Introduction

The use of lipids in ruminant feeding provides increased energy density of the diet at low cost and also promotes an increase in the flow of polyunsaturated fatty acids into the gut with positive effects on quality of products of animal origin for human health (Jenkins, 1993). Various types of oils and fats have been evaluated in diets for ruminants in order to obtain the minimum negative interference in rumen metabolism and the maximum level in the diet. Changing rumen metabolism by fat feeding depends on several factors, including the amount of lipids present in the diet of the animal, the nature of lipids, type of diet, and animal species.

A frequent consequence of lipid supplementation in the diet is the reduction in ruminal fibre and organic matter digestibility (Ben Salem et al., 1993). This interference is related to the type, amount of fatty acids, and diet composition (Oldick and Firkins, 2000). However, Paengkoum et al. (2006) observed that supplementation with palm oil in the diet of goats has increased the use of urea and improved rumen fermentation, microbial yield, and N balance with consequent increase in animal performance. Increase in the potential of degradation of nutrients in the rumen with the addition of dietary fat has been observed more in the increase in bacterial biomass at the expense of protozoa population, than improvement in the activity of proteolytic enzymes (Doreau and Ferlay, 1995).

Palm oil is extracted from the pulp of the fruit of Elaeis guineensis plant, by physical methods (mechanical pressing), and is used primarily by food industries in Brazil and also with great trade for the export market. About 90% of world production of palm oil is used as human food, corresponding to approximately 13% of total world production of oil and fats, with future expectations to overcome the production of the most important product of vegetable oil, soybean oil (Sambanthamurthi et al., 2000). In the refining process of palm oil, a condensate is obtained as a byproduct during the deodorization step, known as palm fatty acid distillate (PFAD) (Machado and Bruner, 1997). The PFAD is used in the soap and cosmetics industries or for biodiesel production, consisting primarily of free fatty acids (>70%), with approximately 42% palmitic acid, 5% stearic acid, 43% oleic and 10% linoleic and 0.1% tocopherols (Agropalma, 2006). Investigations have been conducted with fat by targeting the beneficial effects achieved by the reduction of ciliates and methanogenesis in the rumen and also by increased content of conjugated linoleic acid (CLA) in muscle and fat in ruminants (Ivan et al., 2001; Machmuller et al., 2003). Kamra (2005) highlighted the importance of studies on the rumen microbial ecosystem that have been evaluated in several species of domestic ruminants such as cattle, sheep and goats, but few studies exist on buffaloes and wild species. The aim of this study was to evaluate the effects of adding different amounts of palm fatty acid distillate in the rumen on in situ degradability of dry matter (DM), neutral detergent fibre (NDF) and crude protein (CP) of two grasses with low quality (bromegrass and brachiaria grass) and on rumen ciliate protozoa population in buffalo.

Materials and methods

Four adult buffaloes (Bubalus bubalis) with average weight of 607 kg and rumen cannulas were used in an experiment in 4 x 4 Latin Squares for 96 days. All animal care followed guidelines recommend for use of animal experiments from the university committee. The animals underwent 19 days for adaptation and five sample collections and determination of rumen degradability in each period. Feeding the animals was performed twice daily at 8 h and 16 h with daily feeding, controlled by weighing the amount offered and orts.

The animals were fed a basal diet consisting of grass hay, ground corn grain and soybean meal, keeping a commercial mixture of mineral salt available all the time, composed by kg: 180 g calcium, 100 mg cobalt, 1000 mg copper, 90 g phosphorus, 80 mg iodine, 20 g magne-
sium, 1000 mg manganese, and 2500 mg zinc.

The treatments consisted of adding palm fatty acid distillate (PFAD) in four increasing amounts directly into the rumen via cannula: 0 (no addition), 200, 420 and 500 g/animal/d.

The commercial product was purchased from Agroaluma SA (Sao Paulo, Brazil) looking like solidified fat yellow gold. The bermudagrass hay used contained low crude protein content (6.5% DM) with 79.9% of neutral detergent fibre (NDF). The ground corn grain and soybean meal had an average of 9.1 and 52.2% CP, respectively. The composition of experimental diets with the chemical composition is shown in Table 1.

