Effect of an energy-deficient diet on populations of ciliate protozoans in bovine rumen

[*Efeito da deficiência de energia na dieta sobre a população de protozoários ciliados do rúmen de bovinos*]

P.C. *Soares*[1], I. *Martinele*[2], M. *D'Agosto*[2], C.A. *Maruta*[3], M.C.A. *Sucupira*[4],
A.C. *Antonelli*[4], C.S. *Mori*[4], E.L. *Ortolani*[4]

[1]Departamento de Medicina Veterinária - UFRPE
Rua Dom Manoel de Medeiros, s/n
52171-900 – Recife, PE
[2]Departamento de Zoologia - UFJF – Juiz de Fora, MG
[3]Indústria Vallée – São Paulo, SP
[4]Faculdade de Medicina Veterinária e Zootecnia - USP – São Paulo, SP

ABSTRACT

Ten young rumen-cannulated crossbred steers were randomly divided into two groups: a control group (C; n=4), which was fed a balanced diet for daily weight gain of 900g; and a pronounced energy-deprived group (PED; n=6), receiving 30% less of the required energy for maintenance. After 140 days of these alimentary regimes, rumen fluid and urine samples were collected for biochemical and functional tests, before feeding and at 1, 3, 6, and 9 hours after feeding. The energy-deprivation diet caused a significant reduction in the number of *Entodinium*, *Eodinium*, *Isotricha*, *Dasytricha*, *Eremoplastron*, *Eudiplodinium*, *Metadinium, Charonina, Ostracodinium*, and *Epidinium* protozoa. There was no effect of the time of sampling in both groups on the total number of ciliates in rumen fluid. A higher number of protozoan forms in binary division were recorded in the control group, at the 6th and 9th hours after feeding (P<0.019). There was a high positive correlation between the total count of protozoans in rumen fluid and glucose fermentation, ammonia, and urinary allantoin excretion index; and a negative correlation between the total count of protozoa and metilene blue reduction, and a medium correlation between the total count of protozoa and total volatile fatty acids concentration. The determination of the protozoa populations does not imply in the use of complex and hard-to-execute techniques, although it is time consuming and needs practice. This exam particularly helps in clinical expected diagnosis.

Keywords: bovine, rumen, energy deprivation, ruminal metabolism

RESUMO

Foram utilizados 10 novilhos mestiços com cânula ruminal, distribuídos em dois grupos: no grupo controle (C; n=4) receberam dieta balanceada para ganho diário de 900g; no grupo tratado com carência pronunciada de energia (CP; n=6), receberam dieta com 30% a menos do nível de manutenção em energia. Após 140 dias sob esses regimes de alimentação, foram coletadas amostras do fluido ruminal e urina, para realização de provas bioquímicas e funcionais, antes e às 1, 3, 6 e 9 horas após o fornecimento do alimento. A carência energética resultou em diminuição significativa na quantidade dos protozoários Entodinium, Eodinium, Isotricha, Dasytricha, Eremoplastron, Eudiplodinium, Metadinium, Charonina, Ostracodinium e Epidinium. *Não houve efeito da hora de coleta sobre o total de ciliados nos grupos C e CP. Maior número de formas em divisão binária foi registrado no grupo C, na sexta e nona horas pós-alimentação (P<0,019). Observaram-se altas correlações positivas entre a contagem total de protozoários e a fermentação de glicose, amônia e o índice de excreção urinária de alantoína e negativa entre a contagem total de protozoários e a redução do azul de metileno, e correlação média entre a contagem total de protozoários e os ácidos graxos voláteis totais. A determinação da população de*

protozoários do rúmen é um método simples de avaliação, além de que particularmente auxilia o diagnóstico clínico da função ruminal.

Palavras-chave: bovino, rúmen, carência energética, metabolismo ruminal

INTRODUCTION

Tropical forage plants grow less in the dry season, have lower protein and energy content and higher crude fiber content. Ingestion of dry matter by ruminants decreases significantly in this period of the year, because of the slower growth rate of the forage and its lower digestibility, leading to less energy and protein intake resulting in significant loss of body weight.

