PAPER

Effects of adding a spray-dried polyclonal antibody preparation on ruminal fermentation patterns and digestibility of cows fed high concentrate diets

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Abstract

The present study aimed to evaluate the effects of different doses of a spray-dried polyclonal antibody preparation against specific ruminal bacteria on rumen fermentation parameters as well as protozoa counts, *in situ* degradability of sugarcane neutral detergent fibre (NDF) and total tract apparent digestibility of total diet in cows fed high concentrate diets. Eight ruminally-cannulated cows were used in a replicated 4×4 Latin square design with four experimental periods of 21 days. The treatments were: T1 (control), 0.0 g/d of multivalent polyclonal antibody preparation (PAP-MV); T2, 1.5 g/d of PAP-MV; T3, 3.0 g/d of PAP-MV; T4, 4.5 g/d of PAP-MV. Sample collection for rumen fermentation parameters was carried out the last day of each period at 0, 2, 4, 6, 8, 10 and 12 h after morning meal. For protozoa counts, samples were collected the last day of each period at 0 and 4 h after feeding. *In situ* degradability of sugarcane NDF was performed the last 5 days of each period, while total tract apparent digestibility of total diet was assessed the last 10 days of each period. Regardless of sampling time, there was no linear or quadratic effect on rumen pH, total concentration of short chain fatty acids, molar proportion of acetate, propionate and butyrate, ammonia nitrogen (NH_3-N) or lactate. No treatment effects were detected for protozoa counts, *in situ* degradability of sugarcane NDF and total tract apparent digestibility of total diet. In conclusion, different levels of spray-dried PAP-MV were not sufficient to alter rumen environment.

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Introduction

The recent intensification of beef cattle production arises the necessity of searching for different ways to allow small adjustments in the production system. The ultimate aim of this search is to explore the maximum potential of animals. In this context, an increase in the energy density of feedlot diets is observed. This due to the positive responses of this practice in relation to animal performance, carcass quality, facility of management and economy. However, feeding high-grain diets can predispose animals to metabolic digestive disturbances, such as acidosis (Schwartzkopf-Genswein *et al.,* 2003). This scenario provides the opportunity to develop new feed additives which could improve i) the rumen environment through positive fermentation manipulation; and consequently ii) animal performance. Some studies have shown the potential of immunisation for this purpose. Immunisation of steers through vaccine against lactate-producer bacteria [*Streptococcus (S.) bovis*] has been proved to be effective to maintain feed intake, and to decrease ruminal concentration of lactate and rumen bacteria counts after the high-grain diet challenge (Shu *et al.,* 1999; Gill *et al.,* 2000).

For passive immunisation, DiLorenzo *et al.* (2006) observed that polyclonal antibodies preparations (PAPs) against *S. bovis* or *Fusobacterium* (*F.*) *necrophorum* decreased ruminal counts of target bacteria and increased ruminal pH of steers. Blanch *et al.* (2009) observed positive effects of PAP in controlling the acidosis of heifers during a rapid transition to high-grain diets. Indeed, ruminal pH was higher in the PAP group than in the control, thus allowing fewer animals in the treatment group to have acidosis. All these positive results were obtained in experiments testing PAP in liquid presentation. In field conditions, this system could represent a disadvantage, especially regarding its conservation and administration to a large amount of animals. With the purpose of gathering more information about this feed additive, the present study was carried out to evaluate the effects of different doses of a spray-dried multivalent polyclonal antibody preparation (PAP-MV) against specific rumen bacteria on ruminal fermentation parameters, protozoa counts, *in situ* degradability of sugarcane neutral detergent fibre (NDF) and total tract apparent digestibility of ruminally cannulated dry cows fed high-grain diets.

Materials and methods

All animals were cared for by trained personnel, according to the guidelines established by the São Paulo State University

(Brazil) Ethical Committee for Animal Research (CEEA).

