship between dose, rumen fluid pH and animal size. **Veterinary and Human Toxicology**, v. 37, n. 5, p.462-64, 1995.

ORTOLANI, E. L. Diagnóstico de doenças nutricionais e metabólicas por meio de exame de urina em ruminantes. In: GONZÁLEZ, F. H. D.; ORTOLANI, E. L.; BARROS, L.; CAMPOS, R. Avaliação metabólico-nutricional de vacas leiteiras por meio de fluídos corporais (sangue, leite e urina). **Arquivos do 29º Congresso Nacional de Medicina Veterinária**, Gramado, RS, p. 18-26, 2002. http://www6.ufrgs.br/favet/lacvet/restrito/pdf/avalia_ao%20metabolica%20vacas.pdf#page=5

OWENS, F. N.; SECRIST, D. S.; HILL, W. J.; GILL, D. R. Acidosis in cattle: A review. **Journal of the American Society of Animal Science**, v. 76, p. 275-286, 1998.

PATRA, R. C.; LAL, S. B.; SWARUP, D. Physicochemical alterations in blood, cerebrospinal fluid and urine in experimental lactic acidosis in sheep. **Research in Veterinary Science**, v. 54, p. 217-220, 1993.

PATRA, R. C.; LAL, S. B.; SWARUP, D. Biochemical profile of rumen liquor, blood and urine in experimental acidosis in sheep. **Small Ruminant Research**, v. 19, n. 2, p. 177-180, 1996.

PATRA, R. C.; LAL, S. B.; SWARUP, D. Therapeutic management of experimental ruminal acidosis in sheep. **Indian Veterinary Journal**, v. 74, n. 3, p. 237-240, March 1997.

RADOSTITS, O. M.; HINCHCLIFF, K. W.; BLOOD, D. C.; GAY, C. C. **Veterinary Medicine**. A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. St Louis: Saunders, 2007. 2156p.

TELE, P. P.; PRESTON, R. L. Ovine lactic acidosis: Intraruminal and systemic. **Journal of Animal Science**, v. 33, n. 3, p. 699-705, 1971.

UNDERWOOD, W. J. Rumen lactic acidosis. Part II. Clinical signs, diagnosis, treatment and prevention. **The Compendium – Food Animal**, v. 14, n. 9, p. 1265-1269, 1992.

USAGAWA, T. Effects of monensin and salinomycin on the *In Vitro* foam stability of sheep rumen fluid. **Animal Science and Technology**, Japan, v. 63, p. 16-22, 1992.

VIEIRA, A. C. S. et al. Estudo retrospectivo da acidose láctica em caprinos e ovinos atendidos na Clínica de Bovinos, Campus Garanhuns/UFRPE. **Revista Brasileira de Ciências Agrárias**, v. 1, n. único, p. 97-101, 2006.

VIHAN, V. S.; WANI, G. M.; SAHNI, K. L. Observation on changes in blood serum in experimental rumen acidosis in goats. **Indian Veterinary Journal**, v. 59, p. 998-1000, 1982.

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