At the beginning of the experiment, re inoculation rumen intra-specific was performed, with the aim of greater uniformity of the rumen microbial population among the four experimental animals. Two liters of rumen contents were removed from each animal through the cannula, using a vacuum pump, and then placed in one bucket, homogenized and immediately, two litres of inoculum were introduced into the rumen of each animal.

**In situ** degradability of DM, CP and NDF of two grass hay, bermudagrass (*Cynodon dacty- lon* [L] Pers cv. cross-tress 1) and brachiariagras (*Brachiaria brizantha*) were determined for each collection period, as the **in situ** technical characteristics observed in literature by review of Huntington and Givens (1995).

Nylon bags (10×20 cm) with pores averaging 53 micrometer (Ankom Technology, Macedon, NY, USA) containing approximately 6 g samples of each grass previously dried in an oven at 65°C and crushed to 2 mm in diameter were incubated in the rumen for 6, 12, 24, 48, 72 and 96 hours in reverse sequential schedule with all the bags collected at the same time. The washing was done in plastic buckets with constant water flow, and then dried in an oven of forced air circulation at 65°C for 72 hours and properly weighed. The brachiariagrass presented low quality with 3.3% CP and 80.4% NDF.

With the curves of disappearance of nutrients from the nylon bags obtained in the different incubation periods were estimated the kinetics of degradation by applying the model proposed by Ørskov and McDonald (1979):

\[ PD = a + b \times (1-e^{-kt}) \]

where “a” is the soluble, “b” the potentially degradable fraction and “c” represents the rate constant of degradation.

The effective degradability was estimated by the equation quoted by the same investigators:

\[ ED = a + \left( b \times \left( \frac{1}{c + k} \right) \right) \]

**Table 1. Composition and chemical analysis of experimental diets (% DM) with different amount of palm fatty acid distilled added in the rumen.**

| Feedstuffs                  | 0   | 200 | 420 | 500 |
|-----------------------------|-----|-----|-----|-----|
| Bermuda grass hay           | 74.98 | 72.60 | 69.69 | 68.02 |
| Ground grain corn           | 16.62 | 16.92 | 17.37 | 17.92 |
| Soybean meal                | 8.40  | 8.56  | 8.79  | 9.06  |
| Palm fatty acid destilled   | 0.00  | 1.92  | 4.15  | 4.99  |

The pH of rumen contents was measured in samples collected on the last day of each collection period, at times: 0, 2, 4, 8 and 24 hours after the first daily feeding with a digital pH meter. The rumen liquid outflow rate and rumen volume were determined using 100 g of marker polyethylene glycol (PEG) dissolved in water added in the rumen of each animal via the rumen cannula, an hour before feeding and fluid collections at 0, 1 and 24 h. Samples of rumen fluid were frozen and later analyzed by turbidometric method to quantify the marker, according to Hyden (1956).

The identification and counting of rumen ciliate protozoa were done according to Dehority (2003). Samples of the contents (liquid + solid) were collected manually, via rumen cannula, using a plastic bottle with a capacity of approximately 200 mL, after homogenization of rumen contents on the last day in each period, before the morning feeding. Then, 10 mL samples were fixed in equal volume of 18.5% formaldehyde solution in glass jars properly labeled, sealed and kept at room temperature.

The following genera were counted separately: Entodinium, Epidinium, Isotricha, Dasytricha and ciliates belonging to the subfamily Diplodiniinae, were counted together: Metadinium, Eudiplodinium, Ostracodinium, Elytroplastron and Polyplastron. After thorough homogenization of each sample, one mL was pipetted using special wide aperture pipette and placed in a test tube. Then two drops of 2% of brilliant green solution were added and kept overnight at room temperature. Subsequent dilutions were made with 30% glycerol solution according to concentration of cell number in the sample. The count in each sample was performed in 100 fields, using a Sedgwick-Rafter counting chamber in an optical microscope at magnification x 100, with eyepiece grid containing 0.5 mm².