Polyclonal antibody preparations

The procedure to generate the PAP-MV (RMT Optimize; CAMAS Inc., Le Center, MN, USA) was similar to those previously described by DiLorenzo *et al.* (2006, 2008) and Marino *et al.* (2011). The model organisms for this study were *Streptococcus bovis* (ATCC 9809), which is a main lactic acid-producing bacterium; *Fusobacterium necrophorum* (ATCC 27852), which is involved in the development of liver abscesses in acidotic animals; *Clostridium stricklandii* (ATCC 12662), *Clostridium aminophilum* (ATCC 49906), and *Peptostreptococcus anaerobius* (ATCC 49031), which are hyper-ammonia-producing bacteria; and *Escherichia coli* O157:H7 (ATCC 43895), which is a human pathogen developing in ruminants under acidosis.

The final RMT Optimize product contained approximately 26% of antibodies acting against *Streptococcus bovis*, 12% against *Fusobacterium necrophorum* and 48% against the proteolytic bacteria *Clostridium aminophilum*, *Peptostreptococcus anaerobius* and *Clostridium sticklandii*. The rest of the antibodies (14%) acted against *Escherichia coli* O157:H7.

The product is usually presented in the liquid form. However, for this experiment the product passed through a spray-dried process, thus turning into powder form. This form was maintained in hermetically sealed packages during all its period of utilisation. This change of presentation form of the product was tested in order to facilitate its administration in field conditions. In this experiment, the powder presentation was tested.

Animal and experimental procedures

The trial was conducted at the College of Veterinary Medicine and Animal Science [University of São Paulo (USP), Brazil]. Eight Holstein non-pregnant and non-lactating cows initially weighing 567±104 kg of body weight (BW), fitted with ruminal cannulas were randomly assigned to two Latin squares 4 x 4, each one containing four doses of PAP (T1: 0.0 g/d; T2: 1.5 g/d; T3: 3.0 g/d; T4: 4.5 g/d), in four periods of 21 days each. The dose of 3.0 g was the dose recommended by the manufacturer. One dose lower and one higher plus control group were chosen for this trial. Cows were housed in a tie-stall barn equipped with individual feed bunks, rubber matted floors, and automatic water fountains shared by 2 animals. There were fans on the ceiling to relieve the high temperatures during the day. Body weight was measured at the beginning of period 1 (day 1) and at the end of each of the four periods (day 21) at the same time each day.

Diets

Diets were fed as total mixed rations (TMR) with a ratio of concentrate to forage of 73:27 [dry matter (DM) basis]. Diets were offered twice a day at 8.00 a.m. and 4.00 p.m. throughout the experiment for *ad libitum* consumption (minimum of 10% feed refusal). The forage source was fresh sugarcane chopped with a theoretical average particle size of 1.14 cm. Size measures were taken using the Penn State Particle Size Separator, according to Lammers *et al.* (1996).

The different doses of the feed additive were administered in absorbent tissue paper and delivered directly through the ruminal cannula twice a day, just before the meals. The composition and analysed nutrient content of the experimental diets are presented in Table 1. Diets were formulated using the Cornell Net Carbohydrate and Protein System (model version 5.0.40) (Fox *et al.,* 2004).

Sample collection and laboratory methods

Dry matter intake

All feeders were examined every morning at 6.30 a.m. If there was no feed surplus, feed offered was raised by 10%. If there was a ~10% surplus, the feed was kept at the same level and if the surplus was >10%, the feed offered was reduced by 10%. On the last five days of each period, feed surplus from each cow was collected and weighted to calculate feed intake.

Ruminal fermentation parameters

Ruminal fluid samples were collected at day 21 of each period, through the ruminal cannula with a vacuum pump at 0, 2, 4, 6, 8, 10 and 12 h after the morning meal. On this day, animals were fed once in the morning. The evening meal was offered after the collection of the 12-h sample. Approximately 500 mL of rumen fluid was collected, at each sampling time, from three different parts of the rumen. Immediately after the collection, 100 mL of rumen fluid was used for pH determination with a portable digital pH meter (model HI8424; HANNA Instruments Ltd., Leighton Buzzard, UK), calibrated with solutions of pH 4.0 and 7.0.

