The use of sodic monensin and probiotics for controlling subacute ruminal acidosis in sheep

O uso de monensina sódica e probióticos para o controle de acidose ruminal subaguda em ovinos

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Abstract

It was to validated a protocol for induction of subacute ruminal acidosis (SARA) (Experiment 1) and test the efficiency of probiotic *Saccharomyces cerevisiae* or monensin to avoid pH ruminal drops in sheep (Experiment 2). In Experiment 1, six ewes were fasted for two days and then fed most with concentrate during four days. Ewes in this protocol had ruminal fluid pH below 6.0 and kept it for 75 consecutive hours. In Experiment 2, 18 sheep were distributed into three groups: Control (CG, n = 6), monensin (MG, n = 6) and probiotic group (PG, n = 6). SARA was induced according Experiment 1. PG had lower pH (5.7 ± 0.1) than CG (6.0 ± 0.1) (P = 0.05), while MG (5.7 ± 0.1) was similar to both during SARA induction. SARA induction reduced ruminal protozoa population (P < 0.05) and increased chloride concentrations in ruminal fluid (P < 0.01). In serum, SARA increased concentrations of phosphorus (P < 0.01), AST (P < 0.01) and GGT (P < 0.01), but reduced LDH (P < 0.01). In conclusion, the protocol used for SARA induction was able to maintain ruminal pH between 5.5-6.0 for more than 48 hours. However, monensin and probiotics supplementation was not effective in preventing changes in ruminal and serum parameters during SARA.

Keywords: SARA induction. Sheep. *Saccharomyces cerevisiae*. Ionophores.

Resumo

Foi validado um protocolo para a indução de acidose ruminal subaguda (SARA) (Experimento 1) e testar a eficácia do probiótico *Saccharomyces cerevisiae* ou monensina na prevenção da queda do pH do fluido ruminal em ovinos (Experimento 2). No Experimento 1, seis ovelhas foram mantidas em jejum por dois dias e, em seguida, alimentadas basicamente com concentrado durante quatro dias. Nesse protocolo as ovelhas mantiveram o pH do fluido ruminal abaixo de 6,0 por 75 horas consecutivas. No Experimento 2, 18 ovelhas foram distribuídas em três grupos: controle (GC, n = 6), monensina (GM, n = 6) e o grupo probiótico (GP, n = 6). A SARA foi induzida de acordo com o Experimento 1. GP apresentaram valores de pH mais baixos (5,7 ± 0,1) do que o GC (6,0 ± 0,1) (P = 0,05), enquanto GM (5,7 ± 0,1) foi semelhante durante a indução de SARA. A indução SARA reduziu a população de protozoários no rúmen (P < 0,05) e aumentou a concentração de cloreto no líquido ruminal (P < 0,01). Durante a SARA observou-se aumento das concentrações séricas de fósforo (P < 0,01), AST (P < 0,01) e GGT (P < 0,01), mas reduziu a de LDH (P < 0,01). Em conclusão, o protocolo utilizado para a indução de SARA foi capaz de manter o pH do rúmen entre 5,5-6,0 por períodos superiores a 48 horas. No entanto, a suplementação com monensina e probióticos não foi eficaz na prevenção das alterações nos parâmetros ruminais e séricos durante SARA.

Palavras-chave: Indução SARA. Ovelhas. *Saccharomyces cerevisiae*. Ionóforos.

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Received: 16/01/2014
Approved: 17/11/2014

DOI: 10.11606/issn.1678-4456.v51i4p324-332

Introduction

Subacute Ruminal Acidosis (SARA) is a common disease of cattle subjected to diets containing high amounts of carbohydrates (PLAIZIER et al., 2009). This disease is characterized by ruminal fluid pH bellow 6.0 (DESNOYERS et al., 2008; PLAIZIER, 2004) due to the excessive production of volatile fatty acids (VFA) and insufficient ruminal buffering (PLAIZIER et al., 2009). It is estimated that 20% of dairy cows in commercial herds have SARA (OETZEL, 2004), and its economic losses can reach US$ 1 billion per year in the United States (ENEMARK, 2009). SARA diagnosis is a difficult task, due to the absence of evident clinical manifestations. The correct diagnosis of SARA must consider several points, such as pH of the ruminal fluid, milk fat depression and reduction in urinary pH and these symptoms must be associated to the herd's history (ENEMARK, 2009).

