**Revista Brasileira de Zootecnia**

ISSN 1806-9290
www.sbz.org.br

**R. Bras. Zootec., v.39, n.10, p.2262-2267, 2010**

# The role of pH on the survival of rumen protozoa in steers

**Raul Franzolin[1], Burk A. Dehority[2]**

*[1] Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de Sao Paulo, Pirassununga, SP, Brazil.*
*[2] Ohio Agricultural Research and Development Center, The Ohio State University, Wooster-OH, USA.*

**ABSTRACT** - In order to study the effect of pH on defaunation in the rumen, four rumen fistulated steers were fed a basal roughage diet for a 4-week adaptation period followed by 17 weeks of feeding with three diets and two feeding levels of high concentrate diet. Rumen outflow fluid rate was evaluated in both ration levels. Rumen protozoa population was monitored weekly and when animals became defaunated, protozoa were reinoculated with rumen contents from one of the faunated steers. At every two weeks, during all the experimental period, rumen pH was measured in all animals at 0, 4, 8 and 12 h after feeding. It was observed an individual animal influence on the establishment and maintenance of the rumen ciliate protozoa population. In all sampling times, mean rumen pH values were higher in faunated steers than in the defaunated ones. No differences were observed in rumen outflow fluid rates between the two ration levels. Extended periods of low rumen pH are probably more detrimental to the survival of ciliate protozoa in the rumen than other factors.

Key Words: cattle, ciliate protozoa, rumen microorganism, ruminal fauna

# Importância do pH na sobrevivência de protozoários no rúmen de bovinos

**RESUMO** - Com o objetivo de estudar efeito do pH sobre a defaunação no rúmen, quatro novilhos fistulados no rúmen foram alimentados com uma dieta basal com volumoso por um período de quatro semanas, seguido de 17 semanas com três dietas e dois níveis de alimentação com dieta rica em concentrado. A taxa de passagem do líquido ruminal foi avaliada em ambos os níveis de ração. A população de protozoários do rúmen foi monitorada semanalmente e, quando os animais se tornavam defaunados, protozoários eram reinoculados com conteúdo ruminal de um dos bovinos faunados. A cada duas semanas, durante todo o período experimental, o pH ruminal foi medido em todos os animais às 0, 4, 8 e 12 horas após a alimentação. Foi observada influência individual do animal sobre o estabelecimento e a manutenção da população de protozoários ciliados no rúmen. Em todos os tempos de amostragem, as médias de valores de pH ruminal nos animais faunados foram maiores que nos animais defaunados. Não foram observadas diferenças no rúmen na taxa de passagem do líquido ruminal entre os dois níveis de ração. Longos períodos de baixo pH no rúmen provavelmente são mais prejudiciais para a sobrevivência dos protozoários ciliados no rúmen que outros fatores.

Palavras-chave: bovinos, fauna ruminal, protozoários ciliados, microrganismos rúmen

## Introduction

Maintaining rumen pH is very important for persistence and stability of the gut microbiota. Rumen pH can vary from 5.5 to 7.5 and this variation is influenced by the type of diet and the feeding frequency according to different ruminant species (Franzolin et al., 2010). According to Clarke (1977) rumen ciliated protozoa are sensitive to changes in rumen pH and they cannot survive if the pH goes up above 7.8 or goes down below 5.0. Dehority (2005) reported death of in vitro protozoa at pH values below 5.4.

Animals without rumen protozoa often show an increase in bacterial numbers, a decrease in ammonia and volatile fatty acid (VFA) productions, and a decrease in organic matter digestibility, depending on the quality of the diet consumed by the animal. Biological value of bacterial and protozoal protein is assumed to be similar; however, the digestibility of protozoal protein is considerably greater than bacterial protein (Williams & Coleman, 1992). In addition, protozoa appear to have a buffering effect due to their rapid ingestion of starch granules, which slows the fermentation rate of ruminal bacteria and the subsequent production of organic acids (volatile fatty acid). This buffering effect tends to stabilize ruminal fermentation (Slyter, 1976) and to decrease the redox potential of rumen digesta (Jouany &Ushida, 1999).

