Effects of Enzyme Application Method and Levels and Pre-treatment Times on Rumen Fermentation, Nutrient Degradation and Digestion in Goats and Steers

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ABSTRACT : Present study investigate the effect of enzyme supplementation, methods (applied to rumen or enzyme treated diet) compared with no enzyme diet, on rumen fermentation and apparent nutrient digestibility in a 3×3 Latin square design with three rumen cannulated Korean Native goats. *In situ* rumen degradation kinetics was studied in three rumen cannulated Holstein steers. Three diets were, no enzyme, 1% enzyme in rumen and 1% enzyme in diet. The enzyme was sprayed onto forage, and the forage: concentrate ratio was 30:70. Degradation kinetics was studied with three enzyme levels (0, 1 and 2%, w/w) and four pre-treatment times (0, 1, 12 and 24 h). Results suggested that enzyme application method did not affect rumen fermentation, ruminal enzyme activity and total tract apparent digestibility. Nutrient degradation rate and effective degradability of DM, NDF and ADF increased with increasing enzyme level and pre-treatment times. Degradation of nutrients was affected by enzymes levels or pre-treatment times. Therefore, it is probable that the improved degradation may be due to the supplemented exogenous hydrolytic enzymes under a certain condition. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 389-393)***

Key Words : Enzyme, Digestibility, Rumen, Fermentation, *In situ* Degradation

INTRODUCTION

Enzymes supplements are intensively used to improve the nutritive value of feeds and to remove anti-nutritional factors from feeds for non-ruminants, particularly in broiler diets. However, the use of enzymes in ruminant diets is not common and a technology yet to develop. Beauchemin and Rode (1996) reviewed that studies supplementing dietary enzymes for cattle and sheep improved feed digestibility and animal performance in 1960s, but many of these early preparations were poorly defined and results were often inconsistent. Recently the use of feed enzymes for ruminants showed variable animal responses; the use of feed enzymes improved cattle performance (Beauchemin et al., 1995; Feng et al., 1996; Stokes and Zheng, 1995; Treacher et al., 1997), but no improvement was observed (Chen et al., 1995). Therefore, apparently, the inconsistent results from those studies can be attributed to several factors, including diet composition (Yang et al., 2000), type of enzyme preparation (Morgavi et al., 2001), complement of enzyme activities (Wallace et al., 2001), amount of enzyme (Beauchemin et al., 1997), enzyme stability (Hristov et al., 1998), and method of application (Yang et al., 2000). Several researches demonstrated that enzymes are less effective if infused directly into the rumen than applied directly to feed (Lewis et al., 1996; McAllister et al., 1999; Wang et al., 2001). Beauchemin et al. (2001) recently reviewed that increasing the level of enzyme supplementation is usually not beneficial. Applying enzymes to feed prior to feeding enhances ruminal fibre digestion by altering the structure of the feed thereby making it more susceptible to degradation. In addition, cell wall degrading enzymes are added to the silage to provide more fermentable sugar for silage fermentation and to improve silage DM digestibility (Kung, Jr. et al., 1991; Ridla and Uchida, 1999). In commercial feeding, processed forage and grains are stored prior to feeding, providing an ideal opportunity for the use of enzyme products (Beauchemin et al., 1995).

This study aims to investigate the effects of enzyme supplementation on ruminal fermentation and nutrients digestibilities in ruminants by different enzyme application methods, levels and pre-treatment times.

MATERIALS AND METHODS

Rumen fermentation and nutrient digestibility studies

Animal, experimental design and treatments : Six Korean native goats (live weight 25 kg±3.5 kg) were fitted with a rumen cannula were housed in individual pens and had free access to fresh water. The experiment was conducted in a 3×3 Latin square design. Each experimental period consisted of 10 d adaptation to diets and 5 d experimental measurements of feed intake (5 d), feces collection (3 d) and ruminal fermentation characteristics (2 d). Each period, animals received one of three diets, such that each animal received all diets by completion of the experiment. The amount of feed per day was 2% of body weight of experimental animals. The three diets consisted of 70% concentrate, 30% forage as tall fescue (DM base). The