Several alternatives aiming to reduce SARA incidence in ruminants fed with high amounts of carbohydrates have been tested (BEAUCHEMIN et al., 2003; KAWAS et al., 2007; MUTSVANGWA et al., 2002). One very popular alternative is the use of ionophores antibiotics (e.g. sodic monensin). Its action is based on the inhibition of lactate producing by Gram-positive bacteria (e.g. *Streptococcus bovis*) (RANGEL et al., 2008). The ionophores reduce the proportion of acetate:butirate, reduce methane production and increase propionate production (JOUANY; MORGAVI, 2007; RANGEL et al., 2008). Another alternative for controlling SARA is the use of probiotics such as yeast (*Saccharomyces cerevisiae*), which can remove the oxygen passage in the rumen and increase the number of viable cellulolytic bacteria (WALLACE, 1994). As a result of yeast supplementation, ruminal pH becomes more stable and the production of methane and lactic acid is reduced (QUEIROZ et al., 2004).

Either SARA or its prevention protocols are most extensively studied using the bovine model. However, studies that induced SARA in cattle were not able to maintain this condition for more than 48 consecutive hours (KRAUSE; OETZEL, 2005; ORTOLANI et al., 1995). Moreover, the use of sheep as an experimental model to study SARA could be interesting (MIKEL et al., 2004), since it has a smaller digestive tract and is easier collect ruminal fluid and manipulate than cattle. Furthermore, to our knowledge, there is no validated protocol in ewes to induce SARA in order to test the effects of products on its control. Based on those considerations, the hypothesis of this study is that ewes supplemented with monensin and probiotic, when induced to SARA, have better control of the ruminal pH. To test this hypothesis two experiments have been performed. The aim of experiment 1 was to validate a protocol for long-term SARA induction, and of experiment 2, to evaluate the ruminal and metabolic parameters of ewes supplemented with probiotics and monensin when subjected to SARA.

Material and methods

Animal welfare

The Committee for Ethics in Animal Experimentation from the Universidade Federal de Pelotas has approved all procedures performed in this experiment, under number 10/2006.

Experiment1

Animals and experimental design

Six crossbreed ewes (35.0 ± 8.0 kg initial of body weight- BW, and 2.5 ± 0.5 of body condition score - BCS, 1 to 5 scale, RUSSEL, 1991), housed in two groups of three were used. The ewes were fed a mix of concentrate and forage, corresponding 1% of their BW, for 21 days before the beginning of the experiment. The diet consisted of the Tifton hay (*Cynodon* sp., neutral detergent fiber 77.4%, acid detergent fiber 59.8%, ether extract 1.3%, crude protein 8.0%) and concentrate (crude fiber 13.9%, ether extract 5.68%, crude protein 14.8%). Before the beginning of SARA induction, the ewes were fasted for two days with free access to water. For SARA induction, all ewes received

a concentrate diet, corresponding to 3% of BW, during four days. To stimulate concentrate intake, ewes were kept under artificial light for 24 h/day.

Ruminal fluid collection

Ruminal fluid was collected four times a day (0800, 1200, 1600 and 1900 h) during SARA induction by oro-ruminal intubation. Briefly, ruminal fluid was poured through a filter and the pH of the liquid phase was measured (Phtek®, PHTEK, Germany). The concentrated diet was removed from every ewe that presented ruminal pH bellow 5.2, and tifton hay was offered until pH return to a value above 5.2. Clinical examinations were performed during the entire experiment in order to observe clinical manifestation of acute ruminal acidosis (ENEMARK; JORGENSEN; ENEMARK, 2002).