Franzolin & Dehority (1996a) reported that the rumen environment varies among individual animals, with some

*Received August 14, 2009 and accepted March 24, 2010.*
*Corresponding author: rfranzol@usp.br*

animals showing markedly lower rumen pH values when fed either all-concentrate or 90% concentrate diets. In turn, these animals are either defaunated or have greatly reduced numbers compared to other animals. According to Franzolin & Dehority (1996a), factors other than a low rumen pH are involved in the survival of rumen ciliates, such as the length of time with a low pH, generation time of rumen ciliates and rumen outflow rate. Owens et al. (1998) suggested that on high concentrate diets, the prevalence of protozoa in the rumen typically declines, probably due to the lack of a fibrous floating mat in the rumen where the ciliate remain attached in order to multiply. According to Nagaraja & Titgemeyer (2007), a reduction in the number of the rumen ciliate population may be a good indicator of acute and subacute acidosis due to the accumulation of lactic acid or volatile fatty acids in the rumen, respectively.

The present study was conducted to evaluate if low ruminal pH and/or other "individual characteristics" of an individual animal host are responsible for elimination of the ciliate protozoa from the rumen. Variables used to alter rumen pH were diet and intake level.

## Material and Methods

Four Angus × Hereford steers, at an average body weight of 365.5 kg, were fistulated according to the procedures approved by The Ohio State University Institutional Animal Care and Use Committee. The animals were individually housed in pens with free access to both water and alfalfa hay during the four week adaptation period, as well as free access to water during the 17-week experimental period.

Three diets were used in this study: alfalfa pellets; mixed whole shelled corn (MWC), composed of 87.0% of whole corn, 4.8% of soybean meal, 6.3% ground corn, 1.4% limestone, 0.45% trace mineral salt, 0.05% selenium, 0.01% vitamin A, 0.01% vitamin D and 0.02% of Rumensin-60; and mixed whole corn pelleted in feed mill.

The experimental period was designed with all animals being fed at the same time over all the four periods: (1) 50% mixed whole corn plus 50% alfalfa pellets for six weeks; then (2) 65% mixed whole corn and 35% mixed whole corn pellets for four weeks followed by (3) mixed whole corn for three weeks and (4) mixed whole corn for four weeks. Each animal was fed 4.6 kg/day of dry matter intake (low feeding level) for the first 13 weeks, followed by feeding 7.3 kg dry matter intake/day (high feeding level) for the last four weeks.

Rumen ciliate concentrations were monitored weekly and if an animal became ciliate-free, it would be refaunated by adding 2 L of rumen contents from one of the faunated steers, usually the one with the highest protozoal count. For counting protozoa, rumen contents were manually mixed through the fistula and samples were obtained from the ventral sac approximately 30 minutes before feeding. The samples were fixed by diluting with an equal volume (v/v) of formalin solution (18.5% formaldehyde final concentration). If no protozoa were microscopically found on five separate slides, then the animal was considered to be defaunated. Total counts of protozoa were made in 100 microscopic fields at a magnification of 100 times according to Dehority (1993). Briefly, 1 mL of the fixed rumen contents from faunated steers were pipetted using a wide-mouth pipette into a test tube and two drops of brilliant green dye (2 g brilliant green and 2 mL glacial acetic acid diluted to 100 mL with distilled water) were added. The test tube was mixed thoroughly and allowed to be kept overnight at room temperature. Further dilutions were made with 30% (v/v) glycerol.

Rumen pH was measured at two week intervals over the experimental period, i.e., weeks 1, 3, 5, 7, 9, 11, 13, 15 and 17. Samples of whole rumen contents were taken approximately 30 minutes before feeding (time zero), 4, 8 and 12 h after feeding. The pH of the sample was determined immediately after each collection with a digital pH meter.