The data were statistically analyzed using GLM procedure of Statistica (Statsoft, 1995), according to Latin square design with four treatments assigned, four buffalo and four periods. The model included fixed effects of treatment and periods and effects, randomized animal, according to the model:

\[ Y_{ijk} = \mu + A_k + P_j + T_i + e_{ijk} \]

where:

- \( Y_{ijk} \) = dependent variable,
- \( \mu \) = general mean,
- \( A_k \) = fixed effect of period \( j \) (j=1-4),
- \( P_j \) = fixed effect of period \( j \) (j=1-4),
- \( T_i \) = randomized effect of buffalo \( k \) (k=1-4),
- \( e_{ijk} \) = the residual error randomized.

Analysis of variance (ANOVA) was performed and mean comparison LSD (Least Significant Difference) test for contrasts among means, adopting the minimum level of significance of 0.05 and regression analysis as well.

**Results and discussion**

No significant difference was observed in the dry matter intake (P>0.05), but buffaloes showed a decrease in the absolute DMI values with increasing PFAD in the diet, with average of 86.6, 85.01, 82.88 and 80.28 g DM/kg BW⁰.⁵⁰⁰ or 1.75, 1.71, 1.67 and 1.62% of BW for treatments 0, 200, 420 and 500 g/d of PFAD, respectively.

These values are slightly below the average value cited by Kearl (1982), 97.4 g DM/kg BW⁰.⁵⁰⁰ for buffalo growing and indicated that the animals can tolerate well the PFAD in the rumen up to the studied levels, although the accept-
ability of the product by animals has not been assessed, since the product was added directly into the rumen in order to ensure the intake levels of the desired treatment. Initially, the trial was planned with one treatment with a higher level of PFAD (640 g/d) with mean estimate of 8.2% fat in dry matter. However, after five days of the beginning of the experiment, the animal had ruminal metabolism problems, manifested by the stopping feed intake and rumen becoming liquefied and foamy.

There were differences between treatments (P<0.05) in the disappearance of DM and NDF samples from nylon bags incubated at different times in the rumen in both grasses with a reduction in the percentage of disappearance in treatments with higher levels of PFAD (420 and 500 g/d) compared with diets with lower fat content (0 and 200 g/d), but only after 24 h incubation with the bermudagrass and from 48 h with the brachiariagrass (Table 2). This indicated an action of lesser fermentation by rumen microorganisms with fat increases in the diet after an initial period that is differentiated according to the type of grass. One hypothesis to be raised on this would be a possible toxic effect of fat on certain species of microorganisms after 24 h of fermentation substrate in bermudagrass and 48 h in the brachiariagrass involving the attachment fibre process anyway.

There were significant differences between treatments (P<0.05) in most of the average values observed for degradability kinetics of DM, CP and NDF of bermudagrass and for DM and NDF of brachiariagrass (Table 3).

The buffaloes fed higher levels of PFAD (420 and 500 g/d) showed an increase in soluble fraction (a) and reduction of the potentially degradable (b) of dry matter of both grasses (Table 3), indicating a beneficial effect of fat in the initial stage of degradation at the expense of degradation along the length of stay of substrate in the rumen, resulting in potential and effective degradability lower than animals fed low fat diets (0 and 200 g/d).

No significant difference was observed in the degradability of crude protein among the treatments in both grasses (Table 3). The potential degradability of NDF was reduced with the inclusion of higher levels of PFAD (420 and 500 g/d) in both grasses. Decreased values were also observed in the effective degradability of DM and NDF of brachiariagrass, but with bermudagrass this effect was detected (P<0.05) only treatment with the highest level of PFAD.