Experiment 2

Animals and experimental design

Eighteen crossbreed sheep (seven males and 11 females) with 12 ± 4 months of age, 31.0 ± 8.0 kg of initial BW and 2.5 ± 0.5 of body condition score (BCS, 1 to 5 scale, RUSSEL, 1991) were used. Sheep were housed in pens (six animals each; 2.0 x 3.5 m), and had free access to water. The diet was the same of *Experiment 1*. The sheep were divided homogeneously according to sex and BCS into three groups: 1) Control Group (n = 6); 2) Monensin Group (n = 6), and 3) Probiotics Group (n = 6). The Control Group received the standard diet without any supplementation. Sheep in the Monensin Group received the same diet as control group, plus a supplement of 11 ppm/kg/DM/day of sodic monensin (Rumensin 100®, Elanco Animal Health, United States) (TAGHIPOOR et al., 2011). Finally, sheep from the Probiotics Group received 8.4×10^4 UFC of *Saccharomyces cerevisiae*/ewe/day (3g of Yea-Sacc®, Alltech®, United States) (KAWAS et al., 2007).

The sheep were adapted to the standard diet with supplement for 21 days before the beginning of the trial. SARA induction procedures were performed as described for Experiment 1. During the fasting period ewes received the supplements orally, diluted in distilled water. The treatments were given once a day at the very first meal of the day (0800 h). After the period of SARA induction, ewes returned to the standard diet, and continued receiving the respective treatments (recovery period).

Ruminal fluid collection and analysis

Ruminal fluid was collected four times a day during the SARA induction period (ID1 to ID4) by oro-ruminal intubation (0800, 1200, 1600 e 1900 h). After that, ruminal fluid collection was performed once a day (1200 h), during the recovery period (RD1, RD2 and RD3). The first collection at 0800 h on Induction Day 1 (ID1) was considered as pre-induction period, since all ewes were under fasting and had not yet receive concentrated diet. The ruminal fluid was strained through a filter, and the pH of the liquid phase was measured (pHmetroAn 2000 microprocessadoAnalion®, Analion Aparelhos e Sensores Indústria e Comércio, Brazil). Subsequently, the fluid was strained through gauze to obtain 5 mL samples for protozoa counting, according to Dehority (1977). Clinical examinations were conducted the same as *Experiment 1*.

Blood sampling and Biochemistry analysis

Blood sampling was performed once a day (1200 h) from the jugular vein. Blood samples were placed into tubes without anticoagulant. Serum was obtained by centrifugation (1800 X g, 15 min) and stored at -80°C. Concentrations of aspartate aminotransferase (AST), calcium, chloride, phosphorus, gamaglutamiltransferase (GGT), lactate dehydrogenase (LDH) and magnesium were evaluated by enzymatic colorimetric methods (AST 52, Cálcium 94 Chloride 49, phosphorus 42, GGT 105, LDH 138, Labtest Diagnóstica S. A., Brazil) (TABELEÃO et al., 2008), and quantified in spectrophotometer (FEMTO 700 Plus®, Femto Ind. Com. Instrumentos LTDA,

Brazil). Assays for measuring concentrations of sodium (Human GmbH®, Germany) and potassium (Doles Reagentes e Equipamentos para LaboratóriosLtda, Brazil) in serum were also performed by colorimetric methods. The ruminal fluid chlorides concentration was also evaluated by colorimetric methods (Chloretos 49, **Labtest** Diagnóstica S. A., Brazil).

Statistical analysis

The results are presented as means ± standard error of the mean (SEM). All the statistical analyses were performed using SAS (SAS Institute Inc. Cary, NC, USA). In the initial analysis the statistical model included: age, parity, sex, body condition score and body weight. Since these variables had no significant effects in the model, they were excluded from the final statistical model. Analyses involving repeated measures over time (e.g., AST, calcium, chloride, phosphorus, GGT, LDH, magnesium, and protozoa count) were compared between treatments by analysis of variance for repeated measures using the MIXED procedure to evaluate the main effects of period, treatment, and their interactions (LITTELL et al., 1998). The statistical models and data analysis were designed and performed separately for the pre-induction, induction and recovery period. When the interaction between treatment and period was significant ($P < 0.05$) pair-wise comparison of individual means was carried over. A probability below 0.05 was considered significant.

Results

Experiment 1

Eight hours after the beginning of the SARA induction protocol, ewes presented ruminal fluid pH bellow 6.0 ($P < 0.05$). Ewes responded to the protocol in a homogeneous fashion up to 1900 h from the 4th day of SARA induction. Therefore, the protocol was effective in maintaining SARA for 75 consecutive hours (Figure 1).

