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Original article

Selected ruminal variables and purine urinary excretion rate of steers subjected to feeding, fasting, and re-feeding conditions

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Abstract

The effects of feeding, fasting, and re-feeding on the ruminal profile of growing cattle were studied. Ruminal fluid and urine samples were obtained from 12 crossbred steers weighing approximately 300 kg during the following periods: 11 h of normal feeding (postprandial period), 48 consecutive hours of fasting, and followed by 48 h of re-feeding. Fasting promotes changes in the ruminal profile, such as an increase in ruminal pH, reduction in the number of rumen protozoa and bacteria, and decrease in the urinary excretion of allantoin; however, it does not change the urinary uric acid excretion rate. The overall mean ruminal pH was higher during fasting (7.53 ± 0.27) in comparison to those at normal feeding (6.72 ± 0.25) and re-feeding (6.62 ± 0.31) ($p < 0.05$). During re-feeding, the ruminal profile returned to normal, except for the protozoa count, which despite a slight increase only after 48 h of re-feeding, did not recover to baseline values.

Key words: rumen, feed deprivation, pH, allantoin excretion, cattle

Introduction

In veterinary medicine ambulatory practice, lack of appetite is a common symptom and manifests as different severities, ranging from hyporexia to anorexia. However, in many cases, the illnesses affecting the animals do not trigger anorexia *per se*, but other circumstances associated with animal hospitalization, such as travel stress, intensive management, adaptation to the new environment and food can possibly cause the changes in appetite (Radostits et al. 2007). Added to these circumstances is the re-feeding effect, which usually occurs after a short period of fasting, or even during recovery, after the primary condition is resolved (Ortolani et al. 2020).

Anorexia or hyporexia may generate changes in the ruminal profile and urinary metabolite excretion rate. Anorexia has a complex origin and is characterized by the complete absence of appetite and evidenced by the non-ingestion of food. The hypothalamus, especially through its centers of hunger and satiety, controls food ingestion. The causes of anorexia are diverse, and ruminal fluid examination can indicate the effects of anorexia on ruminal metabolism (Rosenberger 1993, Nagadi et al. 2000, Soares et al. 2006).

The sudden interruption in food consumption interferes negatively in the ruminal profile, especially in the microbial population and function. The main variable impacted by diet is the ruminal pH, which varies according to the type and quantity of food ingested, the time of ingestion, the amount of secreted saliva, and the absorption of volatile fatty acids produced in the rumen (Russell and Rychlik 2001, Phesatcha et al. 2020). Another way of assessing the normality of the ruminal microbiota is to determine the count of bacteria and protozoa in the rumen content. The determination of the number of protozoa is carried out in a simple way, however, the determination of the number of bacteria requires more complex methodologies (Hobson and Stewart 1997, Saro et al. 2014). Nagadi et al. (2000) found a high correlation between rumen bacteria with an indirect determination of the microbial dry matter, a simple but precise technic. Another method that indirectly estimates the total amount of ruminal microorganisms that left the rumen and were digested in the intestine, is the urine excretion index of purines, especially that of allantoin and uric acid (Soares et al. 2006). Digested ruminal microorganisms release fragments of DNA and RNA that are absorbed and catabolized in allantoin and uric acid and since that most of purine derivatives (about 85%) are from the ruminal microbiota, their estimate in urine is considered a reliable indicator of the microbial population present in the rumen (Chen et al. 1990).

Although the effects of fasting on animal metabolism have been studied previously (Kim et al. 2019, Rabaza et al. 2019), there are obscure points that are not properly understood till date, especially those related to ruminal parameters and excretion of some metabolites by the urinary route. Therefore, we studied the influences of the postprandial period, non-prolonged fasting, and subsequent re-feeding on ruminal variables, related to the fermentative and functional alterations in the rumen microbial ecosystem.

