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INFLUENCE OF TREATING SOME LOW-QUALITY FORAGES WITH EXOGENOUS FIBROLYTIC ENZYMES ON RUMINAL FERMENTATION.

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SUMMARY

A study was undertaken to assess appropriate fibrolytic enzymes inclusion effects of three fibrolytic enzymes treatments on the In vitro ruminal fermentation of five low-quality forages (three cereal straws (barley, wheat and rice), corn stover and Rhodes grass hay) were investigated using batch cultures of mixed ruminal microorganisms. Four different treatments were investigated: no additive (control; CON), cellulase from Aspergillus niger (CEL; Fluka Chemie GmbH), xylanase from ruminal microorganisms (XYL; Megazyme International Ireland Ltd), and a 1:1 mixture cellulase:xylanase (MIX). Enzymes (20 IU/g forage dry matter (DM)) were applied directly into the forages 24 h before incubation with buffered ruminal fluid at 39°C for 24 h. The treatment with CEL increased ($P<0.05$) gas production after 24 h of incubation for wheat straw, barley straw and grass hay. Meanwhile, the treatment with MIX and XYL did not affect ($P>0.05$) gas production for any forage. For all forages, there was no change ($P>0.05$) in NH3-N concentration with added enzymes, indicating no differences in protein degradability and/or ammonia-N incorporation by ruminal microorganisms. Also, Natural detergent fiber degradability (NDFD) concentration were not affected ($P>0.05$) by the addition of enzymes. The treatment of low-quality forages with CEL increased the production of propionate for Rhodes grass hay, corn stover and barley straw, and increased ($P<0.05$) total VFA production for wheat straw and corn stover. No effects ($P>0.05$) of MIX and XYL treatments on VFA production were observed for any substrate. Under the conditions of the present experiment, the pre-treatment of low-quality forages with cellulase and xylanase enzymes produced subtle effects on In vitro ruminal fermentation, suggesting that the used enzymes contributed little, if any, to ruminal fibrolytic activity.

***Keywords**: cellulase, xylanase, cereal straws, batch cultures, in vitro degradability*

INTRODUCTION

In some small ruminant production systems, forages constitute the major portion of all available feed resources. In the case of low-quality forages (high fibre content and low digestibility), any improvement in their nutritive value would increase the productivity of the animals. The use of feed enzymes for ruminants has been viewed with considerable scepticism, but in recent years a considerable number of studies on this topic have been conducted. The majority of these experiments were designed with the expectation that a fibrolytic enzyme should increase the degradability of feed in the rumen, and this response has been observed in many of these studies (McAllister *et al.*, 2001; Phipps *et al.*, 2002).

Recent research indicates that a blend of cellulase and xylanase is more effective than cellulase alone (Pritchard *et al* ., 1998, Carro *et al.*, 2003 and Wang *et al.*, 2004) reported that adding the enzyme mixture just prior to feeding is as effective as treating the forage 2 week (Yang *et al* ., 1999) or 1 or 3 days (Lewis *et al.*, 1996 and Nussin *et al* ., 1997) before feeding. Concerning milk production, (Nussin *et al.*, 1997) reported that different responses in the performance of cows fed enzyme treated diets observed at different stages of lactation . Yang *et al.*, (1998) observed a 10 % increase in milk production for dairy cows in early lactation , when a different fiberolytic enzyme additive was included in diets. Also, Schingoethe *et al.*, (1999) reported greater production responses during early than during late lactation.

In this context, treatment of paddy straw with fibrolytic enzymes is gaining researcher's attention. It has been demonstrated that (Morgavi *et al* 2000 and Mohamed *et al.*, 2005) exogenous fibrolytic enzymes work in synergy with the endogenous rumen microbial enzymes to enhance the digestion of high fibrous feed. Therefore, supplementation of fibrolytic enzymes to ruminant diets or pre-treatment of diets containing high levels of crop residues with fibrolytic enzymes is expected to enhance the digestibility and nutritive value of the diet. In addition use of fibrolytic enzymes would also pave way for effective utilization of paddy straw leading to increased economic benefits for the farmer. The objective of this study was to evaluate the effects of three different fibrolytic enzymes treatments on the in vitro ruminal fermentation of five low-quality forages.