Liquid outflow rate and dry matter of rumen contents were measured on the 100% mixed whole corn diet at both low and high intake levels (4.6 and 7.3 kg/d of dry matter intake). It was used 100 g of polyethylene glycol (PEG) 4000 as marker and added into the rumen via fistula 1 h before feeding and samples of rumen contents were removed immediately before feeding, and at 1 h and 24 h after feeding. The concentration of polyethylene glycol in the samples was measured by turbidimetry according to the procedure of Hyden (1955). Five aliquots of rumen contents, composed of both liquid and solid parts, were taken from different rumen sites of each steer just before polyethylene glycol was added. The percentage of dry matter was determined from approximately 20 g of each sample by drying in an oven at 100$^{o}$C for 24 h. The data of rumen pH, liquid outflow rate and dry matter or rumen contents were analyzed by repeated measures analysis of variance (ANOVA), using the Statistica computational software (Statsoft, Statistics for Window ver. 6.0). The model included effects caused by the animal, rumen sampling time and feeding level.

## Results and Discussion

Individual animal differences affected the establishment and maintenance of the rumen ciliate population (Table 1).

Steer 1 had a high number of protozoa during the first two weeks. However, it became defaunated after week 2 and it was unable to maintain very high numbers of protozoa thereafter. Steer 2 had high numbers of protozoa for the first eight weeks, but it became defaunated at week 10. After that, ciliate numbers remained low throughout the experiment. Steer 3 became defaunated after the first three weeks, remained defaunated during three weeks and it was then able to reestablish and maintain a high number of rumen ciliate for the next nine weeks before becoming defaunated again. Steer 4 began the experiment ciliate-free and it was unable to establish a stable population over the 17 weeks of the experiment. Towards the end of the experiment, these animals showed symptoms of clinical acidosis, specifically swelling of the joints.

Average rumen pH values differed (P<0.01) between faunated and defaunated animals at all times after feeding (Table 2). The overall rumen pH was higher in faunated than in defaunated steers with mean pH values of 5.98 and 5.50, respectively. In both faunated and defaunated animals, the pH was the highest before feeding, but was lower 4 h after feeding.

Results suggest that rumen pH plays an important role on the survival of rumen ciliated protozoa as researchers have observed differences in rumen protozoa under different pH levels in several feeding systems (Williams & Coleman, 1992; Dehority, 2003). The present data support this finding (Tables 1 and 2). For example, daily rumen pH values at 0, 4, 8 and 12 h ranged from 5.73 to 6.28 in faunated animals as compared to pH 5.31 to 5.81 in defaunated animals. These

Table 1 - Weekly monitoring of rumen ciliate population in steers offered three different diets and two feed intake levels on mixed whole shelled corn diet[1]

| Weeks | | Protozoa number (× $10^5$)/mL rumen content | | | |
|---|---|---|---|---|---|
| | Feed intake (kg dry matter intake/animal/day) | Steer 1 | Steer 2 | Steer 3 | Steer 4 |
| | 50% mixed whole corn:50% alfafa pellets | | | | |
| 1 | 4.6 | 24.8 | 16.8 | 28.2 | Defaunated |
| 2 | 4.6 | 39.6 | 26.7 | 10.1 | Defaunated |
| 3 | 4.6 | Defaunated | 13.6 | 18.6 | Defaunated |
| 4 | 4.6 | 1.75 | 17.2 | Defaunated | 9.4 |
| 5 | 4.6 | 2.69 | 13.5 | Defaunated | 13.3 |
| 6 | 4.6 | 16.9 | 12.0 | Defaunated | 1.5 |
| | 65% mixed whole corn:35% mixed whole corn pellets | | | | |
| 7 | 4.6 | 14.2 | 21.8 | 26.0 | 0.12 |
| 8 | 4.6 | 0.79 | 23.0 | 22.3 | Defaunated |
| 9 | 4.6 | Ddefaunated | 1.2 | 48.4 | 12.6 |
| 10 | 4.6 | Defaunated | Defaunated | 16.7 | Defaunated |
| | 100% mixed whole corn | | | | |
| 11 | 4.6 | 0.46 | 14.4 | 26.8 | 0.02 |
| 12 | 4.6 | 0.29 | 12.1 | 17.8 | Defaunated |
| 13 | 4.6 | 0.86 | 3.9 | 9.3 | 0.62 |
| 14 | 7.3 | Defaunated | 3.7 | 12.5 | 0.28 |
| 15 | 7.3 | Defaunated | Defaunated | 32.8 | Defaunated |
| 16 | 7.3 | 0.53 | Defaunated | Defaunated | 0.06 |
| 17 | 7.3 | 0.12 | 2.3 | 0.67 | 8.5 |

[1]Each time a steer became defaunated, it was inoculated with rumen contents from one of the faunated steers.
All diets were fed at 4.6 kg per day from week 1 through week 13. Intake was increased to 7.3 kg per d for weeks 13-17.