Materials and Methods

This study received the approval from the Ethics Commission on Animal Use from the School of Veterinary Medicine and Animal Science, University of São Paulo (protocol no. 595/2004) and was conducted with best practices of animal welfare. A total of twelve male crossbred steers (18 months-old) were used. The animals were healthy and weighted around 300 kg. The experiment design was already published by Ortolani et al. (2020) that focuses only on the biochemical profile variable. None of the variables already published were included in this study which focus exclusively on ruminal variables. The animals were kept in individual stalls with individual feeder and water supply and underwent a 30 days adaptation period to new feeding and housing conditions. Their diet comprised a total mixed ration calculated by 2.7% (dry matter basis) of the live weight (corrected weekly). The diet was composed by 70% of coast-cross (*Cynodon dactylon*) grass hay and 30% of concentrate. The diet was offered twice a day at morning and afternoon. Water and mineral mixture (Fosbovi 20[®], Tortuga, São Paulo, SP, Brazil) were supplied *ad libitum*. The composition of the diet was the following: % DM (dry matter) 86.3; % CF (crude fiber) 23.7; % CP (crude protein) 10.3; % EE (ether extract) 2.8; % MM (mineral matter) 7.3; % NNFE (non-nitrogen free extract) 55.9; % TDN (total digestible nutrients) 60.5; % NDF (neutral detergent soluble fiber) 61.4; % ADF (acid detergent soluble fiber) 30.4; CE (crude energy) (kcal/kg) 4240 as previously reported (Ortolani et al. 2020).

The study was divided into three separate and consecutive phases. The animals were simultaneously subjected to treatments following a study design for continuous flow response. The 1st phase, feeding, the steers were evaluated through normal feeding. Samples were obtained at the following time-points: before food supply and after one, three, five, seven, nine, and eleven hours from the morning feeding. The 2nd phase was the fasting period. This phase was initiated at the following day from the 1st phase by the removal of food leftovers from the feeders in the morning and without the supply

of the daily ration. Although free access to water was maintained, mineral mixture was not offered. The fasting lasted for 48 h, and the samples were obtained at the following time-points: before food removal and after twelve, 24, 36, and 48 h from the food removal. The 3rd phase was the re-feeding period. It was initiated at the end of the 48 h of fasting. The animals received the same diet used during the 1st phase with the same amount and feeding condition. At the re-feeding phase, samples were obtained at the following time-points: before the diet supply (which was the same sampling point of the last time-point of the 2nd phase) and after six, twelve, 24, and 48 h from the diet supply.

At all the evaluated moments, the ruminal fluid and urine samples were obtained from steers. The ruminal fluid was collected directly from the ruminal cannula in wide-mouthed plastic bottles with a lid (universal collector). Immediately after collection, the ruminal fluid pH was measured using a digital bench meter (PH-10, Celm®). For the counting of protozoa, a 10-mL aliquot with equal amount of 50% formaldehyde solution was utilized. This sample remained at room temperature until analysis. Counting of protozoa was performed according to the technique described by Ortolani and Takimoto (1987). Briefly, 5 ml of ruminal fluid were sampled and added to 5 ml of 20% formaldehyde. From this solution 1 ml was stained with 2 drops of 2% bright green dye for 4 hours, then 9 ml of 30% glycerol were added, and the mixture was homogenized (20 times dilution). Differential counts of rumen ciliated protozoa were determined using a Neubauer counting chamber with a 100x magnification. For each analysis, a total of 50 randomly selected squares were counted. The determination of bacterial dry matter (DM) was done indirectly by measuring the absorbance at 600 nm, based on the technique described by Nagadi et al. (2000) that consist simply in reading the absorbance at 600 nm the ruminal fluid that had been diluted fifty fold with buffer.

To obtain the urine samples the preputial area was massaged to induce urination. Prior to each sampling the preputial area was cleaned and dried with paper towel and the first amounts of urine were avoided. The urine samples were stored at 4°C immediately after sampling and kept under refrigeration. Subsequently, the urine samples were centrifuged at 500 × g in a refrigerated centrifuge for five min. These samples were then transferred to plastic tubes and stored in a freezer at -20°C until biochemical analyses were carried out. Following this, urinary biochemical determinations of urea, creatinine, and uric acid were performed. Biochemical analyses were performed using an automatic biochemical analyzer (Liasic, AMS Alliance, Rome, Italy) according to previously published protocols (Araújo et al. 2018, Araújo et al. 2020).

The urinary urea concentration was determined using the commercial kit Bayer® n° T-01 1821-56, following the technique described previously (Talke and Schubert 1965). The urinary creatinine concentration was determined using a commercial kit (Sigma® no 555) following the kinetic method (Lustgarten and Wenk 1972). The urinary allantoin concentration was determined using a colorimetric method (Borchers 1977). The readings were taken on a digital Spectrophotometer. The urinary concentration of uric acid was determined using the BioSystems® commercial kit No. 11821, following the technique described previously (Fossati et al. 1980). Urinary concentrations (urea, allantoin, and uric acid) were corrected by the concentration of urinary creatinine and the metabolic weight (live weight^{0.75}) of the animals, using the following formula: Concentration of the metabolite in question (mmol/L) × metabolic weight/urinary creatinine concentration (mmol/L) (Minervino et al. 2014a).