Ciliated protozoa constitute an important fraction of the total microbial population in the ruminal ecosystem. Because ciliated protozoa are preferentially retained in the rumen and do not significantly contribute to the postruminal nutritive supply, their overall value to the host is debatable. In animals fed low-protein and energy diets, ciliated protozoa apparently have a negative effect on growth and performance (Nagaraja et al., 1992).

The lower intake of nutrients affects cattle along with their ruminal microbiota (Sucupira, 2003). The number of microorganisms decreases as does the amount of theirs final fermentation products, which are used as a source of energy by the hosts (Oetzel, 1988). Rumen ciliate protozoa play diverse and important roles in ruminal metabolism of nutrients (Williams and Coleman, 1992; Hristov et al., 2001) and the feed level has been suggested as one of the factors which influence the ciliate protozoan population in the rumen (Potter and Dehority, 1973; Leng et al., 1981; Franzolin and Dehority, 1996).

Although the significance of ciliated protozoa in feedlot cattle is uncertain, high average protozoan concentrations imply a beneficial contribution to the fermentation process (Towne et al., 1990; Franzolin et al., 2000; Siqueira, 2002). There is now considerable information indicating that the energy-deficient diet decreases the amount of ruminal microbial.

A study concerning the metabolic activities of the rumen in the interval after feeding and its possible consequences, both for the ruminant and the rumen microbiota, found that there are changes in the number and diversity of ciliate protozoa because of their behavior in response to the physiological alterations that occur in their host after feeding (Hobson and Wallace, 1982).

The objectives of the present study were to evaluate the effects of feeding time on the ciliate protozoa of the rumen of bovines fed diets deficient or not in energy, and to estimate their correlations with biochemical and functional tests on the ruminal fluid and urine.

MATERIAL AND METHODS

Ten healthy Girolando steers, aging approximately 10-month-old and an averaging live weight of 165kg in the beginning of the experiment, were used in this study. Sixty days before the start of the assays, latex cannulas were implanted in the rumens. The steers were kept in individual stalls during the entire experiment, receiving water and mineral salt[1] *ad libitum*.

The steers were randomly allocated in two groups: a control group (C; n=4) and a group with pronounced energy deficient diet (PED; n=6). The control group received a balanced diet for an average weight gain of 900g/d, which provided an average of 17.7Mcal/d of digestible energy (DE). The PED group received a diet with a 30% energy deficit (an average of 5.25Mcal/d of DE) in relation to the requirement content for their maintenance. Although the dietary percentage of crude protein was lower in the PED group (7%) than in the control (13%), the amount of protein offered in the former group still met the requirements for maintaining steers (Nutrient…, 1996), and was not as limited as the energy content was. The diets were formulated using the following food elements: A - coast-cross hay; B – hydrolyzed sugarcane bagasse; C – commercial concentrate for dairy cattle. The breakdowns of these ingredients for the C and PED groups were 27%, 14%, and 59% and 4%, 70%, and 26% to A, B, and C, respectively. All the rations were mixed before being offered to the animals. Table 1 shows the bromatological composition of the foods. The quantity of dry matter daily offered corresponded to 2.8% of each live weight of the animals.

---

[1]Fosbovi 20 – Tortuga® - São Paulo, Brazil.

Arq. Bras. Med. Vet. Zootec., v.60, n.1, p.148-155, 2008

149

Table 1. Bromatological composition of coast-cross hay, concentrated feed, and hydrolyzed sugarcane bagasse (HSB) during the experimental period

Composition	Foods		
	Coast-cross hay	Feed concentrated	HSB
% DM	87.17	84.27	63.57
% CF	31.21	6.44	42.65
% CP	7.05	17.63	1.42
% EE	1.75	5.11	1.27
% MM	6.38	9.45	3.08
% NFE	53.61	61.37	51.58
% TDN	54.33	74.88	49.75
% NDF	76.20	27.01	90.02
% ADF	38.13	12.42	62.86

DM: dry matter; CF: crude fiber; CP: crude protein; EE: ether extract; MM: mineral matter; NFE: nitrogen free extract; TDN: total digestible nutrients; NDF: soluble neutral detergent fiber; ADF: soluble acid detergent fiber.