These changes may be related to the type of fat used (Oldick and Firkins, 2000) and/or change in microbial population in the rumen.

| Table 2. In situ disappearance of dry matter, neutral detergent fibre and crude protein of bermudagrass and brachiariagrass in buffalo with different amount of palm fatty acid distilled added in the rumen. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Time, h | Bermudgrass | Brachiariagrass |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|         | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d |
|         | 0 | 200 | 420 | 500 | 0 | 200 | 420 | 500 |
| Dry matter | | | | | | | | |
| 12 | 28.58% | 26.26% | 28.98% | 26.61% | 21.58% | 22.15% | 22.09% | 22.23% |
| 24 | 65.47% | 33.59% | 35.71% | 32.24% | 28.76% | 26.50% | 29.33% | 26.95% |
| 48 | 51.43% | 45.45% | 45.12% | 40.62% | 46.48% | 42.48% | 38.73% | 37.06% |
| 72 | 50.39% | 57.14% | 52.18% | 49.59% | 56.55% | 57.44% | 47.11% | 48.13% |
| 96 | 64.11% | 60.4% | 56.38% | 54.08% | 67.00% | 67.29% | 56.79% | 53.83% |
| Neutral detergent fibre | | | | | | | | |
| 12 | 6.15% | 14.36% | 18.89% | 14.24% | 10.53% | 12.52% | 12.00% | 11.40% |
| 24 | 27.24% | 23.08% | 26.88% | 21.80% | 18.78% | 15.80% | 19.27% | 15.67% |
| 48 | 45.26% | 37.45% | 37.57% | 32.49% | 39.95% | 34.74% | 31.16% | 27.61% |
| 72 | 54.44% | 51.71% | 46.24% | 42.78% | 53.78% | 52.67% | 40.84% | 41.31% |
| 96 | 57.44% | 56.83% | 49.33% | 44.64% | 60.07% | 59.85% | 50.23% | 50.38% |
| Crude protein | | | | | | | | |
| 12 | 30.31% | 27.71% | 28.93% | 32.81% | 26.55% | 25.63% | 28.74% | 33.71% |
| 24 | 31.60% | 29.28% | 36.86% | 36.31% | 32.32% | 32.23% | 29.88% | 34.47% |
| 48 | 52.26% | 47.22% | 50.90% | 45.35% | 51.21% | 51.98% | 28.71% | 31.01% |
| 72 | 66.38% | 61.16% | 59.26% | 56.54% | 59.28% | 47.22% | 37.33% | 35.94% |
| 96 | 59.76% | 59.36% | 51.14% | 49.05% | 63.05% | 63.82% | 52.97% | 49.73% |

*Time of incubation in the rumen, h; abdifferent letters in the same row in the same grass are different (P<0.05).*

| Table 3. Kinetics of in situ degradability of dry matter, neutral detergent fibre and crude protein of bermudagrass and brachiariagrass in buffalo with different amount of palm fatty acid distilled added in the rumen. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Time, h | Bermudgrass | Brachiariagrass |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|         | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d | PFAD added in the rumen, g/d |
|         | 0 | 200 | 420 | 500 | 0 | 200 | 420 | 500 |
| Dry matter | | | | | | | | |
| 6 | 45.36% | 42.64% | 50.35% | 49.39% | 56.55% | 57.44% | 47.11% | 48.13% |
| 12 | 51.43% | 45.45% | 45.12% | 40.62% | 46.48% | 42.48% | 38.73% | 37.06% |
| 24 | 50.39% | 57.14% | 52.18% | 49.59% | 56.55% | 57.44% | 47.11% | 48.13% |
| 48 | 64.11% | 60.4% | 56.38% | 54.08% | 67.00% | 67.29% | 56.79% | 53.83% |

* a, soluble fraction; b, insoluble potentially degradable fraction; c, degradation rate; PD, potential degradability; ED 0.02, effective degradability for turnover rate of 0.02 h⁻¹. abdifferent letters in the same row in the same grass are different (P<0.05).*
(Doreau and Ferlay, 1995). In contrast, no experimental evidence had been identified from extensive literature review done by Doreau and Ferlay (1995) of the action of lipids on rumen degradability of nitrogen, although some experiments have shown an increase in the degradability of nitrogen, but not in the synthesis and efficiency of rumen microbial.

