

Influence of a polyclonal antibody preparation on the *in situ* degradability of three energy sources

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Abstract

The objective of this study was to evaluate the effect of a polyclonal antibody preparation (PAP) against specific ruminal bacteria on the *in situ* degradability of dry-grounded maize grain (DMG), high moisture maize silage (HMMS) starch and citrus pulp (CiPu) pectin. Nine ruminally cannulated cows were used in a 3 x 3 Latin square design, replicated three times in a factorial arrangement of treatments of two rumen modifiers represented by monensin and PAP plus a control group, and the three energy sources (DMG, HMMS and CiPu). Each period had 21 days, where 16 were used for adaptation to treatment and five for data collection. The group treated with PAP showed an effect on the soluble fraction ("a") of DMG starch, decreasing it by respectively 45.3% and 45.4% compared to the CON and MON groups. No effect of PAP was observed for any *in situ* degradability parameters of starch from HMMS or pectin of CiPu. It was concluded that the polyclonal antibody preparation had limited effect on the *in situ* degradability of the tested energy sources.

Keywords: *In sacco* degradability, ionophores, polyclonal antibody preparation, ruminal digestion

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Introduction

The use of ionophores for ruminal fermentation modulation has been employed with great success for better utilization of diets. However, the possible health effects of the use of these additives are a cause for concern and new methods of ruminal fermentation manipulation are beginning to be tested. The European Community, a major importer of meat from Brazil, by Regulation (EC) 1831/2003 (Europe, 2003), banned the use of antibiotics and coccidiostats as feed additives for cattle. This regulation reinforces the need of new feed additive development. The objective of this study was to evaluate the *in situ* degradation of the dry matter and starch from dry-grounded maize grain (DMG), high moisture maize silage (HMMS) and pectin from citrus pulp (CiPu), as influenced by a polyclonal antibody preparation against specific rumen bacteria in cows fed a high concentrate diet. The bacteria species are *Streptococcus bovis*, *Fusobacterium necrophorum*, *Clostridium aminophilum*, *Peptostreptococcus anacrobis* and *Clostridium sticklandii*.

Materials and Methods

The trial was conducted at the College of Veterinary Medicine and Animal Science at the University of São Paulo (USP), Brazil. Nine ruminally cannulated Holstein x Zebu non-pregnant dry cows (690 ± 44 kg BW) were used in 3 x 3 Latin square experimental design with three periods of 21 d each. Treatments were arranged as a 3 x 3 factorial arrangement of two all-feed additives monensin ([MON] or polyclonal antibody preparation [PAP]), plus a control group and three energy sources in the diet (dry-grounded maize grain [DMG], high moisture maize silage [HMMS] and citrus pulp [CiPu]). Cows were housed in a tie-stall barn equipped with individual feed bunks, rubber-matted floors and automatic water fountains common to two animals. There were fans in the ceiling in order to relieve the high temperatures during the day. Body weight was measured at the beginning of period one (d 1) and at the end of each of the three periods (d 21) at the same time each day.

Diets were fed as total mixed rations (TMR) with a ratio of concentrate to forage of 70 : 30 (DM basis, Table 1). Diets were offered twice daily at 08:00 and 16:00 for *ad libitum* consumption (minimum of 10%

Table 1 Ingredients proportions and chemical composition of experimental diets (dry matter basis)

Ingredients (%)	Experimental Diets		
	DMG	HMMS	CiPu
Sugarcane fresh and chopped	30.4	30.2	30.4
Dry-grounded maize grain (DMG)	64.2	15.7	11.2
High moisture maize silage (HMMS)	-	48.1	-
Citrus pulp (CiPu)	-	-	50.0
Soyabean meal	3.1	3.7	6.7
Urea	0.74	0.65	0.65
Vitamin and mineral premix ¹	0.74	0.75	0.56
Limestone	0.83	0.84	-
Dicalcium phosphate	-	-	0.47
Chemical composition			
Dry matter (%)	59.0	53.5	61.1
Ash (g/kg DM)	27.7	29.1	53.9
Crude protein (g/kg DM)	117	108	108
Degradable protein in rumen (% CP) ²	73.0	73.0	71.0
Non degradable protein in rumen (% CP) ²	27.0	27.0	29.0
Ether extract (g/kg DM)	28.4	30.0	20.9
Neutral detergent fibre (g/kg DM)	249	232	213
Acid detergent fibre (g/kg DM)	163	143	213
Non-structural carbohydrate (g/kg DM)	576	601	503
Starch (g/kg DM)	498	467	122
Pectin (% DM)	3.3	3.4	112
Total digestible nutrients (g/kg DM) ²	780	790	730
Calcium (g/kg DM)	4.9	5.7	14.1
Phosphorus (g/kg DM)	3.0	2.6	2.5

¹ Composition of vitamin and mineral premix per kg of product: 230 g calcium; 90 g phosphorus; 15 g sulphur; 20 g magnesium; 48 g sodium; 100 mg cobalt; 700 mg copper; 2.000 mg iron; 80 mg iodine; 1.250 mg manganese; 20 mg selenium; 2.700 mg zinc; 900 mg fluorine (maximum); 200.000 IU vitamin A; 60.000 IU vitamin D3; 60 IU vitamin E. ²Value estimated by CNCPS programme, version 5.0.40.