Table 2 - Average rumen pH values at 0, 4, 8 and 12 h after feeding in rumen samples collected during all experimental period in both faunated and defaunated steers

| Hours after feeding | Rumen pH in faunated steers | SEM | Rumen pH in defaunated steers | SEM | P-value |
|---|---|---|---|---|---|
| 0 | 6.27 ± 0.35 | 0.079 | 5.81 ± 0.37 | 0.106 | 0.0015 |
| 4 | 5.73 ± 0.30 | 0.067 | 5.31 ± 0.28 | 0.082 | 0.0005 |
| 8 | 5.85 ± 0.30 | 0.068 | 5.37 ± 0.28 | 0.080 | 0.0001 |
| 12 | 5.98 ± 0.31 | 0.069 | 5.52 ± 0.31 | 0.088 | 0.0003 |
| Mean | 5.98 ± 0.37 | 0.041 | 5.50 ± 0,36 | 0.052 | 0.0001 |

SEM = standard error of mean.

values were lower than those observed by Veira et al. (1983) from 6.3 to 6.6 for faunated and 5.95 to 6.85 for defaunated sheep receiving corn silage:corn (1:1) diets. Santra & Karim (2002) also observed that the pH and ammonia concentration of rumen liquor decreased while production of total VFA and total nitrogen increased in the absence of rumen ciliate protozoa in lambs.

There was no difference (P>0.05) in the outflow rate of rumen fluid and dry matter (DM) of rumen contents, measured on the mixed whole shelled corn diet among the four steers with an intake of either 4.6 kg dry matter intake/day (mean 4.3%/hour and 11.84%) or 7.3 kg DMI/d (mean 4.5%/h and 11.91%), respectively. However, the steers with the highest dry matter intake had a larger (P<0.05) rumen volume and total DM of rumen contents (24 L and 38 kg) than those fed the lower level (17.8 L and 28 kg), respectively.

For many years, it was assumed that the decrease in numbers or complete disappearance of protozoa in animals fed high concentrate diets was the result of the drastic reduction in rumen pH caused by these types of diets (Eadie, 1962; Vance et al., 1972; Mackie et al., 1978). Mendoza et al. (1993) observed no differences in rumen fluid pH between defaunated and faunated sheep, when pH was measured three hours after feeding. However, these authors observed higher amylolytic activity and osmotic pressure in defaunated animals. Owens et al. (1998) also cited that rumen protozoa have high amylase activity and when protozoa lysis occurs, due to changes in pH or osmolarity, amylase is released from the lysed cells into the surrounding environment. This can contribute to accelerate glucose production from starch which potentially may exacerbate acidosis. Veira et al. (1983) also observed a less abrupt decrease in rumen pH in faunated sheep compared to ciliate free animals.

In spite of other factors involved in the survival of rumen ciliate protozoa such as osmotic pressure (Mendoza et al., 1993), quality and the quantity of the dry matter intake, solid and fluid outflow rates, diurnal variation (Dehority, 2003) and individual animal variability on rumen environment (Franzolin & Dehority, 1996a), the present experiment only focused on rumen pH. During the 13 weeks when the steers were fed different diets at low level (4.6 kg DM/animal/day), they were able to maintain relatively high number of protozoa reaching up to $4.8 \times 10^6$ cells per mL of rumen contents, except in those cases when they became defaunated (Table 1). In general, on a higher feeding level (7.3 kg dry matter intake/animal/day), low population of protozoa and more defaunation were observed, except in steer 3. However, the liquid outflow rate, which could be a factor in the washout of ciliates from the rumen in animals fed on high concentrate diet (Dehority, 2003), was not different between the faunated and defaunated animals. The average rumen liquid outflow rate (11.8 %/hour) was similar to the values reported by Goetsch & Owens (1985) with cattle on high concentrate diets (10.3 %/h). So, in the present experiment, the amount of feed intake had more influence than the type of diet on the ciliate protozoa, probably promoting increase of the bacterial fermentation rate on high concentrate diets and consequently decreasing the rumen pH.

