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Alfalfa Ruminal Degradation Using Xylanases

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Abstract: Animal production is improved by the use of exogenous enzymes during feeding that modify solubility and digestibility of fiber, which favors a greater ruminal microorganisms activity, this improves profitability of ruminant diets based on grain and forages. There are evidences of the use of these enzymes on diets with a 78% of concentrate, where there was an increase of 25% of NDF ruminal digestion, better than 18% when using 25% of forage. In this research a xilanase enzyme was evaluated Fibrozyme (Alltech, Inc.) which is composed of fermentation extracts of *Aspergillus niger* and *Trichoderma longibrachatium*, in four treatments: T1 = only alfalfa or control (1), T2 = alfalfa plus Fibrozyme (1), T3 = alfalfa plus Fibrozyme (2) and T4 = 40% concentrate plus 60% of alfalfa without Fibrozyme (2), the first two with a basal diet 1 = 100% alfalfa and the last two with 2 = 40% alfalfa plus 60% concentrate for 10 days. The enzyme was given at a dose of 14 g d^{-1}, 7 g at 0800 and 7 g at 2000. Two male animals ¾ Holstein-Gyr 480 kg average and ruminally cannulated were used. Dry matter offered was adjusted with this equation: 95 g DM ($W^{0.75}$). A total of 32 nylon bags 10×20 cm, 50±15 micron (Ankom), weighted and identified were introduced in the rumen. Evaluation time was 48 h after bag introduction. Variables evaluated were: ruminal disappearance of Dry Matter, (DM), Neutral Detergent Fiber (NDF) and net energy gain (NEg Mcal kg^{-1} DM), pH and ruminal temperature. A randomized complete block design, X^2 test and Tukey means comparison were performed. Results show that for dry matter, T4 had less residues (30.888^a) ($p<0.05$) in comparison with the other treatments, which didn't show significant differences. The lowest NDF residues after ruminal degradation was in T3 (57.979^a) ($p<0.05$) with basal diet 2; T1 had the highest values (68.316^c). The greatest net energy gain was on T3, being significantly different to control. Results of comparing treatments including Fibrozyme *versus* control in relation to NDF 48 h after bag introduction show that when adding Fibrozyme, NDF percentages are in general lower than when Fibrozyme was not added. In the same sense, the highest change percentage (15.13%) was when animals were given alfalfa plus. Fibrozyme with basal diet 2. When comparing means of treatments without Fibrozyme *versus* the ones with it; superiority of these last was of 10.207 % ($p<0.01$).

Key words: Alfalfa degradation, ruminal digestion, exogenous enzymes, xilanases

INTRODUCTION

Animal production is improved by the use of exogenous xilanases during feeding, they modify solubility and digestibility of fiber, permitting this way a greater activity of ruminal microorganisms and finally improving the use of diets for ruminants based on grains and forages (Mendoza, 2000). There exist evidence of the use of these enzymes in dairy cows which gained more weight in early lactation (Knowlton *et al*., 2002) and during fattening, using a 78% of concentrate, obtaining a increase of 25% in ruminal digestion of NDF (Zinn and Salinas, 1999), even higher than the 18% found when using the same enzyme but with a 35% of forage

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(Ambrozio *et al*., 2000). The objective of this research was to evaluate the effect of the xilanase Fibrozyme (Alltech, Inc.) on rations with basal diets with and without concentrate.

MATERIALS AND METHODS

The xilanase Fibrozyme (Alltech, Inc.) composed of extracts of fermentation of *Aspergillus niger* and *Trichoderma longibrachtium* was evaluated in four treatments: T1 = alfalfa without Fibrozyme (1), T2 = alfalfa plus Fibrozyme (1), T3 = alfalfa plus Fibrozyme (2) and T4 = 60% of alfalfa without Fibrozyme plus 40% of concentrate; the first two with the basal diet 1 = 100% of alfalfa and the last two with basal diet 2 = 60% of alfalfa plus 40% of concentrate. Both for ten days. The enzyme was given at a dose of 14 g d^{-1}, 7 g at 0800 and 7 g at 2000 h. Two male animals ¾ Holstein-Gyr 480 kg average and ruminally cannulated were used. Dry matter offered was adjusted with this equation: 95 g DM ($W^{0.75}$). Thirty two nylon bags 10×20 cm, 50±15 micron (Ankom), weighted and identified were introduced in the rumen. Evaluation time was 48 h after bag introduction. Variables evaluated were: Ruminal Disappearance of Dry Matter (RDDM%) and Neutral Detergent Fiber (NDF%) and Net Energy gain (NEg Mcal kg^{-1} DM) and pH and Ruminal Temperature (RT°C). For the *in situ* estimation of DM and NDF the Schneider and Flatt (1975) formula was used. DM and NDF were determined in the laboratory according to AOAC (1975) and the Neg with the equation proposed by Zinn and Salinas (1999). A randomized complete block design was used and X^2 test and Tukey means comparisons (SAS, 1998) were performed.