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contents of crude protein, ether extract and NDF in the concentrate were 12.7%, 3.1% and 48.4% (DM basis), respectively. In addition, forage contained crude protein, ether extract and NDF were 5.6%, 0.9% and 69.7% (DM basis), respectively. The enzyme mixture consisting of mainly cellulase and xylanse was applied in different methods of application : no enzyme, 1% enzyme in rumen- The enzyme was infused into rumen via cannula in a liquid form twice on feeding time; and 1% enzyme treated diet- Enzyme was sprayed onto forage. The enzyme was dissolved in distilled water, made concentration 1% (w/w), and the actual supplemented enzyme level was 0.01% of diet. The guaranteed enzyme activity was 1,400,000 BXU (=Enzyme development corporation in house activity unit). The available xylanase activity in a feed sample is determined by the ratio method. The delta absorbances are calculated and a ratio was set up against the known activity of the control sample. Commercial information observed that 1 unit of activity is defined as the amount of enzyme that is required to release one micromole of xylose reducing sugar equivalents from wheat arabinoxylan (1% w/v) in one minute at 400°C and pH 4.6. It has been determined experimentally that 1 unit=100 BXU.

Experiment procedures and analytical methods : Ruminal fluid was collected for two days (d 4 and d 5) from multiple sites in the rumen. Samples were immediately measured for pH using a pH meter and then squeezed through four layers of cheesecloth with a mesh size of 250 μm. Nine ml of filtrate was preserved with 1 ml of 1% sulfuric acid to determine VFA and NH_3. The samples were stored frozen at -20°C until analyses. Estimation of VFA contents was performed by Hewlett Packard® 6890 GC System (Erwin et al., 1961). NH_3 concentration was analyzed by using Milton Roy® Spectronic 21D spectrophotometer at 630 nm (Chancy and Marbach, 1962). The enzyme activity assay was based on the method of Groleau and Forsberg (1981). An aliquot of 0.5 ml supernatant of cultures were added to the tubes containing 0.5 ml of 1% carboxymethylcellulose (CMC), which was suspended with 0.5 M citrate buffer (pH 5.5), and 0.5 ml of 1% xylan solution, and then, fixed for 30 min at 40°C. After adding 0.6 ml of dinitrosalicyclic acid (DNS), samples were boiled for 5 min. CMCase and xylanse activity were calculated by converting the quantity of separated glucose, which were determined by Milton Ray Spectronic 21D at 550 nm with μmol/min unit. Apparent total tract digestibilities of nutrients were determined using collected fecal samples. Fecal samples were collected for three days (d 1, d 2 and d 3). The fecal samples were dried in a forced-air oven at 60°C till constant weight and ground in a Willey mill (1mm screen). Chemical composition of diet and feces were determined according to AOAC (1990). Additionally, the amounts of NDF and ADF in diet and feces were determined (Van Soest et al., 1991) and the expressions were not including residual ash.

Degradation study

To determine the *in situ* degradation characteristics of nutrients, 2 g of dry sample milled through a 2.5 mm screen were placed in nylon bags (140×75 mm, pore size 40 to 60 μm). The bags were incubated in the rumen of three ruminally-cannulated Holstein steers. The animals were offered a diet consisted of forage and concentrate with 60:40 ratio. Animals had free excess to water and mineral/vitamin licks. Nylon bags were incubated at 0, 6, 12, 24, 48 and 72 h. The measurement of 0 h bags was obtained by soaking the two bags of each sample in warm water (37°C) for 1 h. The 0 h measurement and incubated bags were then washed with running cold water until the rinse out was clear, and dried till constant weight. The DM, NDF and ADF degradation data were fitted to the exponential equation $y=a+b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979) to determine the degradation constants (a, b and c). Effective degradability of DM (ED) was determined using equation $ED=a+[bc/(c+k)]$. The constant k value was assumed to be 0.05/h in equation for ED value.

Statistical analysis

Data obtained from an *in vivo* experiment were analyzed using the General Linear Model (GLM) procedure of SAS package (1990). Individual *in situ* nutrient degradation from incubation was analyzed using marquardt method of proc NLIN (nonlinear regression) to determine a constants (a, b and c) by Ørskov and McDonald (1979)'s exponential model $\{y=a+b\ (1-e^{-ct})\}$. *In situ* data were analyzed by the same statistical procedure as those of *in vivo* experiment.