Nagaraja & Towne (1990) also observed fluctuation of the rumen ciliate population in cattle fed high concentrate diets as a response to the dynamic rumen environment. These authors considered the fluctuation in rumen ciliate number under relatively low pH conditions as the main cause of reduction or defaunation of ciliates in the rumen.

In general, rumen pH is higher in faunated than defaunated animals (Williams & Coleman, 1992). Veira et al. (1983) observed a less abrupt decrease in rumen pH in faunated sheep compared to ciliate free animals. Because starch granules are engulfed by protozoa, especially on high concentrated diets, starch degradation is slower (Hristov et al., 2001), resulting in less production of organic acids in the rumen of faunated steers (Santra & Karim, 2002; Santra et al., 2007). The stabilization effect of rumen pH attributed to rumen ciliates appears to have a significant effect on bacterial growth and enzyme activity since they can be inhibited at low pH values (Williams & Coleman, 1992). The moderation of the ruminal fermentation rate by ciliated protozoa was attributed to reduced bacterial population (Nagaraja et al., 1992). To test the influence of rumen pH on survival of rumen ciliates, investigations using different levels of dietary sodium bicarbonate ($NaHCO_3$) could be performed. Previous studies indicated a linear increase of rumen pH with increasing dietary supplementation of $NaHCO_3$ in lambs fed high concentrate diets (Tripathi et al., 2004).

Even though the present study focused on the total number of rumen ciliates, in vitro studies have been performed to evaluate the effect of rumen pH on the viability of different species of protozoa (Dehority, 2005). Some researchers have concluded that *Entodinium* species are more tolerant to low pH than other genera of rumen protozoa (Mackie et al, 1978; Lyle et al., 1981). However, Dehority (2005) did not find evidence to suggest *Entodinium* as an acid-tolerant species in the rumen and suggested the possibility of an unknown change in the rumen environment favorable for the species of *Entodinium*.

Franzolin & Dehority (1996b) observed that individual variation in the consumption of diets affected the fauna and rumen pH in sheep fed high concentrated diets. In general,

animals became defaunated when rumen pH values were lower than 5.5 for at least 15 hours per day. Dehority (2005) was able to maintain four species of rumen ciliate in vitro circa pH 5.8, and concluded that defaunation occurred when pH fell below 5.4 for an extended period of time.

There is usually a decrease in rumen pH after feeding, up to 8 to 12 h, followed by an increase in rumen pH until the next feeding (24 h) (Nagaraja & Titgemeyer, 2007). The same daily rumen pH was observed in animals with feeding frequencies from two to six times per day. However, at the higher feeding frequency, the rumen pH was lower as a result of higher feed intake (Krause & Oetzel, 2006). The largest drop in rumen pH occurred from 0 to 4 h after feeding in both cases being of 0.55 pH units to faunated and 0.50 to defaunated status (Table 2) contrasting with Veira et al. (1983) who observed a less abrupt decrease in rumen pH in faunated sheep compared to protozoal free animals. Variation of 0.5-1.0 rumen pH units represents a five to 10 fold change in hydrogen ion concentration in the rumen (Krause & Oetzel, 2006). Mendoza et al. (1993) observed no differences between defaunated and faunated sheep on rumen fluid pH, measured 3 hours after feeding.

In the present experiment, the daily rumen pH values were higher in faunated animals with a mean pH of 5.98 than in defaunated animals with a mean pH around 5.50. These values were lower than those reported by Nagaraja et al. (1992), who also observed that faunated steers had higher ruminal pH values (mean pH 6.45) than ciliate-free steers (mean pH 5.97) fed on high grain diets. So, the extension of rumen pH over the 24 hour is probably the most relevant factor in the survival of ciliate protozoa in the rumen.