RESULTS AND DISCUSSION

The obtained results, they are shown in the Table 1 and 2.

Results show that for *in situ* estimation of DM T4 had less residues (30.888^a) ($p<0.05$) than the other treatments, which were not statistically different among them (Table 1). This does not coincide with Bowman *et al.* (2002). After ruminal degradation of NDF, there were less residues in T3 (57.978^a) with basal diet 2 ($p<0.05$). Control (T1) had the highest values with 68.316^c (Table 1). These results are important because a decrease of 24-30% in NDF in ruminal degradation may reduce the intake of dry matter in 8% and average gain in 12% (Zinn *et al*., 2002). The highest support of NEg was present in T3 and statistically differs from the control ($p< 0.05$). Table 1 shows that when considering T3 as 100%, there exist differences of up to a 36.64 % in NEg with respect to the other treatments, even higher to the 5.1 and 1% obtained by Pereira and Zinn (2001) and Ware *et al.* (2002), respectively. There were no differences in pH and ruminal temperature ($p< 0.05$). However, as the optimum ruminal pH for cellulose bacteria is >6.5 and when decreases from this value up to 6, the specific growth rate decreases in 14% h^{-1} by every 0.1 units of ruminal pH decrease (Zinn *et al*., 2002).

In Table 2 we can see that when comparing treatment with Fibrozyme *versus* control in relation to NDF after 48 h, values are in general lower as Fibrozyme increases ($p<0.05$). The same occurred for the percentage of change when animals were fed with alfalfa plus Fybrozyme in basal diet 2 with the highest increase of 15.13 %. When comparing means of treatments without Fibrozyme *versus* the ones with it; superiority of these last was of 10.207 % ($p<0.01$) (Table 2).

Table 1: *In situ* estimation of Dry Matter content (%), Neutral Detergent Fiber (%), Net Gain energy (%), pH and Ruminal Temperature (°C) after 48 h

	Content *In situ* (%)					
Treatments	DM%	NDF%	Neg (Mcal kg^{-1} DM)	NEg%	pH	RT°C
T1 = Alfalfa without Fibrozyme[1]	40.244^b	68.316^c	0.384^c	-36.64	6.30^a	35.87^a
T2 = Alfalfa plus Fibrozyme[1]	44.577^b	63.300^b	0.491^b	-18.98	6.26^a	36.31^a
T3 = Alfalfa plus Fibrozyme[2]	40.316^b	57.978^a	0.606^a	100.00	6.38^a	36.60^a
T4 = 40% de concentrate plus 60% alfalfa without Fibrozyme[2]	30.888^a	63.005^b	0.498^b	-17.82	6.31^a	36.65^a
CV%	10.24	2.87	2.00	1.96		

1) Basal diet 100% alfalfa., 2) Basal diet 40% concentrate plus 60% alfalfa. Values with unlike letters are significantly different, ($p<0.05$)

Table 2: Comparison of *in situ* content of Neutral Detergent Fiber of control vs Fibrozyme 48 h after applying treatments*

	In situ (%) NDF content			
Feed evaluated	Control	Fibrozyme	% change	p = value
Alfalfa[1]	68.316^b	63.30^a	+ 7.34	0.001
Alfalfa[2]	68.316^b	57.978^a	+ 15.13	0.001
40% concentrate plus 60% alfalfa[2]	63.005^b	57.978^a	+ 7.978	0.001
Control vs. Fibrozyme	66.546^b	59.753^a	+10.207	0.001

1) Basal diet 100% alfalfa, 2) Basal diet 40% concentrate plus 60% alfalfa, Values in the same row with different superscripts differ ($p<0.01$), *X^2 values

Overall conclusions show that the use of Fibrozyme doesn't improve dry matter degradation but do the gain of net energy up to a 36.64 % ($p<0.05$) and decreases NDF ($p<0.01$), therefore may be use in animal production but new research is necessary for specific cases.

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