For short-chain fatty acids (SCFA) analyses, a fraction of approximately 100 mL of ruminal fluid was centrifuged at 2000 x g for 20 min and 2 mL of the supernatant was added to 0.4 mL of formic acid and frozen at -20°C for further analyses, according to Erwin *et al.* (1961). Short-chain fatty acids were measured by gas chromatography (model Focus GC; Thermo Scientific, West Palm Beach, FL, USA) with an automatic injector of samples, equipped with a glass column of 2 m of length and 1/5" of diameter packed with 80/120 Carbopack B-DA/4% (Sigma-Aldrich, St. Louis, MO, USA) and a flame ionisation detector maintained at 270°C. The carrier gas was high purity H_2 maintained in flux of 30 mL/min. Lactic acid concentration was measured by a colorimetric technique, according to Pryce (1969).

In order to determine ammonia nitrogen (NH_3-N) concentration, 2 mL of the supernatant was added to 1 mL of 1 N of sulphuric acid (H_2SO_4) solution and the centrifuge tubes were immediately frozen at -20°C until the colorimetric analyses, according to the method described by Kulasek (1972) and adapted by Foldager (1977).

Protozoa counts

Rumen content for protozoa counts was collected at 0 and 4 h after morning meal on the last day of each period (day 21) by scanning the ruminal floor and fixed in 50% formalin

Table 1. Composition and analysed nutrient content of experimental diets.

Ingredients, % DM	
Sugarcane fresh and chopped	26.8
HMCS	29.9
Dry-ground CG	20.6
Soybean meal	20.6
Vitamin and mineral premix°	2.06
Nutrient composition	
DM, %	51.9
Ash, % DM	4.99
CP, % DM	15.9
EE, % DM	2.83
NDF, % DM	25.9
ADF, % DM	13.7
NFC, % DM	51.5
Starch, % DM	32.1
TDN#, % DM	78.0
Ca, % DM	0.36
P, % DM	0.19

°Composition of vitamin and mineral premix per kg of product: Ca, 15.5 g; P, 8 g; S, 0.6 g; Mg, 0.6 g; Na, 11.5 g; Co, 7 mg; Cu, 130 mg; Fe, 350 mg; I, 7 mg; Mn, 290 mg; Se, 1.4 mg; Zn, 350 mg. #Value estimated by the Cornell Net Carbohydrate and Protein System software, version 5.0.40 (Fox *et al.*, 2004). DM, dry matter; HMCS, high moisture corn silage; CG, corn grain; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; NFC, non-fibre carbohydrates; TDN, total digestible nutrients.

(1:1) for microscopic counts, as described by Dehority (1993). Rumen fluid (1 mL) was mixed with 9 mL of methyl green:formaldehyde (38% wt/wt) solution. Entodiniomorphs (*Entodinium*, *Diplodinium* and *Epidinium*) and Holotrichs (*Isotricha* and *Dasytricha*) were identified and counted using a Neubauer Improved Bright-Line counting chamber (Hausser Scientific Partnership, Horsham, PA, USA).

In situ degradability

Degradability of sugar cane NDF was accomplished using the *in situ* technique described by Mehrez and Ørskov (1977). Nylon bags with a porosity of 50 μm (10.0×19.0 cm) were filled with approximately 7 g of feed previously dried at 55°C for 72 h. Bags were weighed, tied and stored in a refrigerator (5°C) before use. Nylon bags were attached to the rumen cannula by a nylon thread with a minimum of 50 cm length and incubated during 0, 12, 24, 48, 72, 96 and 120 h. The incubations were done on days 17 to 21 of each experimental period. After incubation, all bags were washed thoroughly by hand and dried at 55°C for 72 h for later weighing and chemical analyses. Degradability at time zero was measured by washing bags in water (39°C) for 15 min (Cummings *et al.,* 1983). Neutral detergent fiber (with heat-stable α-amylase) was determined on bag residues according to Van Soest *et al.* (1991). As the parameter a, *i.e.*, the soluble fraction, was negative for *in situ* degradability of sugarcane due to the lag phase, the model proposed by McDonald (1981) was used to estimate the degradation parameters:

$$p = b\ (1 - e^{-c(t-l)})$$

where
p is the degradation at each time;
b is the potentially degradable fraction of the insoluble fraction that would be degraded at rate c;
c is the rate of degradation of fraction b;
t is the incubation period expressed in hours;
l is the colonisation time.