The statistical analysis was performed using Minitab software (Minitab Inc., La Jolla, CA, USA) considering the significance level of 0.05. All variables were analyzed using a Kolmogorov-Smirnov test to verify whether the distribution of the data was normal. When the data had a non-Gaussian distribution, the variables were analyzed and compared globally using the Mann-Whitney U test, considering the median for each period (feeding, fasting, and re-feeding). To evaluate the influence of the time of collection (intragroup) the normally-distributed data were analyzed by paired T test and for treatment (intergroup) comparison data were analyzed through analysis of variance followed by Tukey multiple comparison test.

Linear regression analysis were used to determine the relationship between pair of variables and to obtain correlation coefficients (*r*). The pairwise variables were selected according to expected or probable biological interaction according to animal metabolism. Differences were considered significant at $p < 0.05$. Correlations were considered high when $r > 0.6$, moderate when $0.3 < r < 0.6$ and low when $r < 0.3$ (Little and Hills 1978).

Results

The global mean ruminal pH was higher during fasting (7.53 ± 0.27) in comparison to those at normal feeding (6.72 ± 0.25) and re-feeding (6.62 ± 0.31) ($p < 0.05$). The indirect bacterial dry matter was high ($p < 0.05$) at normal feeding, decreased to half during fasting and recovered at re-feeding but were lower than that at normal feeding ($p < 0.05$). A different pattern was observed in the median of the protozoa count, which

Table 1. Mean values and standard deviations of ruminal variables: pH, bacterial dry matter, and protozoa count of cattle in the postprandial (PP), fasting (F), and re-feeding (RF) periods.

	pH	Bacterial DM (Absorbance)	Protozoa Count (x10 ⁵ /ml)	
PP	0 h	7.06±0.09 ^a	0.12±0.02 ^b	3.60±4.21 ^a
	1 h	6.92±0.12 ^a	0.22±0.06 ^a	3.24±2.91 ^a
	3 h	6.83±0.18 ^{ab}	0.23±0.06 ^a	2.24±2.12 ^a
	5 h	6.57±0.20 ^b	0.28±0.11 ^a	3.30±3.02 ^a
	7 h	6.50±0.18 ^c	0.28±0.09 ^a	3.05±2.98 ^a
	9 h	6.57±0.17 ^b	0.30±0.05 ^a	3.44±3.94 ^a
	11 h	6.59±0.12 ^b	0.29±0.07 ^a	4.05±4.40 ^a
F	0 h	6.84±0.10 ^c	0.22±0.03 ^a	2.78±2.87 ^a
	12 h	7.20±0.05 ^{bc}	0.16±0.04 ^{ab}	2.19±2.52 ^a
	24 h	7.38±0.06 ^{bc}	0.13±0.05 ^{ab}	1.15±0.78 ^{ab}
	36 h	7.70±0.10 ^a	0.11±0.03 ^b	0.28±0.21 ^b
RF	48 h	7.84±0.13 ^a	0.09±0.03 ^b	0.13±0.05 ^b
	0 h	7.84±0.13 ^a	0.09±0.03 ^b	0.13±0.05 ^a
	6 h	6.76±0.09 ^{bc}	0.21±0.03 ^a	0.12±0.06 ^a
	12 h	6.22±0.24 ^d	0.19±0.02 ^a	0.13±0.05 ^a
	24 h	6.57±0.13 ^c	0.20±0.03 ^a	0.17±0.09 ^a
	48 h	6.94±0.09 ^b	0.18±0.03 ^a	0.37±0.11 ^a

Dist letters in the same column indicate significant differences within the same physiological period ($p < 0.05$).

was highest at normal feeding ($1.54 \times 10^5/\text{mL}$) and decreased during fasting ($0.55 \times 10^5/\text{mL}$) and at re-feeding ($0.14 \times 10^5/\text{mL}$) ($p < 0.05$). The pH of the ruminal fluid was higher in the 1st h of the postprandial period than in the 5th, 9th, and 11th h, and was lowest at the 7th h ($p < 0.01$) (Table 1). Higher ruminal pH values were obtained at 36 h and 48 h compared to those at other times of the fasting period ($p < 0.01$). The pH of the ruminal fluid was higher at 0 h of re-feeding than at 48 h, which was higher than at 24 h, which in turn was higher ($p < 0.01$) than at 12 h.