The effective degradability of DM, CP and NDF of bermudagrass and brachiagrass hays obtained from different types of experimental diets (Table 3) are close to mean values observed by Martins et al. (2007) for Tifton on 90 d of vegetative growth containing 5.6% CP and 79.8% NDF, which were 42.35%, 55.02% and 90.11%, respectively and Silva et al. (2007) with Tifton-85 containing 4.91% of CP and 85.27% of NDF, who found values of 44.29% effective degradability of DM and 38.83% of NDF.

The reduction in pH and increased liquid outflow rate of rumen contents are two important factors related to the decrease of protozoa in the rumen (Franzolin and Dehority, 1996). However, no influence of different diets of buffalo evaluated was observed on rumen pH (P>0.05), maintaining high pH ranging from 6.53 to 6.76 (mean 6.61) at all times evaluated. The average values of rumen pH throughout the day are typical from animals receiving high forage diets (Van Soest, 1994). There was also no significant differences between treatments (P>0.05) in the outflow rate of rumen fluid nor in the ruminal volume (mean 75 L), however, significant decrease in mean absolute value of outflow rates of the liquid rumen was observed with increasing addition of PFAD of 9.47, 9.63, 8.74 and 8.35%/h for the treatments without addition of PFAD, 200, 420 and 500 g/d, respectively, indicating a possible effect of this type of fat on reduction of outflow rate.

The concentration (cell number per mL of rumen content) and composition (% of total) of ciliated protozoa in the rumen of buffaloes increased with the use of hydrolyzed soybean oil (24,0%) in the total rumen protozoa population for most domestic ruminants under different feeding systems. Thus, the addition of PFAD at high levels promoted change in the composition of the ciliate fauna in the direction of those observed in other species of ruminants, since Entodinium accounted for 92.8% in the rumen of buffaloes with daily addition of 420 g of PFAD and 99.6% with addition of 500 g/day, but with no other species of ciliates in the population (Table 4).

There was significant negative correlation of number of rumen ciliate protozoa with increasing values of PFAD in the diet (r=0.78; P<0.01). Thus, it is possible to estimate the number of rumen protozoa with the content of either extract (%EE) in the diet by the equation [Total number/mL rumen content ×10^7] = 4.721-0.576 (%EE).

Assessing the population of protozoa in the rumen in relation to degradability data, we observed a drastic reduction in the overall population of ciliates (Table 4) and decreases in the potential and effective degradability of fibre from grasses with elevated levels of PFAD. These results agree with the experiment performed by Broudiscou et al. (1990), which observed reduction in the straw degradability with the use of hydrolyzed soybean oil in the diet for sheep, but the degradation of cellulose extract was lower in faunated sheep indicating changing in the composition of microbial population in the rumen. We also observed that the soluble fraction was increased, while the potentially degradable fraction was reduced with increased fat in the diet of buffaloes. Uchida and Jouany (1985) observed a higher proportion of Diplodiniinae compared to genus Entodinium in buffalo than cattle fed in the same conditions. According to Dehority (2003), species of protozoa of the genus Entodinium comprise around 88-90% of the total rumen protozoa population for most domestic ruminants under different feeding systems. Thus, the addition of PFAD at high levels promoted change in the composition of the ciliate fauna in the direction of those observed in other species of ruminants, since Entodinium accounted for 92.8% in the rumen of buffaloes with daily addition of 420 g of PFAD and 99.6% with addition of 500 g/day, but with no other species of ciliates in the population (Table 4).

Table 4. Number and composition of rumen protozoa population in buffalo with different amount of palm fatty acid distilled added in the rumen.

| Palm fatty acid distilled added in the rumen, g/d | 0 | 200 | 420 | 500 |
|-------------------------------------------------|---|-----|-----|-----|
| Number, ×10^7/mL ruminal content                |   |     |     |     |
| Entodinium                                      | 1.22| 1.85| 0.75| 0.56|
| Diplodiniinae                                   | 0.84| 0.71| 0.08| 0.06|
| Epidinium                                       | 0.012| 0.024| 0.0| 0.0|
| Holotricha                                      | 0.036| 0.038| 0.0| 0.0|
| Total                                           | 2.84| 2.63| 0.84| 0.57|
| Composition                                     |   |     |     |     |
| Entodinium, %                                   | 68.03| 72.73| 92.83| 99.64|
| Diplodiniinae, %                                | 30.38| 24.01| 7.17| 0.36|
| Epidinium, %                                    | 0.46| 0.75| 0.0| 0.0|
| Holotrich, %                                    | 1.12| 2.51| 0.0| 0.0|