The parameters b, c and l of the exponential equation obtained by the NLIN procedure from SAS (SAS, 2008) were used to calculate the potential degradability [(PD) = a+b]. The effective ruminal degradability (ED) was calculated according the mathematic model proposed by Von Keyserlingk *et al.* (1996):

$$ED = A+(B*C/(C+K))e^{(-K*L)}$$

where K is the ruminal passage rate of solids, defined as 0.05%/h.

In this case the parameter a was considered zero. It is recommended the value of 5%/h for animals receiving high contents of mixed diets (less than two times the maintenance) (AFRC, 1993).

Total tract dry matter apparent digestibility

The digestibility trial consisted of 10 days: from day 11 to 20 of each experimental period. Chromium oxide was used as an external marker to estimate the apparent nutrient digestibility, as described by Bateman (1970). The first five days (day 11 to 15) were used for marker adaptation and the last five days (day 16 to 20) for sample collection. For each animal, DM intake was measured on the last five days of each period and grab samples of faeces (approximately 200 g) were collected from the rectum on the last five days of the sampling period. Cows received 15 g/d of chromium(III) oxide (Cr_2O_3) twice a day (7.5 g at each feeding time), through ruminal cannula. Chromium oxide concentration was determined colorimetrically through its reaction with s-difenilcarbazide, by following Graner (1972). Feed and faecal samples were dried at 55°C for 72 h and ground to pass a 1-mm screen. Composite samples per cow were used to determine DM (method 934.01; AOAC, 1990); organic matter (OM) (method 924.05; AOAC, 1990); crude protein (CP), by total N determination using the micro-Kjeldahl technique (method 920.87; AOAC, 1990); ether extract (EE), determined gravimetrically after extraction by using petroleum ether in a Soxhlet extractor (method 920.85; AOAC, 1990); NDF (with heat-stable α-amylase), acid detergent fibre (ADF) and pectin, according to Van Soest *et al*. (1991). Starch analysis was done according to Pereira and Rossi Jr. (1995), with previous extraction of soluble carbohydrates, as proposed by Hendrix (1993). The value of non-fibre carbohydrates (NFC) was estimated by following the formula: NFC (% DM) = 100 – (CP + NDF + EE + ash), as described by Hall (2001). Calcium (method 968.08; AOAC, 1995) and phosphorus (method 965.17; AOAC, 1990) were only determined in feed samples.

Statistical analysis

Results were analysed by using the Statistical Analysis System software (SAS, 2008), after verifying the residue normality by the Shapiro-Wilk test (PROC UNIVARIATE). Feed intake, *in situ* degradability of sugarcane NDF and total tract apparent digestibility of total diet were analysed by the MIXED procedure of SAS. The model included the effects of treatment, period, animal nested within square as well as the effect of square. The effect of treatment was considered a fixed factor, while period, animal nested within square and square were considered random factors. The variables ruminal pH, total concentration and molar proportion of SCFA, lactate and NH_3-N concentration as well as protozoa counts were analysed by the MIXED procedure of SAS with repeated measures (Littell *et al.,* 1998). The model accounted for the same effects as described above plus time and its interactions with treatment, period, square and animal nested within square. The effect of time was considered a fixed factor. The matrix best fit to data by the lowest corrected akaike information criteria (AICC) was an autoregressive covariance structure. Effects were considered significant at P≤0.05. All means presented are least-square means and effects of treatments (doses of PAP) were evaluated by polynomial regression by the MIXED procedure of SAS, separating the effects in linear, quadratic and cubic.

Results and discussion

Data on DM intake (kg/d) are shown in Table 2. Regardless of the PAP dose, the mean DM intake was around 13.5 kg/d (2.11% BW) along the experimental period and no effect (P>0.05) of PAP doses was observed for this variable. Contrary to these results, feedlot steers fed high-grain diets and orally dosed with a PAP against *S. bovis*, *F. necrophorum* and several strains of proteolytic bacteria had similar performance to those fed monensin. This is probably related to the lower incidence of rumenitis (Pacheco *et al.,* 2012).