MATERIALS AND METHODS

Samples of barley, wheat and rice straws, corn stover and Rhoades grass hay were ground through a 1-mm screen and fermented In vitro with buffered ruminal fluid. The chemical composition of forages is given in Table 1.

Table (1): Chemical composition (g/kg DM) of forages incubated in vitro with buffered ruminal fluid.

Forage	Neutral-detergent fiber (NDF)	Acid-detergent fiber (ADF)	Crude Protein (CP)	Organic matter (OM)
Barley straw	707	387	54	929
Wheat straw	757	544	35	931
Rice straw	708	407	37	920
Rhodes grass hay	710	419	57	950
Corn stover	653	450	53	940

Three different enzymes treatments preparations were tested: cellulase from Aspergillus niger (CEL; Fluka Chemie GmbH), xylanase from ruminal microorganisms (XYL; Megazyme International Ireland Ltd), and a 1:1 mixture cellulase:xylanase (MIX). Enzymes (20 IU/g forage dry matter (DM)) were applied directly into the forages 24 h before incubation with buffered ruminal fluid at 39°C for 24 h. Solutions of each enzyme containing 5 units per ml were prepared in 0.1 M sodium phosphate buffer (pH 6.5). Samples of 500 mg of forage dry matter (DM) were accurately weighed into 120-ml glass bottles and 2 ml of the corresponding solution (20 IU/g forage DM) were added into each bottle 24 h before starting the incubation. Bottles were kept at room temperature (21-23°C) until incubation. This pre-treatment of forages with enzymes as previous in vitro studies, have shown that this enzyme-feed interaction enhanced the efficacy of enzymatic treatments (Giraldo *et al.*, 2004a). Two ml of buffer were added to bottles corresponding to control treatment.

Rumen fluid was obtained from four rumen-cannulated Merino sheep fed forage (medium-quality alfalfa hay) ad libitum. Rumen contents of each sheep were obtained before the morning feeding, mixed and strained through four layers of cheese-cloth into an Erlenmeyer flask with an O_2-free headspace. Particle-free fluid was mixed with the buffer solution of Goering and Van Soest (1970) in a proportion 1:4 (v:v) at 39 °C under continuous flushing with CO_2. Bottles were pre-warmed (39°C) prior to the addition of 50 ml of buffered rumen fluid into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39 °C for 24 h. Four incubation runs were performed on different days, so that each treatment was conducted in quadruplicate. In each incubation run, two blanks (bottles without substrate but with the corresponding enzymatic treatments) were included. Bottles were withdrawn from the incubator 24 h after inoculation and gas production was measured with a calibrated syringe and a pressure transducer. Bottles were uncapped, the pH was measured immediately and the fermentation was stopped by swirling the bottles in ice. One ml of the bottle content was added to 1 ml of deproteinizing solution (10% of metaphosphoric acid and 0.06% crotonic acid; w/v) for volatile fatty acids (VFA) analysis and another 1 ml was added to 1 ml of HCl for NH_3-N analysis. The contents of the bottles were then transferred to previously weighed filter crucibles. Each solid residue of incubation was washed with 50 ml of hot (50°C) distilled water and crucibles were dried at 50°C for 48 h. Residues were analyzed for neutral-detergent fiber (NDF) to estimate fiber degradability (NDFD). Dry matter, ash and N were determined according to the A.O.A.C. (1999). Neutral- and acid-detergent fiber analyses were carried out according to Van Soest *et al.* (1991). NH3-N concentration was determined according to Conway, (1963) and VFA's concentrations were determined according to Warner, (1964). The amounts of VFA produced were obtained by subtracting the amounts present initially in the incubation medium from those determined at the end of the incubation period. Data were analysed as an one-way ANOVA with four enzymatic treatments (control (CON), CEL, MIX and XYL) and four rumen inocula as main effects. Statistical analysis were performed using the GLM procedures of the statistical Analysis System (SAS, 1988). When a significant effect of the treatment ($P<0.05$) were detected, differences between means were assessed by the LSD test.

RESULTS AND DISCUSSION

Influence of different enzymatic treatments on production of gas (ml) and volatile fatty acids (VFA µmol), NH3-N concentration (mg/l) and neutral-detergent fibre

degradability (NDFD %) after in vitro fermentation of forage samples (500 mg) in batch cultures of mixed rumen microorganisms for 24 h (n=4) are shown in Table (2.).