Table 3 - Average rumen pH values in the individual steers during faunation and defaunation in rumen sample collected during experimental period

| Steer | Rumen pH in faunated | SEM | Rumen pH in defaunated | SEM | P value |
|---|---|---|---|---|---|
| 1 | 5.72b +/- 0.30 (20)* | 0.067 | 5.52ab +/- 0.42 (12) | 0.122 | 0.07 |
| 2 | 6.18a +/- 0.32 (24) | 0.065 | 5.76a +/- 0.37 (8) | 0.130 | 0.002 |
| 3 | 6.00a +/- 0.36 (28) | 0.068 | 5.19b +/- 0.21 (4) | 0.106 | 0.0001 |
| 4 | 5.75b +/- 0.28 (8) | 0.099 | 5.46ab +/- 0.29 (24) | 0.060 | 0.01 |

*Number of observation of the steers with or without protozoa. Two sampling days per period, four samples per day for a total of 32 measurements.
Values following different lower case letters in same column differ (P<0.05).
SEM = standard error of mean.

Individual average rumen pH measured in all rumen samples during the 17 week experiment were significantly different in steers 1, 2 and 3 when they were either faunated or defaunated (Table 3).

Individual animal variation was observed in the present experiment (Table 3). When faunated, the rumen pH of steers 2 and 3 were alike, whereas the rumen pH of steers 1 and 4 were similar to each other. This individual animal variation was similar to that reported by Franzolin & Dehority (1996a) where two steers showed a tendency to have lower daily pH and no protozoa, whereas the inverse occurred in the three other steers. During the last four weeks of the experiment, when the animals had a higher level of feed intake (7.3 kg/day dry matter intake) they became defaunated for most of the time, or had very low ciliate numbers, except the steer 3 which maintained a high number of protozoa for two weeks. According to Krause & Oetzel (2006) total dry matter intake is a major determinant of rumen pH and individual animal difference in rumen pH depends on the capacity of the animal to buffer and to absorb organic acids produced in the rumen which determines the drop of rumen pH after feeding large amounts of fermentable carbohydrates.

## Conclusions

There are individual animal differences regarding to the re-establishment and maintenance of the rumen ciliate population. The maintenance of low pH in the rumen environment throughout the day appears to be a major contributor to eliminate ciliated protozoa. However, the ability of the individual animal to control rumen pH has a marked influence on the rumen ciliate population, but this effect still needs to be clarified.

## References

CLARKE, R.T.J. Protozoa in the rumen ecosystem. In: CLARKE, T.T.J.; BAUCHOP, T. (Eds). **Microbial ecology of the gut**. New York: Academic Press, 1977. p.251-275.

DEHORITY, B.A. **Laboratory manual for classification and morphology of rumen ciliate protozoa**. Boca Raton: CRC Press, 1993. 96p.

DEHORITY, B.A. **Rumen microbiology**. Thrumpton, Nottingham: Nottingham University Press, 2003. 372p.

DEHORITY, B.A. Effect of pH on viability of *Entodinium caudatum*, *Entodinium exiguum*, *Epidinium caudatum*, and

*Ophryoscolex purkynjei* in vitro. **Journal of Eukaryotic Microbiology**, v.52, p.339-342, 2005.

EADIE, J.M. The development of rumen microbial populations in lambs and calves under various conditions of management. **Journal of General Microbiology**, v.29, p.563-578, 1962.

FRANZOLIN, R.; DEHORITY, B.A. Effect of prolonged high-concentrate feeding on rumen protozoa concentrations. **Journal of Animal Science**, v.74, p.2803-2809, 1996a.

FRANZOLIN, R.; DEHORITY, B.A. Effects of rumen pH and feed intake on defaunation in sheep fed high concentrate diets. **Revista Brasileira de Zootecnia**, v.25, p.1207-1215, 1996b.

FRANZOLIN, R.; ROSALES, F.P.; SOARES, W.V.B. Effects of dietary energy and nitrogen supplements on rumen fermentation and protozoa population in buffalo and zebu cattle. **Revista Brasileira de Zootecnia**, v.39, p.549-555, 2010.

GOETSCH, A.L.; OWENS, F.N. Effects of sampling site on passage rate estimates in heifers fed alfalfa hay or a high concentrate diet. **Journal of Dairy Science**, v.68, p.914-922, 1985.