Ruminal fermentation parameters are reported in Table 2. No interaction between time and PAP-MV doses (P>0.05), as well as no effect of PAP-MV doses (P>0.05) were observed for ruminal pH. Contrary to this observation, DiLorenzo *et al.* (2006) observed that steers receiving a PAP against *S. bovis* (PAP-Sb) in high-grain diets increased ruminal pH at 5.5 h *post*-feeding when compared with control (6.08 *vs* 5.67). In addition, *S. bovis* counts were reduced in steers fed PAP-Sb. Blanch *et al.* (2009) also reported an increased ruminal pH in heifers fed PAP-Sb when compared with control 6 h *post*-feeding at day 16 (6.70 *vs* 6.11), 18 (6.54 *vs* 5.95) and 19 (7.26 *vs* 6.59) of the experimental period. Marino *et al.* (2011) reported a higher ruminal pH, 4 h *post*-feeding, in groups supplemented with monensin or PAP against *S. bovis*, *F.*

necrophorum and several strains of proteolytic bacteria when compared with control. No effect of interaction between time and PAP-MV doses (P>0.05) was observed for total concentration of SCFA. Regardless of measurement time, a quadratic deviation (P=0.0010) was observed for this variable. In line with these results, Dahlen *et al.* (2003) did not observe any difference in the total concentration of SCFA in lactating cows supplemented with PAP against ruminal proteolytic bacteria. However, Blanch *et al.* (2009) verified higher total concentration of SCFA for heifers supplemented with PAP against *Streptococcus bovis* (147.1 m*M*) compared with the control group (132.9 m*M*), 6 h after feeding high-grain diets. No effect of interaction between time and PAP-MV doses (P>0.05), as well as no effect of PAP-MV doses were observed for molar proportion of acetate, propionate, butyrate or acetate:propionate (Ac:Pr) ratio.

Although the PAP-MV used in this experiment was against proteolytic species, no effect of interaction between time and PAP-MV doses (P>0.05), as well as no effect of PAP-MV doses (P>0.05) were observed for NH_3-N too (Table 2). The same was observed by Marino *et al.* (2011) in an experiment testing a PAP against *S. bovis*, *F. necrophorum* and several strains of proteolytic bacteria in three different energy sources diets. Irrespective of treatment, the mean concentration of NH_3-N was 9.04 mg/dL which is higher than expected for maximum microbial protein production (5.0 mg/dL) proposed by Satter and Slyter (1974). The mean values of lactate concentration (0.16 m*M*) obtained in this study were expected as the mean pH value was around (6.10). Lactate began to accumulate when ruminal pH falls below 5.0 and lactate catabolism bacteria was inhibited (Nagaraja and Titgemeyer, 2007). No interaction between time and PAP-MV doses (P>0.05) and no effect of PAP-MV doses (P>0.05) were observed for lactate concentration (Table 2).

Results of *in situ* degradability parameters of sugarcane NDF are shown in Table 3. A quadratic effect of PAP doses (P<0.05) on variables b ($Y=50.2-16.4x+3.3x^2$, $R^2=0.20$), c ($Y=0.01+0.01x-0.003x^2$, $R^2=0.24$) and PD ($Y=50.2-16.4x+3.3x^2$, $R^2=0.20$) of sugarcane NDF was observed. For estimating the *in situ* degradation parameters, an alternative method proposed by McDonald (1981) was used instead of what was proposed by Ørskov and McDonald (1979). This substitution was made due to the negative values of variable a. This fact could be related to deviations in the prediction of this fraction. Theoretically, the proportion of fraction a in NDF should be close to zero, as it is a readily soluble fraction. Estimations around 6-8% can occur due to the loss of small particles during the washing of nylon bags (Schmidt *et al.,* 2007).The low values of *in situ* degradability parameters of sugarcane NDF were probably due to the substrate effect in highly fermentable carbohydrates diets. In these diets, rumen microorganisms capable to degrade cellulose would not develop spontaneously as carbohydrates were easily fermentable and are fully available (Rotger *et al.*, 2006). There was no effect of interaction between time and PAP doses (P>0.05) for protozoa counts as well as effect of PAP doses (P>0.05) (Table 4). Higher concentrations of *Entodinium* species were expected in high-grain diets (Franzolin and Dehority, 1998). During an acidosis induction,

Table 2. Values of dry matter intake and ruminal fermentation parameters with different doses of multivalent polyclonal antibody preparation in cattle fed high concentrate diets.