*aDifferent letters in the same row in the same grass and are different (P<0.05).*
also observed decrease in the degradation of insoluble fraction of soybean meal with animals defaunated compared with faunated. Probably, high levels of PFAD promoted changes in the composition of the rumen microorganism, both of protozoa (Table 4) and bacteria, favoring bacterial growth of those using carbohydrates and soluble proteins, but being a disadvantage to bacteria using fibre as substrate, since protozoa ciliates are capable of degrading structural polysaccharides by the ingestion and digestion with their own enzymes or by engulfment of cellulosytic bacteria retained in the vacuoles organelles, probably lysosomes (Delfosse-Debusscher et al., 1979a; Thines-Sempoux et al., 1980). In fact, Delfosse-Debusscher et al. (1979b) suggested that the degradation of cellulose by ciliates is the result of a mutualistic relationship between bacteria and protozoa in the rumen.

It is worth mentioning that there is an important symbiotic relationship between different species of microorganisms in the rumen, involving bacteria, protozoa, and fungi that act differently in the process of degradation and fermentation of nutrients in the rumen. Lee et al. (2000) observed that the enzymatic activity of fungal growth combined with rhizobia penetrating the fibrous portion of the material was more important that the activities of bacteria and protozoa in the degradation of plant cell walls in the rumen. Recently, Zhang et al. (2007) found that the cell wall digestion of corn straw was due to the interaction between bacteria, protozoa and fungi in the rumen and that the interaction between bacteria and protozoa showed a significant contribution to degradation of cell wall in finely ground material while bacteria and fungi showed a large synergism in the degradation of cell wall in coarsely ground roughage. In the present experiment, it was not possible to assess the relevant interaction: bacteria, fungi, protozoa.

Investigation has shown that the addition of lipids in the rumen depresses the concentration of protozoa with enhanced efficiency of bacterial growth (Demeyer and Van Nevel, 1995; Onetti et al., 2001; Balieiro-Neto and Melloti, 2007). However, the effect of dietary supplementation with lipids is very variable and depends on the type of oil and fat used and the total fat in the diet (Doreau and Ferlay, 1995). Ueda et al. (2003) observed a reduction in the population of ciliated protozoa in the rumen with a decrease in digestibility of NDF and ADF in dairy cows supplemented with 3% linseed oil when animals were fed high concentrate diets (65%), but not with diets rich in forage (63%). The change in fibre digestion may be caused by changes in the rumen microbial population with increase of cellulosic bacteria due to the fat toxic effect on the protozoa, since these organisms promote engulfment of bacteria in rumen microbial environment. But large entodiniumorphs protozoa, belonging to the subfamily Diplodiniinae, have better cellulosytic activity in fibre degradation in the rumen and the elimination of these protozoa reduce the digestibility of organic matter and cellulose, due to reduction of activity of carboxymethyl cellulase in the rumen (Santra and Karim, 2002). However, according to Doreau and Ferlay (1995) the decline in population of ciliate protozoa in the rumen by addition of oil or fat in the diet, promotes an increase in the potential degradation of nutrients due to increased bacterial biomass.

The significant decrease in the total rumen ciliates population of buffaloes fed with elevated levels of PFAD, promoting significant change in the fauna composition with a expressive reduction in the percentage of protozoa belong the subfamily Diplodiniinae with predominance of Entodinium, allows to raise the hypothesis that there is greater direct participation of large ciliate with specific enzyme secretion in the degradation of fibre than by an indirect increase in bacterial activity due to increased bacterial biomass. The predominance of Entodinium favors the use of soluble nutrients under such conditions. Further research should be developed to clarify the effects of adding different types of oils and fats in the diet of buffaloes on the microorganisms in the rumen and the role of the different species of protozoa in the degradation and digestion of nutrients, as well as the relationship between the protozoa population with the total rumen microbial community.