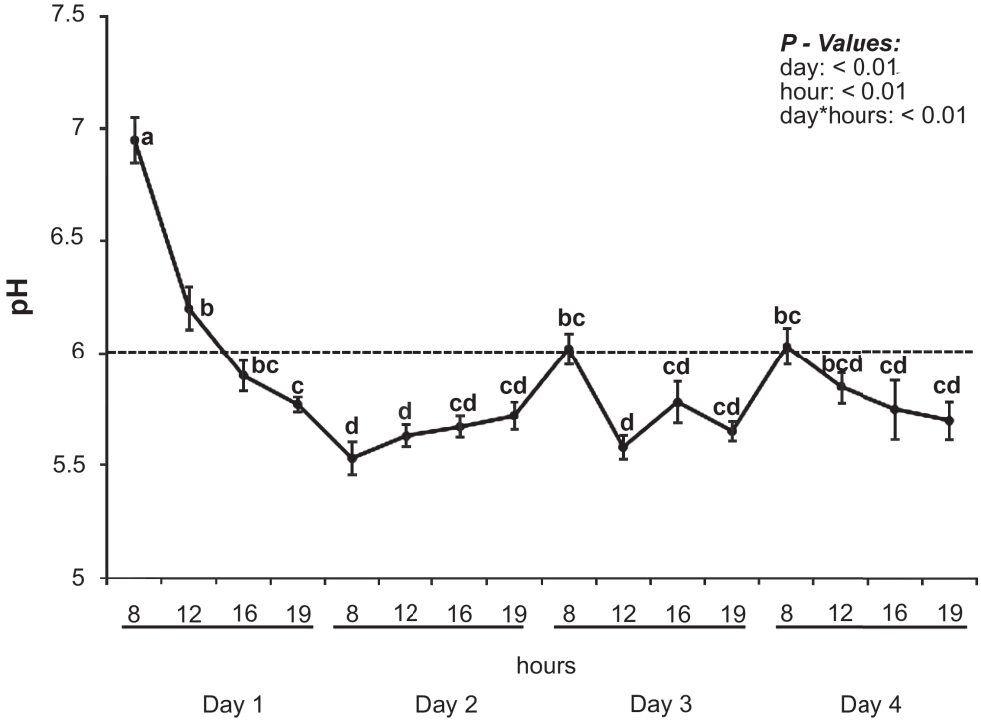

Figure 1 – Changes in pH (means ± SEM) of the ruminal fluid of ewes subjected to SARA induction protocol during four days
Source: (SCHWEGLER et al., 2014)

Experiment 2

There was an effect of the day (P = 0.02), group (P = 0.05) and a tendency for a group*day interaction (P = 0.07) over ruminal pH during the period of SARA induction. During this period, Probiotic Group had a lower ruminal pH (5.7 ± 0.1) than Control Group (6.0 ± 0.1), while Monensin Group was similar (5.7 ± 0.1) to the other two groups (P = 0.05). During the recovery period (RP1, RP2 e RP3), no differences were found for day, group or day*group interaction (P > 0.05) (Figure 2). The treatments also did not affect protozoa counts during the experimental period (Figure 3). During the induction period, protozoa count was lower (30,215 ± 5,665 protozoa/ml) than during the recovery period (77,857 ± 2,886; P < 0.05). The supplements used did not influence the metabolic markers evaluated, as shown in table 1.

Discussion

The protocol of SARA induction used in this study was effective, since the ruminal fluid pH was sustained between 5.2 and 6.0 for 75 consecutive hours, without any ewe presenting clinical manifestation of ruminal acidosis (PLAIZIER, 2004). To our knowledge, this is the first study that validated a protocol for SARA induction for such period. Other protocols for dairy cows have successfully reached at most 48 h of SARA induction (KRAUSE; OETZEL, 2005; KREHBIEL et al., 1995). Furthermore, the similarity between the results of the two experiments (1 and 2) confirms the repeatability of such protocol.