The animals were evaluated every two weeks by visual inspection and blood samples that were collected to define the energy according to some biochemical indicators, such as high fatty acids and low glucose in the blood plasma, besides low weight and overall body condition. The blood samples were drawn from the jugular vein in glass transfer tubes equipped with glide needles[2]. The individual food consumption was weekly recorded.

The animals were considered to be suffering from energy lack 140 days after beginning the energy-deficient diet according to the farmer indicators. At that point, ruminal fluid (RF) samples were taken from the cannula at the mid-part of the ventral sack, along with urine samples (U), after stimulation of the prepuce. This was done just before feeding time and 1, 3, 6, and 9 hours after feeding, to determine the kinetics of the protozoan population in the rumen and its relation with the other ruminal parameters (pH, methyl blue reduction, ammonia, glucose fermentation, and volatile fatty acids), as well as urinary parameters (creatinine and allantoin).

The ruminal fluid samples were fixed in 18.5% (v/v) formalin and evaluated qualitatively and quantitatively for ciliate genera according to the technique of Dehority (1984) with the modifications proposed by D'Agosto and Carneiro (1999). The identification of the ciliates was based on Ogimoto and Imai (1981).

The pH of the RF was determined immediately after collection, using a digital potentiometer. A sample of the RF was promptly frozen at -20^oC

for subsequent ammonia analysis using a diagnosis kit[3], and another was mixed with a phosphoric acid solution for analysis of volatile fatty acids (VFA) by gas chromatography[4] (Erwin et al., 1961). Urine samples were centrifuged at 250g, apportioned into 3-ml plastic test tubes and stored at -20^oC, for analysis of creatinine (Lutsgarten and Wenk, 1972) and allantoin (Borchers, 1977). The creatinine in the urine was determined to enable the correction of the urinary allantoin excretion index (UAEI), according to Chen et al. (1990). The methyl blue reduction (MBR) and glucose fermentation (GF) tests were performed according to Rosemberger (1993).

Data distribution was tested for normality using Kolmogorov-Smirnov test. A simple transformation using natural logarithm was applied to the total count of each protozoan genus. The effects of group and collecting time of samples were evaluated by analysis of variance with repeated measures using the general linear models procedures of SAS computational program (User's …, 2000). The minimum significant differences of the Tukey test were used for the contrasts between logarithmic means, and Pearson's correlation coefficient to study the pair relations (Little and Hills, 1978). Significance was inferred at $P<0.05$.

RESULTS AND DISCUSSION

Organisms of the following genera were identified and counted: *Entodinium*, *Eodinium*,

---

[2]Becton, Dickinson Vacutainer – Franklin Lakes, USA.

[3]Raichem/Sigma (no.735-10) – St. Louis, USA.
[4]Finnigan - Model 9001 gas chromatograph – San Jose, USA.

150

Arq. Bras. Med. Vet. Zootec., v.60, n.1, p.148-155, 2008

Isotricha, Dasytricha, Eremoplastron, Eudiplodinium, Diploplastron, Metadinium, Charonina, Ostracodinium, and *Epidinium.*

The total average number of ciliates per milliliter of rumen content was greater in the control group animals than in the group of animals receiving the energy-deficient diet (P<0.0001). The genus *Entodinium* was predominant, with 73.3% for the control group and 77.3% for the PED group, while *Ostracodinium* was identified the least often (C – 20%; PED – 27%). Exception of the genus *Diploplastron*, which showed no alteration (P>0.17), the energy-deficient diet caused significant reductions of the other protozoa genera. The overall counts and their respective percentages are presented in Table 2.

The time of collection significantly influenced the total protozoan counts between the groups (Table 3), but no differences were identified according to the collection time within the groups (P>0.05).

Table 4 shows the data on the number of protozoa undergoing binary division. As can be seen, there were differences among times (P<0.019) for the control group steers, with the greatest number of divisions recorded 6 and 9 hours after feeding. In the PED group, this number was higher at 1 and 3 hours after feeding. Higher numbers of organisms in binary division were observed in the control group animals than in the PED group steers, with differences between groups at the 6th (P<0.003) and 9th (P<0.0001) hours.