**Conclusions**

The use of palm fatty acid distillate (PFAD) in diets for buffalo at levels above 400 g/animal reduces the potential and effective degradability of DM and NDF of grasses bermudagrass and brachiariagrass, increasing the soluble fraction and reducing the potentially degradable fraction in the rumen. The soluble fraction of crude protein is also increased in high level of PFAD.

The total number of rumen ciliated protozoa is reduced and the fauna composition is changed with increase the proportion of Entodinium in detriment of protozoa belong- ing to the subfamily Diplodiniinae in high levels of PFDA inclusion in the diet. This effect demonstrates an action fat toxicity differential on larger protozoa species with a reduction in the process of degradation of complex carbohydrates of the cell wall of plants in the rumen.

**References**

Agropicalma, 2006. Product specification–palm fatty acid distillate. Agropicalma group, revision 5., Belem, PA, Brazil.

AOAC, 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA, USA.

Balieiro Neto, G., Melloti, L., 2007. Production of volatile fatty acids and rumen protozoal count in cattle supplemented with fat. Braz. J. Vet. Res. An. Sci. 44:115-121.

Ben Salem, H., Krezeminski, R., Ferlay, A., Doreau, M., 1993. Effect of lipid supply on in vivo digestion in cows: comparison of hay and corn silage diet. Can. J. Anim. Sci. 73:547-577.

Broduiscou, L., Van Nevel, C.J., Demeyer, D.I., 1990. Effect of soya oil hydrolysate on rumen digestion in defaunated and faunated sheep. Anim. Feed Sci. Techn. 30:51-67.

Dehority, B.A., 2003. Rumen microbiology. Nottingham Univ. Press, Nottingham, UK.

Delfosse-Debusscher, J., Thines-Sempoux, D., Vanbelle, M., Latteur, B., 1979a. Contribution of protozoa to the rumen cellulosytic activity. Ann. Rech. Vet. 10:255-257.

Delfosse-Debusscher, J., Van Hoof, F., Hellings, F., Thines-Sempoux, D. 1979b. Hydrolytic activities of rumen ciliates. Ann. Rech. Vet. 10:258-260.

Demeyer, D.I., Van Nevel, C.J., 1995. Transformations and effects of lipids in the rumen. Arch. Anim. Nutr. 48:119-134.

Doreau, M., Ferlay, A., 1995. Effect of dietary lipids on nitrogen metabolism in the rumen. Review. Livest. Prod. Sci. 43:57-110.

Firkins, J.L., Hristov, A.N., Hall, M.B., Varga, G.A., St-Pierre, N.R., 2006. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci. 89:E31–E51.

Firkins, J.L., Yu, Z., Morrison, M., 2007. Ruminal nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy. J. Dairy Sci. 90:E1–E16.

Franzolin, R., Dehority, B.A., 1996. Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. J. Anim. Sci. 74:2803-2809.