RESULTS AND DISCUSSION

Nutrient digestibility, rumen fermenttion and enzyme activity

Ruminal parameters such as pH, NH_3 and volatile fatty acids (VFA) contents were not significantly different among three diets (Table 1). No effect of dietary enzyme supplementation on ruminal fermentation parameters was also noted in other studies (Beauchemin et al., 1999; Krause et al., 1998). In fact, it was hypothesized that method of application has an impact on ruminal fermentation. In addition, some studies have shown that enzymes are less effective if infused directly into the rumen than when they are applied to feed (Lewis et al., 1996; McAllister et al., 1999; Wang et al., 2001). However, the present results are not accordance with those results. This is probably because ruminal pH in present study was not in the range between 4.5 and 5.5 (Gashe, 1992). Under these conditions, possibly, exogenous enzymes could make a contribution to rumianl

Table 1. Ruminal fermentation characteristics in Korean Native goats supplemented with exogenous enzyme

Item	Diets			SEM[3]	Significance[4]
	No-enzyme	1% Enzyme in rumen[1]	1% Enzyme in diet[2]		
	Ruminal fermentation				
pH	6.80	6.74	6.65	0.210	NS
NH_3	9.67	3.94	7.75	3.947	NS
Total VFA	55.56	56.69	56.26	12.41	NS
Acetate (A)	34.48	34.94	34.60	7.195	NS
Propionate (P)	14.39	14.74	14.58	4.411	NS
Butyrate	3.91	4.19	4.19	1.155	NS
A:P	2.46	2.50	2.41	0.284	NS
	Enzyme activity				
CMCase (μmol of glucose $min^{-1}ml^{-1}$)	0.61	0.49	0.61	0.099	NS
Xylanase (μmol of xylose $min^{-1}ml^{-1}$)	1.00	1.14	0.99	0.127	NS

[1] Enzyme added via rumen cannula.
[2] Enzyme added onto diets.
[3] Standard error of treatment mean.
[4] NS=Not significant ($p>0.05$).

fibre digestion. Ruminal pH in the present study was in the range of 6.65-6.80. It is also possible that animal types affect enzyme efficacy in ruminants. Yang et al. (2000) reported that supplementing the diet with an enzyme product improved digestion in dairy cows, but not in sheep.

The activities of CMCase and xylanase were not affected by exogenous hydrolytic enzymes. This may be due to sub-optimal ruminal pH for enzyme activity as discussed previously. Unchanged enzyme activity in the rumen by enzyme supplementation may also be explained by the fact that exogenous enzyme was not destroyed by ruminal proteases or enzyme activities in the rumen was high enough, and therefore exogenous enzyme did not add any additional activity.

The digestibilities of nutrients in the total tract of goats were not affected by methods of application of enzyme (Table 2). This result is in accordance with other results

Table 2. Effect of enzyme supplementation on DM, crude protein, NDF and ADF digestibility.

Item	Diets			SEM[3]	Significance[4]
	No-enzyme	1% Enzyme in rumen[1]	1% Enzyme in diet[2]		
	Percentage				
DM	70.3	72.5	71.9	0.45	NS
Crude protein	69.6	70.9	70.1	0.37	NS
NDF	67.3	67.8	68.2	0.57	NS
ADF	43.3	44.9	43.8	0.18	NS

[1] Enzyme added via rumen cannula.
[2] Enzyme added onto diets.
[3] Standard error of treatment mean.
[4] NS=Not significant ($p>0.05$).

(Beauchemin et al., 1995; Chen et al., 1995), but in some studies (Beauchemin et al., 1999; Feng et al., 1996; Rust et al., 1965; Rode et al., 1999; Yang et al., 1999, 2000) nutrient digestibilities were improved by enzyme supplementation. The improved digestibilities of nutrients in total tract indicates that the effects of enzyme supplementation were mostly intestinal. This hypothesis was raised by Hristov et al. (1996) who demonstrated that a significant proportion of exogenous enzymes escaped ruminal digestion and remained active in the small intestine. However, the present results did not support it. This is because adaptation period might be the cause for non significant effect on digestibilities. Trend of NDF digestibility shows that it could have increased if enzyme treatment given for a longer duration.

The present *in vivo* experiment showed that ruminal fermentation, enzyme activities and total tract apparent digestibility of nutrients in the total tract were not affected by supplemented enzymes. Therefore, it seems that exogenous hydrolytic enzymes did not influence ruminal and post-ruminal digestion.