The indirect bacterial dry matter was higher at all times in the post-prandial period than at zero ($p < 0.01$); in fasting, this variable was higher at time zero than at 36th and 48th h ($p < 0.01$). In contrast, at re-feeding, 0 h presented lower ($p < 0.05$) bacterial dry matter than the other periods (Table 1). There was no significant difference in the number of protozoa during the post-prandial period and re-feeding; however, in the fasting period, reduced bacterial counts ($p < 0.05$) were detected in the 36 h and 48 h compared to those at the 0 h and 12 h (Table 1).

Table 2 shows the urinary excretion rates of urea, allantoin, and uric acid. Urinary excretion rate of urea was higher at the 5 h than at the 1 h of the pre-prandial period ($p < 0.05$); during fasting, rate at the 0 h was lower when compared to those at the 24, 36, and 48 h ($p < 0.05$); At re-feeding, higher urinary excretion rate of urea was observed at the 6 h and 12 h when than

those at the 24 h and 48 h ($p < 0.05$). The urinary allantoin excretion rate did not show any significant difference during the normal feeding period; however, a higher value was found at the 0 h compared to those of the other times of the fasting period ($p < 0.01$), and during re-feeding, the mean was higher at the 48 h ($p < 0.01$) compared to those at the 0, 6, and 12 h; higher rates ($p < 0.05$) were observed at the 24 h and 6 h compared to those at the 0 h and 12 h. No differences were observed in the urinary uric acid excretion rate ($p > 0.05$).

Table 3 shows the global evaluation comparison between physiological periods. The global evaluation of different physiologic periods revealed that urinary excretion rates of urea and uric acid did not have differences between normal feeding, fasting, and re-feeding ($p = 0.68$). However, the allantoin urinary excretion rate was highest at normal feeding ($116.3 \pm 26.1 \text{ mmol/L}$), decreased during fasting ($76.0 \pm 25.5 \text{ mmol/L}$) and increased slightly at re-feeding ($97.4 \pm 29.2 \text{ mmol/L}$), with the differences being statistically significant ($p < 0.001$).

Regarding correlations between variables, a high positive correlation was found between fasting time and ruminal pH ($r = 0.96$, $p < 0.0001$). Urinary excretion rate or urea was influenced by fasting time ($r = 0.66$, $p < 0.0001$). The rumen pH and bacterial dry matter had a negative correlation ($r = -0.84$, $p < 0.05$)

Table 2. Mean values and standard deviations of urinary excretion rates of urea, allantoin, and uric acid of cattle in the postprandial periods (PP), fasting (F), and re-feeding (RF).

Times	Urea (mmol/L)	Allantoin (mmol/L)	Uric Acid (mmol/L)	
PP	0 h	1625±893 ^{ab}	98.5±31.4 ^a	11392±3842 ^a
	1 h	1485±852 ^b	122.7±22.7 ^a	7969±3183 ^a
	3 h	1903±785 ^{ab}	119.5±22.8 ^a	7061±3854 ^a
	5 h	2300±1184 ^a	116.6±29.1 ^a	7699±3078 ^a
	7 h	2279±1032 ^{ab}	120.9±20.1 ^a	8099±3426 ^a
	9 h	1995±882 ^{ab}	116.8±19.6 ^a	8535±4677 ^a
	11 h	1768±841 ^{ab}	118.8±32.5 ^a	7366±3910 ^a
F	0 h	1009±371 ^b	125.1±46.5 ^a	9107±6960 ^a
	12 h	1382±442 ^{ab}	88.2±33.6 ^b	10488±7545 ^a
	24 h	1872±358 ^a	82.9±17.7 ^b	7219±4651 ^a
	36 h	1921±436 ^a	62.0±23.0 ^b	7957±8858 ^a
	48 h	2050±461 ^a	71.0±19.0 ^b	8135±5169 ^a
RF	0 h	2050±461 ^{ab}	71.0±19.0 ^c	8135±5169 ^a
	6 h	2188±310 ^a	81.7±18.4 ^b	3919±2603 ^a
	12 h	2164±377 ^a	73.8±10.8 ^c	4809±3622 ^a
	24 h	1243±569 ^{bc}	107.1±24.9 ^{ab}	7371±5237 ^a
	48 h	1189±516 ^c	127.1±24.9 ^a	10834±8207 ^a

Distinct letters in the same column indicate significant differences within the same physiological period ($p < 0.05$).