Franzolin, R., Dehority, B.A., 1999. Comparison of protozoa populations and digestion
rates between water buffalo and cattle fed an all forage diet. J. Appl. An. Res. 16:33-46.
Franzolin, R., Rosales, F.P., Soares, W.V.B., 2010. Effects of dietary energy and nitrogen supplements on rumen fermentation and protozoa population in buffalo and zebu cattle. Rev. Bras. Zootec. 39:549-555.
Goering, H. K., and P. J. Van Soest., 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agricultural Handbook 379. US Dept. Agric., Agricultural Research Service, Washington, DC, USA.
Huntington, J.A., Givens, D.I., 1995. The in situ technique for studying the rumen degradation of feeds: a review of the procedure. Nutr. Abst. Rev. 65:64-93.
Hyden, S.A., 1956. A turbidometric method for the determination of higher polyethyleneglycols in biological materials. K. Lanthar. Hogs Ark Arb. 22:139.
Ivan, M., Mir, P.S., Koenig, K.M., Rode, L.M., Neill, L., Entz, T., Mir, Z., 2001. Effect of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Ruminant Res. 41:215-227.
Jenkins, T.C., 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851-3863.
Kamra, D.N., 2005. Rumen microbial ecosystem. Curr. Sci. India 89:124-135.
Kearl, L.C., 1982. Nutrient requirements of ruminants in developing countries. International Feedstuffs Inst., Logan, Ut, USA.
Lee, S.S., Ha, J.K., Cheng, K.J., 2000. Relative contributions of bacteria, protozoa, and fungi to in vitro degradation of orchard grass cell walls and their interactions. Appl. Environ. Microb. 66:3807-3813.
Machado, N.T., Brunner, G., 1997. High pressure vapor-liquid equilibria of palm fatty acids distillates-carbon dioxide system. Ciencia Tecnol. Alime. 17:354-360.
Machmuller, A., Soliva, C.R., Kreuzer, M., 2003. Effect of coconut oil and defaunation treatment on methanogenesis in sheep. Reprod. Nutr. Dev. 43:41-55.
Martins, A.S., Vieira, P.F., Berchielli, T.T., Prado, I.N., Lempp, B., Paula, M.C., 2007. Degradabilidade in situ e observações microscópicas de volumosos em bovinos suplementados com enzimas fibrolíticas exógenas. Rev. Bras. Zootecn. 36:1927-1936.
Oldick, B.S., Firkins, J.L., 2000. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. J. Anim. Sci. 78:2412-2420.
Onetti, S.G., Shaver, R.D., Mcguire, M.A., Grummer, R.R., 2001. Effect of type and level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. J. Dairy Sci. 84:2751-2759.
Ørskov, E.R., Mcdonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agr. Sci. 92:499-503.
Paengkoum, P., Liang, J.B., Jelan, Z.A., Barsery, M., 2006. Utilization of steam-treated oil palm fronds in growing goats: I. Supplementation with dietary urea. Asian Austral. J. Anim. 19:1305-1313.
Santhanamurthi, R., Sundram, K., Tan, Y., 2000. Chemistry and biochemistry of palm oil. Progr. Lipid Res. 39:507-558.
Santra, A., Karim, S.A., 2002. Influence of ciliate protozoa on biochemical changes and hydrolytic enzyme profile in the rumen ecosystem. J. Appl. Microbiol. 92:801-811.
Silva, E.A., Berchielli, T.T., Reis, R.A., Pires, A.V., Sato, K.J., Paes, J.M.V., Lopes, A.D., 2007. Teores de proteína bruta para bôvios alimentados com feno de capim Tifton 85: parâmetros ruminais, eficiência de síntese microbiana e degradabilidade in situ. Rev. Bras. Zootec. 36:225-236.
Statsof, 1995. Statistic for windows (computer program manual). Statsoft, Inc., Tulsa, OK, USA.
Thines-Sempoux, D., Delfosse-Debusscher, J., Latteur, D., 1980. Mechanism of cellulose degradation by the rumen ciliates. Arch. Int. Phys. Bioch. 88:105-106.
Ueda, K., Ferlay, A., Chabrot, J., Loor, J.J., Chilliard, Y., Doreau, M., 2003. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage:concentrate ratios. J. Dairy Sci. 86:3999-4007.
Ushida, K., Jouany, J.P., 1985. Effect of protozoa on rumen protein degradation in sheep. Reprod. Nutr. Dev. 25:1075-1081.
Valinote, A.C., Nogueira Filho, J.C.M., Leme, P.R., Silva, S.D.E., Cunha, J.A., 2005. Fontes de lipídios e monensina na alimentação de novilhos nelore e sua relação com a população de protozoários ciliados do rúmen. Rev. Bras. Zootec. 34:1418-1423.
Van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell Univ. Press, Ithaca, NY, USA.
Zhang, Y., Gao, W., Meng, Q., 2007. Fermentation of plant cell walls by ruminal bacteria, protozoa and fungi and their interaction with fibre particle size. Arch. Anim. Nutr. 61:114-125.