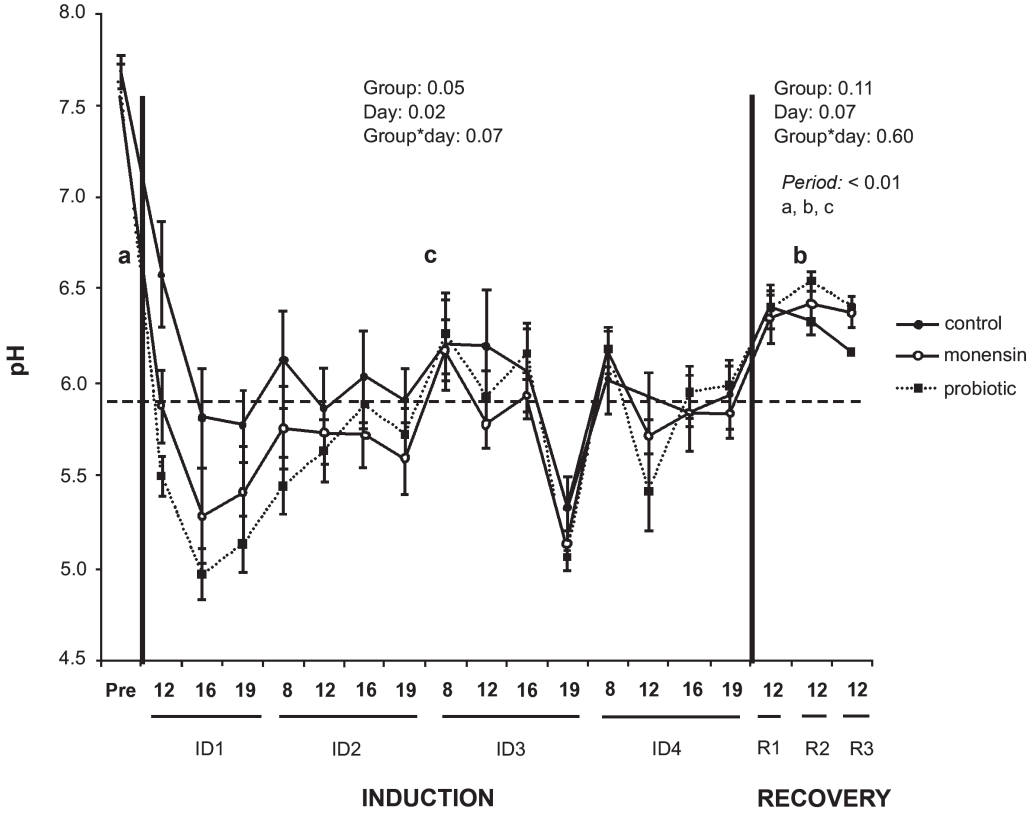

Figure 2 – Changes in pH (means ± SEM) of the ruminal fluid in ewes subjected to SARA induction protocol, from control (n=6), monensin (n=6; 11 ppm/kg/DM/day of sodicmonensin) and probiotics groups (n=6; 8.4×104 UFC of Saccharomyces cerevisiae/ewe/day) during pre-induction (Pre), induction (ID1, ID2, ID3 e ID4) and recovery periods (R1, R2 e R3). Different letters indicates statistical significance among periods (P < 0.05)
Source: (SCHWEGLER et al., 2014)