Table 3. Overall evaluation of biochemical variables in different physiological periods (postprandial, fasting, and re-feeding). Median values of serum concentrations of total protein, globulin, creatinine, urea, cholesterol, aspartate aminotransferase (AST), and plasma volume deficit (PVD) calculated by protein, albumin, and globulin levels.

Variable	Physiological Period			p
	Postprandial	Fasting	Re-feeding	
Rumen pH	6.72±0.25 ^b	7.53±0.31 ^a	6.62±0.31 ^b	0.001
Bacterial DM (abs.)	0.26 ^a	0.12 ^c	0.19 ^b	0.047
Protozoa count ($\times 10^5/\text{ml}$)	1.54 ^a	0.55 ^b	0.14 ^c	<0.05
Urea UER (mmol/l)	1581	1779	1844	0.068
Allantoin UER (mmol/l)	116.3±26.1 ^a	76.0±25.5 ^c	97.4±29.2 ^b	0.001
Uric acid UER (mmol/l)	8598	6856	4645	0.13

Distinct letters in the same row indicate significant differences between physiological periods ($p < 0.05$).

Discussion

The pH of the ruminal fluid in the postprandial period showed fluctuations, becoming more acidic during rumen fermentation, reaching its lowest value in the 7 h, and returning to the more alkaline values, from the beginning of the experiment in the subsequent hours, similar to the behavior described by Ortolani (1981) who measured the ruminal fluid pH of heifers fed with the total ration and at the same time periods as in our study. During the fasting period, the ruminal fluid showed a continuous increase in pH, becoming more alkaline at the end of the test. This result indicates that during this period there was a 10-fold increase

in the concentration of bases. This alkalinity was similar to that described in other studies, which was due to the lower production of acids in the rumen (Rabaza et al. 2019) and the continued secretion of saliva during fasting (Bond et al. 1975, Cole and Hutcheson 1981, Galyean et al. 1981). As the saliva of ruminants is rich in bicarbonate, it has a high pH (8.1), and approximately 36% of the total volume is secreted by the animal during the resting period, when it is not ruminating or feeding (Minervino et al. 2014b), contributing to the increase in the ruminal pH observed.

The lack of food intake significantly reduced ruminal microorganisms as well as its fermentative activity. The bacteria that are the main constituents of ruminal

biomass were indirectly evaluated by the bacterial DM and excretion of purine derivatives in the urine. At the end of the fasting period, bacterial DM was reduced by 59%, which was similar to the drop in rumen fermentation activity (70%) reported by Cole and Hutcheson (Cole and Hutcheson 1981) in steers under 48-h fasting of water and feed. Nagadi et al. (2000) found a high correlation between the bacterial DM and ruminal fermentative activity *in vitro*. The bacterial DM test used in the present study proved to be simple and efficient for estimating rumen bacterial activity. We found a high negative correlation between the bacterial DM and ruminal pH. The lack of substrate into the rumen caused a decrease in the number of bacteria, which in turn decreased the amount of acids produced within the rumen, consequently increasing the ruminal fluid pH. The present study demonstrated that this alkalinity negatively interfered with the activity of rumen bacteria, similar to the findings of Smith and Correa (2004), who supplemented cows with magnesium hydroxide to raise ruminal pH, and found that an increase in ruminal pH decreases rumen microbial activity. Besides, the decrease in the number of ruminal bacteria, the protozoa count also showed a drop during the fasting period, with about 95% reduction within 48 h. In a similar experiment, with water restrictions, Galyean et al. (1981) found a decrease of about 67% in the protozoa count at the 32 h of fasting, while this drop was approximately 90% at the 36 h, in the present experiment. The drastic reduction in the number of protozoa in the present study is linked to the fact that water was supplied during fasting, while Galyean et al. (1981) restricted water. Water availability during fasting resulted in dilution of protozoa (Ortolani and Takimoto 1987).