feed refusal). The forage source was fresh sugarcane chopped to a theoretical average particle size of 1.18 cm; measurement taken by the Penn State Particle Size Separator (Lammers *et al.*, 1996). MON and PAP were offered directly through the rumen cannula twice daily, just before the meals. MON (Rumensin, Elanco Animal Health, Indianapolis, I.N., USA) at 300 mg/animal/day was administered in absorbent tissue paper and PAP (CAMAS Inc., Le Centre, MN) at 10 mL/animal/day using a 10 mL syringe. The latter product contained antibodies against *Streptococcus bovis*, *Fusobacterium necrophorum* and some strains of proteolytic bacteria (*Peptostreptococcus* sp., *Clostridium aminophilum* and *Clostridium sticklandii*).

Each period had 21 days, where 16 days were used for adaptation to treatments and five days for data collection. The *in situ* degradability of DM and starch or pectin from the energy sources was measured by the nylon bag technique (Mehrez & Ørskov, 1977). Dry matter was determined according to AOAC (1990). Starch concentration was determined by the method described by Pereira & Rossi (1995) after extraction of soluble carbohydrates (Hendrix, 1993). Pectin was determined by the method described by Van Soest *et al.* (1991).

For degradation, parameters were estimated using the model proposed by Ørskov & McDonald (1979): $p = a + b(1 - e^{-ct})$, where p is the degradation at each time; “ a ” is the soluble fraction; “ b ”, the potentially degradable fraction of the insoluble fraction that is degraded at a rate “ c ”; “ c ” is the rate of degradation of fraction “ b ”; and “ t ” is the incubation period in hours. The parameters “ a ”, “ b ” and “ c ” from exponential equation were used to calculate the potential degradability ($P_d = a + b$), which represents the quantity of feed that can be solubilized or degraded in the rumen if time is not a limiting factor. The effective ruminal degradability (E_d) was calculated according to the mathematical model proposed by Ørskov &

McDonald (1979): $Ed = a + [(b \times c)/(c + K)]$, where K is the passage rate of solids from the rumen, accepted here as either 0.02, 0.05 or 0.08 %/h.

Degradability data were calculated by the difference in weight of nylon bags before and after rumen incubation and adjusted according to the equation of Ørskov & McDonald (1979). Results were analyzed by the Statistical Analysis System software (SAS, 2001). Firstly, the residue normality was verified by the Shapiro-Wilk test (PROC UNIVARIATE). Data (dependent variable) that did not meet this assumption were submitted to logarithmic transformation [$\text{Log}(X+1)$] or square root adjustment [$\text{RQ}(X+1/2)$]. Original or transformed data, when this last procedure was necessary, were submitted to analysis of variance by PROC GLM (General Linear Models) procedure. The model accounted for the effect of feed additive, energy source, the interaction of feed additive x energy source, period and animal. The effects of the main factors (feed additive and energy source) were separated by Duncan test. Effects were declared significant at $P \leq 0.05$.

Results and Discussion

The results of the influence of polyclonal antibody on the *in situ* degradability parameters of starch from DMG and HMMS and pectin from CiPu are presented on Tables 2, 3 and 4, respectively.

The group treated with PAP showed an effect ($P = 0.037$) in the soluble fraction “a” of DMG starch, decreasing it by 45.3% and 45.4% compared to the CON and MON groups, respectively (Table 2).

It was observed that the treatment with MON decreased the value of the potentially degradable fraction “b” of DM of HMMS by 16.1% compared to the CON group, but there was no difference when PAP was administered (Table 3). No effect of rumen modifier was observed for any of the *in situ* degradability parameters of starch of HMMS. In general, irrespective of treatments, *in situ* degradability values of HMMS were higher than the ones described by Jobim *et al.* (1999). These authors mentioned that high moisture maize silages had higher soluble fractions “a” in comparison to whole plant or ear of maize silages (grain +

Table 2 Effect of rumen modifier on the *in situ* degradability of dry matter and starch of dry- grounded maize grain

Dry matter	Rumen modifier ¹			Mean	s.e.m. ²	Prob. ³
	CON	MON	PAP			
Fraction a	12.84	13.39	12.64	12.96	0.620	0.8531
Fraction b	85.56	84.33	86.95	85.61	1.362	0.8541
Rate c	0.0501	0.0433	0.0483	0.047	0.004	0.8608
Ed ⁴ 2%	73.33	70.86	72.27	72.15	1.246	0.8842
Ed 5%	55.15	52.33	53.68	53.72	1.603	0.8899
Ed 8%	45.43	42.84	44.07	44.12	1.560	0.8986
Pd ⁵	98.40	97.72	99.59	98.57	1.316	0.8699

Starch	Rumen modifier ¹			Mean	s.e.m.	Prob.
	CON	MON	PAP			
Fraction a	4.64 ^a	4.65 ^a	2.54 ^b	3.95	0.596	0.0374
Fraction b	97.25	97.66	100.71	98.54	0.694	0.1699
Rate c	0.0592	0.0505	0.0564	0.0554	0.006	0.8573
Ed 2%	76.65	74.38	74.53	75.18	1.576	0.8210
Ed 5%	56.75	53.55	53.69	54.66	2.128	0.8246
Ed 8%	45.55	42.32	42.44	43.44	2.084	0.8272
Pd	101.89	102.31	103.25	102.49	0.381	0.4833

^{a,b,c} Means without common superscripts differ, $P \leq 0.05$ (Duncan).