Table 5 shows the correlation coefficients between the studied variables. There was a high positive correlation between the total count of protozoa and the variables glucose fermentation and ammonia and urinary allantoin excretion index. Besides this, there was a high negative correlation between the total number of protozoa and methyl blue reduction and a moderate positive correlation between the total number of protozoa and the concentration of total volatile fatty acids.

Table 2. Total (x10^4) and relative (%) counts of protozoa in the rumen of bovines receiving control (C) and pronounced energy-deficient (PED) diets

Protozoan genus	Group		
	C	PED	P
Entodinium	1080.3a (73.34)	231.7b (77.32)	0.0001
Eodinium	52.16a (3.54)	1.28b (0.43)	0.0001
Isotricha	77.28a (5.25)	6.24b (2.08)	0.0001
Dasytricha	56.96a (3.87)	1.12b (0.37)	0.0001
Eremoplastron	95.36a (6.47)	11.36b (3.80)	0.0001
Eudiplodinium	11.04a (0.75)	2.08b (0.70)	0.006
Diploplastron	4.32a (0.29)	1.44a (0.48)	0.169
Metadinium	8.8a (0.60)	1.60b (0.53)	0.009
Charonina	58.24a (3.95)	38.4b (12.81)	0.006
Ostracodinium	2.88a (0.20)	0.80b (0.27)	0.018
Epidinium	25.60a (1.74)	3.68b (1.23)	0.0001
Total	1472.96a	299.68b	0.0001

Different small letters in the same row indicate significant difference between groups.

Table 3. Total count, average values, and percentile cut-off points (x10^4) of protozoa in the rumen of bovine receiving control (C) and pronounced energy-deficient (PED) diets, at different collection times

Group		0h	1h	3h	6h	9h	P
C	MD	5.6a	18.24a	9.92a	9.6a	5.44a	0.962
	(P_{25};P_{75})	2.56;10.0	1.12;22.8	1.92;14.16	1.44;10.72	1.44;8.40	
PED	MD	0.64b	0.48b	1.12b	0.48b	0.0b	0.975
	(P_{25};P_{75})	0.24;1.76	0.32;2.32	0.32;2.88	0.0;1.36	0.0;1.20	
p		0.005	0.0001	0.006	0.014	0.002	

Different small letters in the same column indicate significant difference of the collection times between groups. No significant differences between collection times within the same group were found. MD: Medians; (P_{25}; P_{75}): 25th and 75th percentiles.

Arq. Bras. Med. Vet. Zootec., v.60, n.1, p.148-155, 2008

151

Table 4. Total count, median values, and percentile cut-off points ($x10^4$) of protozoa undergoing binary division in the rumen of bovines receiving control (C) and pronounced energy-deficient (PED) diets, at different collection times

Group		0h	1h	3h	6h	9h	P
C	MD	0.0aB	0.0aB	0.37aB	1.12aA	0.72aA	0.019
	($P_{25};P_{75}$)	0.0;0.0	0.0;0.36	0.0;0.64	0.64;1.64	0.6;0.84	
PED	MD	0.0 aA	0.10aA	0.10aA	0.0bA	0.0bA	0.288
	($P_{25};P_{75}$)	0.0;0.0	0.0;0.16	0.0;0.28	0.0;0.0	0.0;0.12	
P		-	0.858	0.558	0.003	0.0001	

Different small letters in the same column indicate significant difference of collection times between groups. Different capital letters in the same row indicate significant difference within groups. MD: Medians; (P_{25}; P_{75}): 25th and 75th percentiles.

Table 5. Correlation coefficients (r) and significance levels (P) between the protozoa count and ruminal profile and metabolic variables of bovines receiving control (C) and pronounced energy-deficient diets

Statistical parameter	Biochemistry profile					
	pH	MBR	GF	Ruminal NH$_4$	Total VFA	UAEI
r	0.02	-0.66	0.88	0.70	0.55	0.76
P	0.910	0.0001	0.0001	0.0001	0.0001	0.0001

MBR: methyl blue reduction; GF: glucose fermentation; ruminal NH$_4$: ammonia; total VFA: total volatile fatty acids; UAEI: urinary allantoin excretion index.