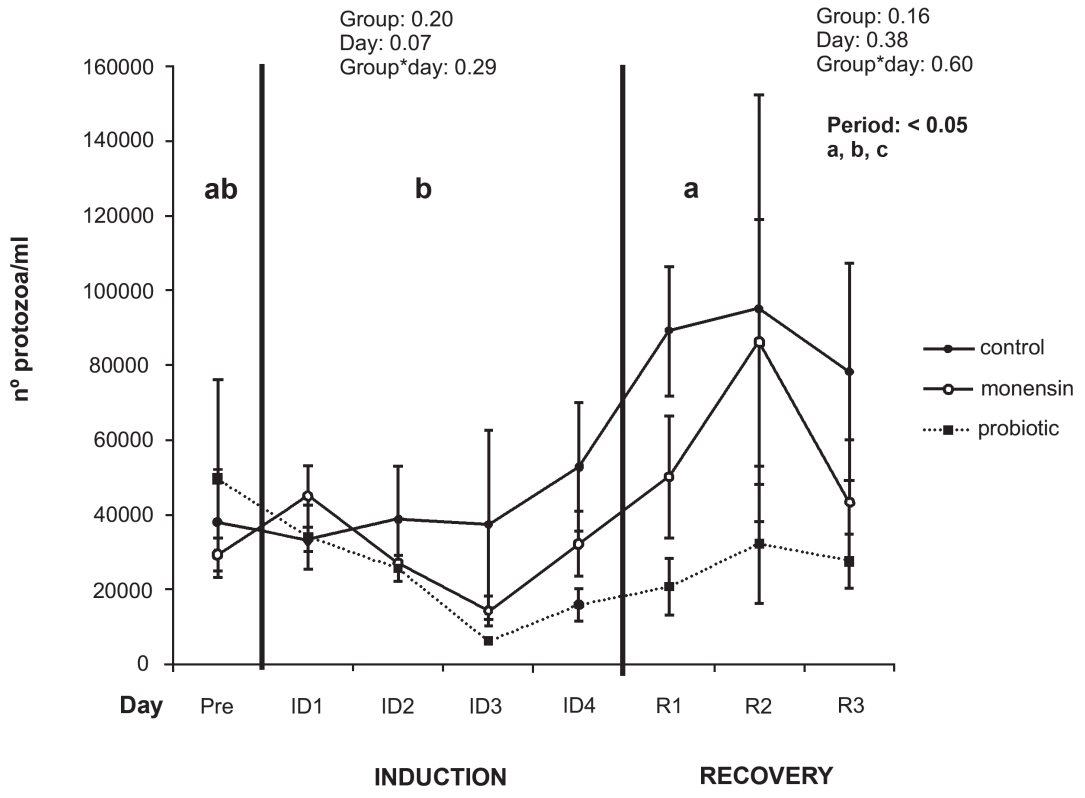

Figure 3 - Number protozoa/ml (means ± SEM) in the ruminal fluid of ewes subjected to SARA induction protocol, from control (n=6), monensin (n=6; 11 ppm/kg/DM/day of sodicmonensin) and probiotics groups (n=6; 8.4×104 UFC of Saccharomyces cerevisiae/ewe/day) during pre-induction (Pre), induction (ID1, ID2, ID3 e ID4) and recovery periods (R1, R2 e R3). Different letters indicates statistical significance among periods (P < 0.05)
Source: (SCHWEGLER et al., 2014)

Table 1 – Concentration of metabolic markers in ewes subjected to SARA induction protocol, during pre-induction, induction (ID1, ID2, ID3 e ID4) and recovery periods (R1, R2 e R3). Means represent an average for all days inside each period for the three groups combined (n = 18, n = 6/group) evaluated by ANOVA – Pelotas – 2014

Parameters	Pre-induction	Induction	Recovery	SEM*	*P values*		
					Period	Treatment	Treat.* Period
Ruminal chloride (mEq/L)	10.3b	19.4a	8.8b	1.0	<0.01	0.70	0.69
Serum chloride (mEq/L)	101.8	95.8	98.7	1.6	0.17	0.98	0.96
GGT†(U/L)	83.3b	105.0a	106.9a	3.2	<0.01	0.85	0.83
AST‡ (U/L)	53.9b	59.2a	59.9a	1.1	<0.01	0.98	0.52
LDH§ (U/L)	244.5a	226.0b	220.6b	4.5	<0.01	0.21	0.71
Sodium (mmol/L)	128.4	129.9	130.7	1.1	0.74	0.53	0.78
Potassium (mmol/L)	5.3	4.9	5.1	0.1	0.35	0.24	0.54
Magnesium (mg/dL)	2.3	2.4	2.3	0.1	0.95	0.57	0.57
Calcium (mg/dL)	9.4a	9.3a	8.3b	0.2	<0.01	0.43	0.93
Phosphorus (mg/dL)	4.9c	7.5a	6.2b	0.3	<0.01	0.90	0.50

* Standard Error of Mean
† Gama glutamil transferase, analyzed at 25ºC
‡ Aspartate amino transferase, analyzed at 25ºC
§ Lactate dehydrogenase, analyzed at 25ºC
Different superscripts in the same line indicate difference between periods (P < 0.05)