Nutrient degradation

The degradation constants "a", "b", "c" and "a+b" fractions, and effective degradability (ED) of DM and NDF were partially affected by the levels of enzyme was increased over the incubation time (Table 3). While, the "a+b" fraction and ED of ADF were increased as levels of enzyme was increased, the constants "a", "b" and "c" of ADF degradation were not affected by levels of enzyme over the incubation time. The increased degradation could be due to the increased damage of feed surface by exogenous enzyme treatment of feed before feeding (Nsereko et al., 2000).

The EDs of DM, NDF and ADF were increased with increasing pre-treatment time of feed with enzyme ($p<0.001$). Also, the degradation constants "b", "c" and "a+b" fractions of DM were increased as pre-treatment time of enzyme was increased. While, the degradation constants "a", "b", and "a+b" fractions of NDF and ADF were not affected by pre-treatment times of enzyme. Recent research indicates that adding the enzyme mixture just prior to feeding is as effective as treating the forage for 2 wk (Yang et al., 1999) or 1 to 3 d (Lewis et al., 1996; Nussio et al., 1997) before feeding. Enzyme applied to forages immediately before *in vitro* incubation also improved digestion of DM and NDF (Feng et al., 1996), suggesting that fibrolytic enzymes applied at feeding (direct fed) may enhance digestion of forages by cattle. Beauchemin et al. (2001) reported that applying fibrolytic enzymes to feed prior to feeding enhances ruminal fibre digestion by altering the structure of the feed thereby making it more susceptible to degradation. Exogenous enzymes treatment, prior to

Table 3. Effect of enzyme supplementation on degradation kinetics of DM, NDF and ADF in steers

	Control	1% Enzyme			2% Enzyme			SEM	Significance[9]		
	0 h	1 h	12 h	24 h	1h	12 h	24 h		L[6]	P[7]	L*P[8]
					DM						
a[1]	6.12	7.03	8.12	8.24	8.01	8.32	8.21	0.763	***	NS	NS
b[2]	61.01	66.41	67.87	68.10	68.12	68.99	72.95	0.838	***	***	***
c[3]	0.05	0.04	0.05	0.06	0.05	0.05	0.05	0.004	NS	*	**
a+b[4]	67.13	73.44	75.99	73.34	76.13	77.32	81.16	0.960	***	***	**
ED[5]	36.79	38.21	42.65	44.02	43.31	44.20	44.69	0.362	***	***	***
					NDF						
a[1]	4.46	6.37	7.57	7.23	7.29	7.31	7.35	0.837	***	NS	NS
b[2]	63.03	66.08	65.91	66.35	67.17	66.10	72.53	3.246	**	NS	NS
c[3]	0.03	0.04	0.05	0.06	0.05	0.06	0.05	0.004	***	*	**
a+b[4]	67.49	72.44	73.48	73.57	74.45	73.41	79.87	3.480	***	NS	NS
ED[5]	29.55	36.97	40.90	42.54	42.31	42.66	43.82	0.734	***	***	***
					ADF						
a[1]	3.15	4.22	3.46	3.27	3.91	4.57	5.02	1.280	NS	NS	NS
b[2]	31.55	36.60	33.03	33.07	33.61	33.72	38.28	3.305	NS	NS	NS
c[3]	0.05	0.04	0.06	0.06	0.06	0.06	0.04	0.008	NS	NS	**
a+b[4]	34.71	40.82	36.49	36.34	37.52	38.29	43.30	3.215	*	NS	NS
ED[5]	18.74	19.23	21.38	21.76	21.43	22.04	22.50	0.890	***	*	NS

[1] Immediately degradable fraction.
[2] Potentially degradable fraction.
[3] Degradable rate per hour of fraction.
[4] Fermentable fraction.
[5] Effective degradability (ED)=a+[bc/(c+k)] (assumed rumen outflow rate (k) of 0.05 h^{-1}).
[6] Level of enzyme.
[7] Pre-treatment time of enzyme.
[8] Interaction between level and pre-treatment time of enzyme.
[9] NS=Not significant ($p>0.05$), *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

ingestion. increase bacterial colonization and thereby improves DM disappearance of forage (Yang et al., 1999) because exogenous enzymes increased microbial attachment of ruminal microbes to feed and increased activity of enzymes associated feed particles (Wang et al., 2001)

There was no interaction effect between level and pre-treatment time of enzyme on "a" fraction of DM. but "b", "c", "a+b" and ED was increased as level and pre-treatment time of enzyme were increased. The constant "c" of NDF and ADF were increased as level and pre-treatment time of enzyme were increased. Also. ED of NDF was highest for 24 h pre-treatment time and 2% enzyme treatment ($p<0.001$). Degradation of nutrients was affected by enzymes levels or pre-treatment times. Therefore, it is probable that the improved degradation may be due to the supplemented exogenous hydrolytic enzymes under a certain condition.