¹ Rumen modifier: CON – control; MON – monensin; PAP – polyclonal antibody preparation.

² s.e.m. – standard error of the mean; ³ Prob. – statistical probabilities for rumen modifier effect;

⁴ Ed – effective degradability; ⁵ Pd – potential degradability.

Table 3 Effect of rumen modifier on the *in situ* degradability of dry matter and starch of high moisture maize grain

Dry matter	Rumen modifier ¹			Mean	s.e.m. ²	Prob. ³
	CON	MON	PAP			
Fraction a	58.66	61.14	59.36	59.72	0.480	0.3248
Fraction b	35.13 ^a	29.46 ^b	32.78 ^{ab}	32.45	1.028	0.0383
Rate c	0.1350 ^b	0.2203 ^a	0.1371 ^b	0.164	0.020	0.0169
Ed ⁴ 2%	88.85	88.01	87.90	88.25	0.508	0.7728
Ed 5%	83.69	84.92	83.27	83.96	0.632	0.6979
Ed 8%	80.08	82.49	79.95	80.84	0.782	0.5032
Pd ⁵	93.79 ^a	90.60 ^b	92.14 ^{ab}	92.18	0.714	0.0810

Starch	Rumen modifier ¹			Mean	s.e.m.	Prob.
	CON	MON	PAP			
Fraction a	75.36	75.77	74.52	75.21	0.502	0.7730
Fraction b	23.99	23.31	25.23	24.17	0.629	0.6715
Rate c	0.2633	0.0573	0.1995	0.3234	0.504	0.1579
Ed 2%	97.49	98.18	97.11	97.60	0.244	0.3947
Ed 5%	95.21	96.97	94.12	95.43	0.583	0.3583
Ed 8%	93.38	95.87	91.87	93.71	0.805	0.3485
Pd	99.35	99.08	99.74	99.39	0.165	0.4619

^{a,b,c} Means without common superscripts differ, $P \leq 0.05$ (Duncan).

¹ Rumen modifier: CON – control; MON – monensin; PAP – polyclonal antibody preparation.

² s.e.m. - standard error of the mean; ³ Prob. - statistical probabilities for rumen modifier effect;

⁴ Ed - effective degradability; ⁵ Pd - potential degradability.

Table 4 Effect of rumen modifier on the *in situ* degradability of dry matter and pectin of citrus pulp

Dry matter	Rumen modifier ¹			Mean	s.e.m. ²	Prob. ³
	CON	MON	PAP			
Fraction a	14.26	15.54	16.62	15.47	0.601	0.2118
Fraction b	85.09	85.46	87.10	85.88	0.950	0.7207
Rate c	0.0386	0.0411	0.0287	0.036	0.005	0.3223
Ed ⁴ 2%	67.74	72.54	67.88	69.39	2.181	0.3023
Ed 5%	49.54	53.75	48.35	50.55	2.209	0.2883
Ed 8%	40.79	44.31	39.70	41.57	1.917	0.2925
Pd ⁵	99.34	101.00	103.72	101.35	0.952	0.2703

Starch	Rumen modifier ¹			Mean	s.e.m.	Prob.
	CON	MON	PAP			
Fraction a	14.88	14.20	16.30	15.13	1.450	0.9026
Fraction b	84.93	88.23	79.55	84.24	2.870	0.3610
Rate c	0.0462	0.0452	0.0596	0.050	0.007	0.6048
Ed 2%	72.45	74.56	72.94	73.32	2.138	0.8787
Ed 5%	54.46	55.44	56.56	55.49	2.51	0.9305
Ed 8%	45.20	45.60	47.75	46.18	2.522	0.8884
Pd	99.81	102.43	95.85	99.36	1.708	0.1313

^{a,b,c} Means without common superscripts differ, $P \leq 0.05$ (Duncan).

¹ Rumen modifier: CON – control; MON – monensin; PAP – polyclonal antibody preparation;

² s.e.m. - standard error of the mean; ³ Prob. - statistical probabilities for rumen modifier effect;

⁴ Ed. - effective degradability; ⁵ Pd - potential degradability.

maize cob), which was attributed to lower cell wall fractions in grain silages. A high fraction “a” in association with a high potentially degradable fraction “b” of the feed supports rapid disappearance of grain silages from the rumen.

Moreover, there was no observed effect of rumen modifier on any *in situ* degradability parameters of the DM or pectin of CiPu (Table 4).

Conclusion

The polyclonal antibody preparation showed limited effect on the *in situ* degradability of energy sources. On the other hand, monensin affected dry matter *in situ* degradability of some energy sources, but not in others.

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