A high positive correlation between glucose fermentation with the genera *Entodinium* (r = 0.76; P<0.0001), *Eodinium* (r= 0.62; P<0.0001), *Isotricha* (r= 0.87; P<0.0001), *Dasytricha* (r= 0.77; P<0.0001), *Eremoplastron* (r= 0.62; P<0.0001), and *Epidinium* (r= 0.60; P<0.0001) was observed. A moderate correlation between the genus *Entodinium* was observed with the ammonia concentration (r= 0.52; P<0.0001), total volatile fatty acids (r= 0.33; P<0.018), urinary allantoin excretion index (r= 0.43; P<0.002), and methyl blue reduction (r= - 0.40; P<0.011).

A high positive correlation between ammonia concentration with *Eodinium* (r= 0.63; P<0.0001), *Isotricha* (r= 0.80; P<0.0001), *Dasytricha* (r= 0.68; P<0.0001), and *Epidinium* (r= 0.71; P<0.0001) was also observed.

The predominance of the genus *Entodinium* (Table 2) is similar to the findings of other studies, both in cattle and sheep. This genus can comprise up to 95% of the total ciliates in the rumen (D'Agosto et al., 1996; D'Agosto et al., 1998). Depending on the influence of the tested diet or the individual variation of the host, the genus *Entodinium* may not predominate among the ciliates in the rumen, being outnumbered by *Isotricha*, as well as by *Epidinium* (Towne et al., 1990), or *Ostracodinium* (Siqueira, 2002).

The energy-deficient diet (Table 1) determined a sharp decrease in the protozoan populations. This decline is closely associated with the low digestibility of sugarcane bagasse, which made up 70% of the dry matter eaten by the PED group. Franzolin et al. (2000) found that the total number of protozoa tended to decrease when sheep received diets based on sugarcane, although the number of *Entodinium* increased in these conditions. This result was confirmed in the present study also for cattle, where despite the decrease in the total number of ciliates in the PED diet, rich in sugarcane, the number of *Entodinium* increased from 73.3% in the control group to 77.3% in the PED group animals. By considering the relationship that exists among the different forms of organisms in the rumen, such as protozoa and bacteria, Sucupira (2003) showed that steers receiving a pronounced energy-deficient diet presented a noticeable reduction in the ruminal microbial protein content, analyzed in this case by the indices of allantoin and uric acid excretion in the urine. Not only was the bacterial microbiota compromised in these animals, the population of protozoa was also. This is confirmed in the present study by the correlation between the total protozoa count and the urinary allantoin excretion index (r= 0.76), i.e., the lower the number of protozoa, the lower the urinary allantoin excretion index (Table 5).

152

Arq. Bras. Med. Vet. Zootec., v.60, n.1, p.148-155, 2008

A study with ovines (Chen et al., 1990) indicates that there is a positive correlation between the number of microorganisms in the rumen and excretion of purine derivatives, particularly allantoin, in the urine. In the study by Sucupira (2003), the UAEI was always less, by an order of 2.65, in animals not receiving energy supplements.

Although the difference in the number of protozoa was not significant in relation to the sample collection times (Table 3), it was clear that the average number of ciliates increased in the first hour in the control group animals and in the third hour in the PED group animals, and then decreased thereafter.

Nagaraja et al. (1992) estimated the overall average number and the number of each genus of ciliate protozoa in the rumen of heifers at 0, 1, 2, 4, 6, 8, and 12 hours after feeding and found no influence of the sample collection time on the number of protozoa. Results of this study are similar to the ones of Nagaraja et al. (1992), even considering factors that, because of the time elapsed since feeding, can influence the number of protozoa, such as escape and migration behavior of Isotrichidae ciliates as observed by D'Agosto et al. (2001).

The lower frequency of the other protozoan genera, except for *Entodinium*, in that study was probably due to the dilution of these organisms in the food mass ingested or the ruminal flow, in addition to the lysis that all microorganisms undergo inside the rumen (Ankrah et al., 1990). These authors cited that the ciliates suffered a significant decrease in population density around 10 to 14 hours after host feeding, and then increased again due to the lower dilution and influence of forms undergoing division as was also observed by Michalowski (1977). In contrast, in that study, the population decrease occurred after the sixth hour for both group animals.