HRISTOV, A.N.; IVAN, M.; RODE, L.M. et al. Fermentation characteristics and rumen ciliate protozoal populations in cattle fed medium or high barley based diets. **Journal of Animal Science**, v.79, p.515-524, 2001.

HYDEN, S. A turbidimetric method for the determination of higher polyethylene glycols in biological materials. **Kungl Lanthbrukshogskolans Annaler**, v.22, p.139-145, 1955.

JOUANY, J.P.; USHIDA, K. The role of protozoa in feed digestion - Review. **Asian-Australasian Journal of Animal Science**, v.12, p.113-128, 1999.

KRAUSE, K.M.; OETZEL, G.R. Understanding and preventing subacute rumen acidosis in dairy herds: a review. **Animal Feed Science and Technology**, v.126, p.215-236, 2006.

LYLE, R.R.; JOHNSON, R.R.; WILHITE, J.V. et al. Rumen characteristics in steers as affected by adaptation from forage to all concentrate diets. **Journal of Animal Science**, v.53, p.1383-1390, 1981.

MACKIE, R.J.; GILCHRIST, A.M.; ROBERTS, A.M. et al. Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. **Journal of Agriculture Science**, v.90, p.241-254, 1978.

MENDOZA, G.D.; BRITTON, R.A.; STOCK, R.A. Influence of rumen protozoa on site and extent of starch digestion and rumen fermentation. **Journal of Animal Science**, v.71, p.1572-1578, 1993.

NAGARAJA, T.G.; TOWNE, G. Ciliated protozoa in relation to rumen acidosis and lactic acid metabolism. In: HOSHINO, S.; ONODERA, S.; MINATO, R.H. et al. (Eds). **The rumen ecosystem**: the microbial metabolism and its regulation. New York: Springer-Verlag, 1990. p.187-194.

NAGARAJA, T.G.; TOWNE, G.; BEHARKA, A. Moderation of ruminal fermentation by ciliated protozoa in cattle fed a high-grain diet. **Applied and Environmental Microbiology**, v.58, p.2410-2414, 1992.

NAGARAJA, T.G.; TITGEMEYER, E.C. Rumen acidosis in beef cattle: the current microbiological and nutritional outlook. **Journal of Dairy Science**, v.90(E. Suppl.), p.E17-E38, 2007.

OWENS, F.N.; SECRIST, D.S.; HILL, W.J. et al. Acidosis in cattle: a review. **Journal of Animal Science**, v.76, p.27-286, 1998.

SANTRA, A.; KARIM, S.A.; CHATURVEDI, O.H. Rumen enzyme profile and fermentation characteristics in sheep as affected by treatment with sodium lauryl sulfate as defaunating agent and presence of ciliate protozoa. **Small Ruminant Research**, v.67, p.126-137, 2007.

SANTRA, A.; KARIM, S.A. Influence of ciliate protozoa on biochemical changes and hydrolytic enzyme profile in the rumen ecosystem. **Journal of Applied Microbiology**, v.92, p.801-811, 2002.

SLYTER, L.L. Influence of acidosis on rumen function. **Journal of Animal Science**, v.43, p.910-929, 1976.

STATSOFT INC. **Statistica for Windows**. Tulsa: Statsoft, 1995, v.1. 878p.

TRIPATHI, M.K.; SANTRA, A.; CHATURVEDI, O.H. et al. Effect of sodium bicarbonate supplementation on rumen fluid pH, feed intake, nutrient utilization and growth of lambs fed high concentrate diets. **Animal Feed Science and Technology**, v.111, p.27-39, 2004.

VANCE, R.D.; PRESTON, R.L.; KLOSTERMAN, E.W. et al. Utilization of whole shelled and crimped corn grain with varying proportions of corn silage by growing-finishing steers. **Journal of Animal Science**, v.35, p.598-605, 1972.

VEIRA, D.M.; IVAN, M.; JUI, P.J. Rumen ciliate protozoa: effects on digestion in the stomach of sheep. **Journal of Dairy Science**, v.66, p.1015-1022, 1983.

WILLIAMS, A.G.; COLEMAN, G.S. **The rumen protozoa**. New York: Springer-Verlag, 1992. 423p.