Since the induction protocol was able to maintain pH at SARA levels for a long period, it was used to test the effectiveness of probiotics and monensin to prevent SARA in experiment 2. In Experiment 2, none of the treatments were able to prevent the drop in the ruminal pH and damage to the ruminal flora caused by SARA induction. Mutsvangwa, Edwards and Topps (1992) observed similar results when supplementing probiotics to healthy dairy cows fed high amounts of concentrate, and attributed this reduction in ruminal fluid pH to an increased dry matter intake. Although it was demonstrated that probiotics stimulates the utilization of ruminal lactate (GIRARD et al., 1993), its effectiveness to prevent SARA is still variable (HADDAD; GOUSSOUS, 2005), and results depend on the intensity of production system. Regarding the use of monensin for controlling ruminal fluid pH, results are also not conclusive (GONZALEZ-MOMITA et al., 2009). These results are similar to those described by Gonzalez-Momita et al. (2009), indicating that there is no stability in the rumen pH fluid with the supplementation of monensin during SARA.

The reduced number of protozoa in the rumen during the SARA induction period agrees with a previous observation that there is a negative correlation between number of protozoa and SARA occurrence (NAGARAJA; TITGEMEYER, 2007) due to the rapid drop in ruminal pH (SUN; MAO; ZHU, 2010). The ruminal chloride concentration increased during the induction period, increasing the osmolality of the ruminal fluid. Such differences in osmolality relaxes the abomasum, by acting in osmoreceptors and affect the frequency of afferent impulses in the vagus nerve, affecting negatively the rumen-abomasum transit, disrupting the efficiency of fluid and acids removal from the ruminal fluid (OWENS et al., 1998). Nevertheless, it was not observed any changes in serum chlorides during SARA. In this regard, ruminal chloride concentration together with the pH has been shown to be an efficient marker of SARA occurrence in sheep.

Regarding serum biochemical parameters, there were no differences among groups during the SARA induction period, but some markers were different between periods. During metabolic acidosis, the blood cation-anion balance may be altered, and can change the acid-base balance. It was not observed changes in concentrations of Ca, Na, Mg, K or Cl during SARA induction. Phosphorous concentration was increased during SARA induction. Corroborating with our results, Fürll (1994) also observed high P concentrations in cattle with clinical acidosis, and used it as a potential marker for the disease state. The current results show that P can also be a marker for the sub-clinical form of acidosis, and can play a role in regulation of pH homeostasis. In ruminants, large amounts of phosphorus are recycled by saliva and by the rumen, leading to variations in levels of this mineral. The presence of this mineral in the rumen is necessary to maintain the activity of ruminal flora and consequently a proper digestion of food (BORGES et al., 2011).

There was an increased in enzyme activity of AST and GGT during SARA induction and recovery periods, indicating that there was some level of liver overload (BIONAZ et al., 2007). Although GGT is found in many tissues, the main source is the liver (primarily biliary epithelium), thus GGT is used mainly as a sensitive indicator of cholestasis (TENNANT, 1997). SARA induces pathological alterations in the rumen wall such as chronic inflammation, ulceration and/or hyperkeratosis (GABEL, 1990), which can lead to increased absorption of endotoxins (ERDMAN, 1993) causing liver damage (AINMALAMALI et al., 1992). On the other hand, LDH is produced by rumen bacteria and its function is to reverse the reaction lactate - pyruvate (BEAUCHEMIN et al., 2003), reducing serum LDH levels and lactate absorption at ruminal level (OWENS et al., 1998). In the current experiment, serum LDH concentrations during induction and recovery periods were lower than pre-induction period, indicating that ruminal metabolism was trying to reverse the SARA.

In conclusion, the SARA induction protocol was effective in reducing and keeping the ruminal fluid pH bellow 6.0. SARA induction lead to alterations in ruminal environment and systemic metabolism; however, the supplements used to control SARA were not effective in stabilizing ruminal fluid pH or avoid the changes in serum parameters.

Acknowledgements

The authors thank the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship provided to Elizabeth Schwegler.

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