The number of protozoa in the rumen took longer to increase in the PED group animals (Table 3), considering that in the control group steers occurred in the first hour after feeding. This could be explained considering that the energy-deficient diet reduces significantly the ruminal microbiota along with its fermentation activity (Maruta, 2005).

Just as for the density of ciliate protozoa, the number of organisms undergoing binary division tended to increase after feeding, as occurred in the control group animals (Table 4), where there was a pronounced increase 6 and 9 hours after feeding. No dividing forms were observed before feeding.

Michalowski (1977) made similar observations, but in water buffalo fed with different types of diets, verifying that the division rates varied mainly in function of the time elapsed since feeding and suggesting that this factor could influence the daily variations in population density of ciliate protozoa. The higher nutrient concentration in rumen content one hour after feeding resulted in a higher binary division in the control group animals, while in the PED group steers the increase in the binary division was noticed after 6 to 9 hours after feeding. Protozoa uses the metabolites produced by the rumen bacteria and fungi, and also predates these microorganisms in order to obtain nutrients for their own reproduction.

The data in Table 5 show that the number of protozoa varied with the metabolic and rumen profile variables, except for ruminal pH. Although these variables are classified as functional, there is still no information that well correlates these results with the number of protozoa in the rumen. Vashishta (1976), by examining samples of the ruminal fluid of cattle submitted to different diets, also did not find a positive correlation between the concentration of ruminal protozoa and the pH. It is important to consider that the rumen protozoa need an optimum pH to develop, and either higher or lower variations in this parameter will interfere in the dynamic of these microorganisms in the rumen (Maruta, 2005).

This study found that the different levels of correlation confirm that the reduced population of protozoa is reflected in the metabolic profile and the production of gases in the rumen of cattle. Under PED treatment, there was a sharp drop in the activity of the ruminal microorganisms, evidenced by the lower UAEI and changes in the CF and MBR, respectively.
Diverse factors are responsible for negatively interfering in the ruminal profile, especially in the quantities, functional activity and composition of the microbial populations in the

Arq. Bras. Med. Vet. Zootec., v.60, n.1, p.148-155, 2008

153

rumen (Maruta, 2005). One of the ways to evaluate the ruminal microbiota is by quantitative and qualitative evaluation. The determination of the protozoan populations does not imply use of complex and hard-to-execute techniques, although it is time consuming and needs practice. This exam particularly helps in clinical expected diagnosis.

REFERENCES

ANKRAH, P.; LOERCH, S.C.; DEHORITY, B.A. Sequestration migration and lysis of protozoa in the rumen. *J. Gen. Microbiol.*, v.136, p.1869-1875, 1990.

BORCHERS, R. Allantoin determination. *Anal. Biochem.*, v.79, p.612-613, 1977.

CHEN, X.B.; ORSKOV, E.R.; HOVELL, F.D.D. Excretion of purine derivates by ruminants: endogenous excretion, differences between cattle and sheep. *Br. J. Nutr.*, v.63, p.121-129, 1990.

D'AGOSTO, M.; CARNEIRO, M.E.; NETTO, C.M.M. et al. Avaliação de ciliados do rúmen de bovinos mantidos com duas dietas. *Arq. Bras. Med. Vet. Zootec.*, v.48, p.353-361, 1996.

D'AGOSTO, M.; SANTA-ROSA, M.R.; AROEIRA, L.J.M. et al. Influência da dieta no comportamento da população de ciliados do rúmen. *Arq. Bras. Med. Vet. Zootec.*, v.50, p.153-159, 1998.

D'AGOSTO, M.; CARNEIRO, M.E. Evaluation of lugol solution used for counting rumen ciliates. *Rev. Bras. Zool.*, v.16, p.725-729, 1999.

D'AGOSTO, M.; SIQUEIRA, I.C.V.; ESPÍRITO-SANTO, N.B. Comportamento e distribuição de protozoários ciliados (Protista, Ciliophora) no rúmen e no retículo de bovinos submetidos ao jejum. *Rev. Bras. Cien. Vet.*, v.8, p.16-18, 2001.

DEHORITY, B.A. Evaluation of subsampling and fixation procedures used for counting rumen Protozoa. *Appl. Env. Microbiol.*, v.48, p.182-185, 1984.