	Treatment				SEM	P-value			
	0.0	1.5	3.0	4.5		L	Q	C	Tr x Ti
DM intake, kg/d	13.4	13.2	14.0	13.3	0.70	ns	ns	ns	-
Rumen pH	6.03	6.14	6.03	6.13	0.03	ns	ns	ns	ns
Total SCFA, mM	103.0	95.9	110.0	97.8	2.70	ns	ns	0.0010	ns
Individual SCFA, mol/100 mol									
Acetate	60.0	60.1	60.6	61.1	0.98	ns	ns	ns	ns
Propionate	26.1	26.4	27.0	24.9	1.41	ns	ns	ns	ns
Butyrate	13.8	13.5	12.4	14.0	0.91	ns	ns	ns	ns
Acetate:propionate	2.30	2.28	2.24	2.45	0.17	ns	ns	ns	ns
Ammonia N, mg/dL	10.0	9.24	8.12	10.2	0.89	ns	ns	ns	ns
Lactate, mM	0.17	0.18	0.16	0.14	0.02	ns	ns	ns	ns

L, linear effect; Q, quadratic effect; C, cubic effect; Tr x Ti, treatment*time interaction effect; DM, dry matter; ns, not significant; SCFA, short-chain fatty acids; Ammonia N, ammonia nitrogen.

Table 3. *In situ* degradability of sugarcane neutral detergent fibre of treatments composed of different doses of a multivalent polyclonal antibody preparation in cattle fed high concentrate diets.

	Treatment				SEM	P-value				
Item	0.0	1.5	3.0	4.5		L	Q	C	Equation	R^2
b	51.1	30.5	33.5	39.0	3.62	ns	0.0158	ns	$Y= 50.2 - 16.4x + 3.3x^2$	0.20
c	0.0120	0.0277	0.0207	0.0134	0.002	ns	0.0085	ns	$Y= 0.01 + 0.01x - 0.003x^2$	0.24
l	2.9	4.0	2.7	4.0	0.65	ns	ns	ns	-	-
ED 5%	6.6	6.7	7.3	7.9	0.81	ns	ns	ns	-	-
PD	51.1	30.5	33.5	39.0	5.48	ns	0.0158	ns	$Y= 50.2 - 16.4x + 3.3x^2$	0.20

L, linear effect; Q, quadratic effect; C, cubic effect; b, potentially degradable fraction of the insoluble fraction degraded at a rate c; ns, not significant; c, rate of degradation of fraction b; l, colonisation time; ED, effective ruminal degradability calculated by the formula $ED=A+(B*C/(C+K))e^{(-K*L)}$ (Von Keyserlingk et al., 1996); PD, potential ruminal degradability.

Table 4. Total and relative counts of protozoa of treatments composed of different doses of a multivalent polyclonal antibody preparation in cattle fed high concentrate diets.

	Treatment				SEM	P-value		
	0.0	1.5	3.0	4.5		L	Q	C
Total counts, $\times 10^3$/mL								
Dasytricha	1.00	0.00	0.20	0.00	0.13	ns	ns	ns
Isotricha	0.33	0.80	0.87	0.40	0.16	ns	ns	ns
Epidinium	0.13	0.00	0.07	0.00	0.04	ns	ns	ns
Entodinium	23.53	27.93	17.07	33.27	3.70	ns	ns	ns
Diplodinium	0.20	0.00	0.00	0.00	0.04	ns	ns	ns
Relative counts, %								
Dasytricha	0.68	0.00	2.53	0.00	0.56	ns	ns	ns
Isotricha	3.55	6.00	4.56	1.88	1.08	ns	ns	ns
Epidinium	0.17	0.00	0.37	0.00	0.10	ns	ns	ns
Entodinium	95.11	94.00	92.54	98.12	1.20	ns	ns	ns
Diplodinium	0.49	0.00	0.00	0.00	0.09	ns	ns	ns

L, linear effect; Q, quadratic effect; C, cubic effect; ns, not significant.