Table (2): Influence of different enzymatic treatments on rumen fermentation parameters.

Item	Treatment				±SE
	MIX	XYL	CEL	Con	
Barley straw					
Gas ml	71.9 [a]	69.3 [ab]	72.8 [a]	70.4 [ab]	1.25
Total VFA umol	1703	1675	1692	1656	18.1
Acetate	1165	1144	1139	1129	15.5
Propionate	404 [a]	399[ab]	412[a]	394[ab]	4.54
Butyrate	113	114	118	111	3.0
NH3-N mg/100l	171	180	182	202	7.2
NDFD	45.4	45.6	46.4	46.7	1.31
Wheat straw					
Gas ml	53.4 [ab]	52.6 [ab]	54.5[a]	50.9 [b]	31.1
Total VFA umol	1347[a]	1323[ab]	1395[a]	1298[b]	19.5
Acetate	923[ab]	890[b]	958[a]	895[b]	10.6
Propionate	313	317	324	302	5.16
Butyrate	94.0	96.5	95.0	82.3	8.9
NH3-N mg/100l	184[b]	183[b]	194 [ab]	207[a]	1.30
NDFD	41.3	42.3	41.7	40.9	0.96
Rice straw					
Gas ml	48.8	48.6	50.2	48.5	32.3
Total VFA umol	1242[ab]	1222[b]	1270[a]	1253[a]	21.4
Acetate	834[b]	807[b]	862[a]	843[ab]	8.8
Propionate	308	309	312	313	3.46
Butyrate	80.0	84.5	78.8	77.8	9.1
NH3-N mg/100l	208	187	197	185	0.92
NDFD	43.5	42.8	43.1	42.1	0.89
Rhodes grass hay					
Gas ml	59.7	59.5	60.6	58.1	35.2
Total VFA umol	1476	1454	1493	1419	26.1
Acetate	1010	1003	1013	967	7.7
Propionate	335[ab]	325[a]	343 [b]	324[a]	4.4
Butyrate	106	102	110	102	8.8
NH3-N mg/100l	193	193	213	212	1.22
NDFD	36.0	36.4	34.5	34.8	2.76
Corn stover					29.5
Gas ml	72.7	72.0	73.7	71.6	
Total VFA umol	1820[a]	1826[a]	1907 [b]	1833[a]	26.8
Acetate	1196	1208	1235	1205	10.9
Propionate	461[a]	448[a]	492 [b]	463[a]	3.2
Butyrate	138[a]	140[a]	151 [b]	139 [a]	8.3
NH3-N mg/100l	215	217	224	224	1.73
NDFD	43.2	40.2	42.3	42.6	1.25

a, b Means in the same row with different superscripts differ (P<0.05).

Effect of exogenous fibrolytic ezymes on in vitro rumen fermentation of forages are shown in Table (2). The treatment with CEL increased (P<0.05) gas production for barley straw, wheat straw and grass hay, but no effects (P>0.05) of MIX and XYL treatments were observed for any forage. The increase in gas production for CEL treatment could not stem from enzyme fermentation itself, as there were no differences (P>0.05) between CON and CEL treatments in the amount of gas produced in the blanks (mean values of 5.7 and 5.5 ml, respectively); therefore, these results might indicate that CEL treatment enhanced the in vitro fermentation of these forages. Indeed, the treatment of barley straw, grass hay and corn stover with CEL increased (P<0.05) propionate production by 4.6, 5.9 and 6.3%, respectively, but did not affect (P>0.05) the production of acetate. Wang *et al.* (2004) showed that the treatment of wheat straw with an enzyme preparation containing xylanase and β-gluconase activities increased VFA at 4 h of incubation, but no differences due to the enzyme treatment were observed after 30 h of incubation. In agreement with these results, previous research conducted by Giraldo *et al.*, (2004b) showed that the effects of treating substrates with fibrolytic enzymes on In vitro VFA production by rumen microorganisms were more marked at 6 than at 24 h of incubation. In the present experiment, VFA production was measured at 24 h and only CEL treatment increased significantly (P<0.05) this parameter for wheat straw and corn stover. . For all forages, there was no change (P>0.05) in NH3-N concentration with added enzymes, indicating no differences in protein degradability and/or ammonia-N incorporation by ruminal microorganisms. Although all forages presented a low protein content (35-57 g crude protein/kg DM), NH3-N concentrations after 24 h of incubation were in the range of those considered as optimal for ruminal microbial growth (Mehrez *et al.*, 1977) due to the use of a N-enriched buffer (Goering and Van Soest, 1970).