ERWIN, E.S.; MARCO, G.J.; EMERY, E.M. Volatile fatty acid analyses of blood and rumen fluid gas chromatography. *J. Dairy. Sci.*, v.44, p.1768-1771, 1961.

FRANZOLIN, R.; DEHORITY, B.A. Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. *J. Anim. Sci.*, v.74, p.2803-2809, 1996.

FRANZOLIN, M.H.T.; LUCCI, C.S.; FRANZOLIN, R. Efeitos de rações com níveis crescentes de cana-de-açúcar em substituição à silagem de milho sobre a população de protozoários ciliados no rúmen de ovinos. *Rev. Bras. Zootec.*, v.29, p.1452-1457, 2000.

HRISTOV, A.N.; IVAN, M.; RODE, L.M. et al. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. *J. Anim. Sci.*, v.79, p.515-524, 2001.

HOBSON, P.N.; WALLACE, R.J. Microbial ecology and activities in the rumen: Part 1. *CRC Crit. Rev. Microbiol.*, v.9, p.165-225, 1982.

LENG, R.A.; GILL, M.; KEMPTON, T.J. et al. Kinetics of large ciliate protozoa in the rumen of cattle given sugar cane diets *Br. J. Nutr.*, v.46, p.371-384, 1981.

LITTLE, T.M.; HILLS, F.J. *Agricultural Experimentation:* Design and Analysis. New York: John Wiley & Sons, 1978. 350p.

LUTSGARTEN, J.A.; WENK, R.E. Simple, rapid, kinetic method for serum creatinina measurement. *Clin. Chem.*, v.18, p.1419-1422, 1972.

MARUTA, C.A. *Perfil metabólico e ruminal de garrotes submetidos às condições de alimentação normal, jejum e realimentação.* 2005. 93f. Tese (Doutorado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo.

MICHALOWSKI, T. Diurnal changes in concentration of rumen ciliates and in occurrence of dividing forms in water buffalo (*Bubalus bubalus*) fed once daily. *Appl. Environ. Microbiol.*, v.33, p.802-804, 1977.

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

NUTRIENT requirements of beef cattle. 7.ed. Washington: National Academy of Sciences, 1996. 242p.

OETZEL, G.R. Protein energy malnutrition in ruminants. *Vet. Clin. N. Am.: Food Anim. Pract.*, v.4, p.317-329, 1988.

OGIMOTO, K.; IMAI, S. *Athas of rumen microbiology*. Tokyo: Japan Scientific Societies Press, 1981. 231p.

POTTER, E.L.; DEHORITY, B.A. Effects of changes in feed level, starvation, and level of feed after starvation upon the concentration of rumen protozoa in the ovine. *Appl. Microbiol.*, v.26, p.692-698, 1973.

ROSENBERGER, G. *Exame clínico dos bovinos*. 3.ed. Rio de Janeiro: Guanabara Koogan, 1993. 419p.

SIQUEIRA, I.C.V. *Influência da dieta sobre o comportamento e a dinâmica populacional de protozoários ciliados do rúmen de bovinos*. 2002. 68f. Dissertação (Mestrado) - Universidade Federal de Juiz de Fora, Juiz de Fora, MG.

SUCUPIRA, M.C.A. *Estudos comparativos de exames clínico-laboratoriais no diagnóstico de carência energética prolongada em garrotes*. 2003. 173f. Tese (Doutorado) – Universidade de São Paulo, São Paulo.

TOWNE, G.; NAGARAJA, T.G.; BRANDT Jr., R.T. et al. Dynamics of ruminal ciliated protozoa in feedlot cattle. *Appl. Environ. Microbiol.*, v.56, p.3174-3178, 1990.

USER'S guide statistics. Cary, NC: SAS Institute, 2000.WILLIAMS, A. G.; COLEMAN, G.S. *The rumen protozoa*. New York: Springer-Verlag, 1992. 136p.

VASHISHTA, M.S. Relation of ruminal protozoal numbers to per cent dry matter of pH in catlle. *Indian J. Anim. Health*, v.15, p.51-59, 1976.

Arq. Bras. Med. Vet. Zootec., v.60, n.1, p.148-155, 2008

155