Table 5. Digestibility coefficients for dry matter and its fractions, and mean values of total digestible nutrients (TDN) obtained with treatments composed of different doses of a multivalent polyclonal antibody preparation in cattle fed high concentrate diets.

Nutrient	Treatment				Mean	SEM	P-value		
	0.0	1.5	3.0	4.5			L	Q	C
DM, %	66.0	66.8	67.1	66.3	66.9	1.57	ns	ns	ns
OM, %	68.3	69.4	69.8	68.7	69.3	1.53	ns	ns	ns
CP, %	62.5	63.6	66.1	64.1	64.3	2.39	ns	ns	ns
EE, %	72.8	71.7	73.2	75.1	73.2	10.2	ns	ns	ns
NDF, %	29.1	36.3	26.4	25.7	29.4	5.19	ns	ns	ns
ADF, %	35.1	38.4	40.2	41.3	39.0	2.99	ns	ns	ns
Nitrogen-free extractive, %	84.7	83.3	87.2	84.4	84.9	3.04	ns	ns	ns
Starch, %	81.9	83.9	83.1	79.4	82.4	1.72	ns	ns	ns
TDN, %	69.3	70.2	70.9	69.8	70.3	1.42	ns	ns	ns

L, linear effect; Q, quadratic effect; C, cubic effect; ns, not significant. DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; TDN, total digestible nutrients.

Blanch *et al.* (2009) observed an increase in entodiniomorph counts, but they did not observe any effect of PAP for protozoa counts. Digestibility coefficients for DM and its fractions along with total digestible nutrients (TDN) of diets are presented in Table 5. No effects were observed for PAP doses (P>0.05) on DM digestibility or any of its fractions as well as TDN. Independently from treatment, observed data for DM and CP digestibility were in the range expected for diets with a forage:concentrate ratio between 40:60 and 25:75, with variations between 65.0 to 74.0% for DM digestibility and 58.0 to 74.0 for CP digestibility (Cardoso *et al.,* 2000; Borges *et al.,* 2008). Data observed for NDF digestibility in the present study (20.0%) was lower than that described in the literature (41.0% to 47.0%) (Tibo *et al.,* 2000; Borges *et al.,* 2008). This effect is probably related to high contents of highly fermentable carbohydrates in diets and to the low quality fibre of sugarcane, which is not much available to ruminal degradation probably because of its low content of protein (Carmo *et al*., 2001). Also, NDF digestibility could be reduced when ruminal pH remained four hours at values below 6.0 (De Veth and Kolver, 2001).

The lack of significant difference between the treatments and the control group could be related to the presentation of the product. In previous experiments of the same research group and others (DiLorenzo *et al.,* 2006, 2008; Blanch *et al.,* 2009; Marino *et al.,* 2011), the positive effects observed were obtained in trials made with PAP presented in liquid form. Maybe, the passage of the product from liquid to powder presentation could modify some of its properties.

Conclusions

In the present experimental conditions, different doses of a spray-dried PAP-MV against *Streptococcus bovis*, *Fusobacterium necrophorum*, *Clostridium aminophilum*, *Peptostreptococcus anaerobius* and *Clostridium sticklandii* were not efficient in altering DM intake, ruminal fermentation parameters, *in situ* degradability of sugarcane NDF and total tract apparent digestibility of total diet of cattle fed high concentrate diets. New feed additives evaluation is an opportunity to enhance ruminal fermentation and the knowledge of its mechanisms. We can conclude, then, that further research is needed to determine an ideal processing method to convert the liquid product into powder since it seems to be the most limiting factor for its efficacy.

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