Another test that reveals changes in ruminal biomass is the excretion of purine catabolites (allantoin and uric acid) in the urine. These catabolites originate from the degradation of endogenous DNA or exogenous (DNA of bacteria and rumen protozoa) are digested and with their nucleic acids absorbed in the intestines. In ruminants, approximately 85% of the purines come from the ruminal microbiota, and those without developed ruminal microbiota show a significant decrease in the excretion of endogenous allantoin in the urine during fasting (Fujihara et al. 2003). In the present study, allantoin urinary excretion was reduced as early as at the 12 h of fasting; however, similar amounts of allantoin were eliminated later, demonstrating that it is not a sensitive and practical marker of bacterial biomass that is evacuated from the rumen during fasting. The rate of uric acid excretion in the urine did not prove to be a good indicator of ruminal biomass in this experiment, due to unknown reasons.

In short, fasting, compared to the postprandial period, caused an increase in the ruminal fluid pH, with an intense reduction in the bacterial fermentative activity in this organ, which was verified by the decrease in the bacterial DM, less excretion of allantoin in the urine, an increase in the reduction time of the methylene blue test, and the drastic reduction in the number of protozoa. An expected decrease in the urinary excretion rate of uric acid did not occur.

During the re-feeding period, the pH of the ruminal fluid showed a significant drop compared to that of the initial moment, indicating that adequate fermentation activity occurred in the rumen, causing pH values to return to those observed during the postprandial period. However, attention should be given to the 12 h moment, where mean pH (6.22 ± 0.24) was much lower ($p < 0.01$) than that detected throughout the post-prandial period, especially in relation to that in the 11 h (6.59 ± 0.12). In experiments with similar designs, this pattern was also observed (Cole and Hutcheson 1981, Galyean et al. 1981). This may be due to the lower production of saliva due to stress during the fasting period. Stress causes an increase in the release of sympathomimetic hormones, which negatively interferes with the production and secretion of saliva by ruminants, partially explaining the low buffer capacity in the rumen during the initial re-feeding period (Crookshank et al. 1979, Ortolani 1981).

The indirect determination of bacterial DM showed a very rapid increase in the 6 h (133% higher than the baseline value), remaining at this level throughout the entire re-feeding period. Nevertheless, the global median of re-feeding was lower than that obtained in the postprandial period. Similar results have been reported previously (Cole and Hutcheson 1981, Galyean et al. 1981).

The results of protozoa had a similar pattern but with a different intensity from those obtained by Galyean et al. (1981) who found a decrease in the protozoan population of about 70% in the 32 h of fasting. In our study, after 36 h of fasting, we found a decrease of 90%. Considering the protozoa count at the end of fasting, at the 48th h of re-feeding, the protozoa count was increased by 185%. The protozoan population of the rumen had a completely different behavior from that of bacteria in the re-feeding period. While the former presented a limited growth after the offering of food (9%), the bacterial growth increased markedly (73%). Bacteria tend to multiply more quickly and do not show a drastic decrease in number during fasting, as they present large deposits of polysaccharide granules in their cytoplasm (Hobson and Stewart 1997).

The urinary allantoin excretion rate increased during the re-feeding period, and in the last two

moments (36 and 48 h), their mean values were similar to those obtained in the post-prandial period. The majority (85 %) but not all of purine derivatives are from fragments of DNA and RNA of digested ruminal microorganisms that are absorbed and catabolized in allantoin and uric acid. As re-feeding does not increase the excreted amount of endogenous allantoin (Fujihara et al. 2003), we assume that this increase in allantoin elimination is due to the exogenous component as there was a concomitant increase in ruminal bacterial dry matter. Additionally, the urinary uric acid excretion index was not efficient in quantifying rumen microbiota.

In short, during the 48 h of re-feeding, the pH of the ruminal fluid returned to normal, although in the 12th h, its value was temporarily lower; the bacterial DM had a significant increase and almost reached normal values; the protozoa count showed a slow, significant recovery in its number but it was far from normal values. The urinary allantoin excretion rate, at the end of this period, showed normal values; this did not occur with the uric acid excretion rate, which was not efficient in quantifying the ruminal microbiota.

One limitation of the present study was the absent of a complete ruminal profile, lacking the results from important ruminal variables such as volatile fatty acids concentrations and individual proportions and ammoniacal nitrogen (NH₃-N). Further studies with a more detailed ruminal profile and with identification and quantification of ruminal microorganism are required to better understand the impacts of abrupt changing in cattle feeding on ruminal ecosystem.

Conclusions

This study revealed that steers subjected to 48 h of fasting presented an increase in ruminal pH and reduced microbial activity in the rumen with a severe decrease in the ruminal protozoa count and a mild decrease in allantoin excretion. Although re-feeding resulted in the recovery of ruminal pH, the fermentative activity was less with limited recovery of protozoa count.

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