The sterified bonds between cellulose, hemicellulose and lignin restrict the digestion of cell walls by ruminal microorganisms; however, it has been shown (Nsereko *et al.*, 2000; Giraldo *et al.*, 2004a) that exogenous fibrolytic enzymes could potentially improve fiber degradation through a hydrolytic action prior to feeding or In vitro incubation. On the other hand, the 24 h pre-treatment of a good-quality substrate (mixture grass hay:concentrate; 60:40) with the cellulase used in this study (cellulase from Aspergillus niger; Fluka Chemie GmbH) at rates of 15 and 30 IU/g substrate DM decreased (P<0.05) by 7.6 and 10.2% its NDF content . These results would indicate that effectiveness of fibrolytic enzymes varies with the substrate (McAllister *et al.*, 2001). The ability of cellulases and xylanases to increase the extent of fibre digestion may be limited by the lack of enzymes that degrade the core structure of lignin-cellulose complexes in low-quality forages. Krueger *et al.* (2003) showed that an enzymatic complex containing high esterase, cellulose and endogalacturonase activities enhanced the digestion of low-quality tropical hays and suggested that the use of enzymes such as ferulic acid esterases could made the digestible xylans in the cell wall more susceptible to enzymatic degradation. In the present study, the lack of effects of enzymes on forages NDF content was in agreement with the observed inefficacy of enzymatic treatments to increase forages NDFD (P>0.05).

CONCLUSION

Under the conditions of the present experiment, the pre-treatment of low-quality forages with cellulase and xylanase enzymes produced subtle effects on In vitro ruminal fermentation, suggesting that the used enzymes contributed little, if any, to ruminal

fibrolytic activity. Future work is required to investigate the possible contribution of these enzymes to forage degradation and to find the ideal combination of highly active enzymes for optimizing the degradation of low-quality forages.

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تأثير معاملة الأعلاف الخشنة الفقيرة بالانزيمات المحللة للالياف على تخمرات الكرش ومعدل اختفاء المادة الجافة

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استخدمت بعض التخمرات المعملية لدراسة تأثير بعض الانزيمات المحللة للألياف (Cellulase & Xylanase) منفردة او مجتمعة فى معاملة الأعلاف الخشنة الفقيرة على تخمرات الكرش ومعدل اختفاء المادة الجافة. لقد تم معاملة (تبن القمح، تبن الشعير ، قش الأرز، حطب الأذرة، دريس الحشائش). وكانت مستويات المعاملة بمعدل ٢٠ وحدة دولية / جم مادة جافة بأحد الانزيمات او مخلوطهما معا وتركهما لمدة ٢٤ساعة قبل ان يتم تحضينها مع سائل الكرش.

لقد وجد ان حجم الغاز الناتج من المعاملة Cellulase قد زاد مع (تبن القمح، تبن الشعير ، قش الأرز) بينما لم يتغير حجم الغاز الناتج مع كل من (حطب الأذرة، دريس الحشائش). كذلك لم يحدث تغير معنوى فى كمية الأمونيا الناتجة مع الأعلاف الخشنة الفقيرة نتيجة المعاملة بالانزيمات. كما ان المعاملة لم تؤثر على معدل اختفاء الألياف الذائبة فى المحلول المتعادل. ولقد أدت المعاملة بواسطة Cellulase الى زيادة حامض البروبيونات الناتج مع كل من تبن الشعير، حطب الأذرة، دريس الحشائش)، كما أدت المعاملة الى زيادة كمية الأحماض الدهنية الطيارة مع كل من (تبن القمح ، حطب الأذرة). بينما لم توجد فروق معنوية فى إنتاج الأحماض الدهنية الطيارة فيما بين مواد الاعلاف الخشنة (تبن القمح، تبن الشعير ، قش الأرز، حطب الأذرة، دريس الحشائش) المعاملة بانزيم Xylanase أو مخلوطهما (Cellulase & Xylanase) . ويتضح من هذه المعاملات بالإنزيمات ان التغيرات الناتجة غير معنوية .