IMPLICATION

Presumably enzyme activity within the rumen was not easily increased by a simple addition of exogenous enzyme products. *In vivo* study with Korean Native goats showed that methods of enzyme application did not affect ruminal fermentation and enzymes activity. and digestibility of nutrients in the total tract. While. *in situ* study showed that levels and pre-treatment times of enzyme affect DM. NDF and ADF degradation, indicating that this is still possibility for improved nutrient digestibility by exogenous hydrolytic enzymes. More detailed studies on method of application and. dietary and animal factors are warranted. as improved nutrient degradation resulted by exogenous enzyme under certain condition.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th Edition. Association of Official Analytical Chemists. Washington DC.

Beauchemin, K. A., L. M. Rode and V. J. H. Sewalt. 1995. Fibrolytic enzymes increase fibre digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. 75:641-644.

Beauchemin, K. A. and L. M. Rode. 1996. Use of feed enzymes in ruminant nutrition. In: Animal Science Research and Development-Meeting Future Challenges (Ed. L. M. Rode). Minister of Supply and Services Canada, Ottawa, ON. pp. 104-159.

Beauchemin, K. A., S. D. M. Jones, L .M. Rode and V. J. H. Sewalt. 1997. Effects of fibrolytic enzyme in corn or barley diets on performance and carcass characteristics of feedlot cattle. Can. J. Anim. Sci. 77:645-653.

Beauchemin, K. A., W. Z. Yang and L. M. Rode. 1999. Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. J. Dairy Sci. 82:378-390.

Beauchemin, K. A., D. P. Morgavi, T. A. McAllister., W. Z. Yang and L . M. Rode. 2001. The use of enzymes in ruminant diets. In: Recent Advances in Animal Nutrition (Ed. P. C. Garnsworthy and J. Wiseman). Nottingham University Press. Nottingham. pp. 298-322.

Chancy, A. L. and E. P. Marbach. 1962. Modification reagents for determination of urea and ammonia. Clin. Chem. 8:130.

Chen, K. H., J. T. Huber., J. Simas, C. B. Theurer, P. Yu, S. C. Chan., F. Santos, Z. Wu, R. S. Swingle and E. J. DePeters. 1995. Effect of enzyme treatment or steam-flaking of sorghum grain on lactation and digestion in dairy cows. J. Dairy Sci. 78:1721-1727.

Erwin, E. S., G. J. Marco and E. M. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1770.

Feng, P., C. W. Hunt, G. T. Pritchard and W. E. Julien. 1996. Effect of enzyme preparations on *in situ* and *in vitro* degradation and *in vivo* digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci.. 74:1349-1357.

Gashe, B. A. 1992. Cellulase production and activity by *Trichoderma* sp. A-001. J. Appl. Bact. 73:79-82.

Groleau, D. and C. W. Forsberg. 1981. Cellulolytic activity of the rumen bacterium *Bacteroides succinogenes*. Can. J. Microbiol. 27:517-530.

Hristov, A. N, L. M. Rode, K. A. Beauchemin and R. L. Wuerfel. 1996. Effect of a commercial enzyme preparation on barley silage *in vitro* and *in sacco* dry matter degradability. Proceedings, Western Section, American Society of Animal Science, Rapid City, South Dakota, Vol. 47:282-284.

Hristov, A. N., T. A. McAllister and K-J. Cheng. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci. 76:3146-3156.

Krause, M., K. A. Beauchemin, L. M. Rode, B. I. Farr and P. Norgaard. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high grain diets fed to growing cattle. J. Anim. Sci. 96:1010-1015.

Kung, L., Jr., R. S. Tung, K. G. Maciorowske, K. Buffem and K. Knutsen. 1991. Effecs of plant cell-wall-degrading enzymes and latic acid bacteria on silage fermentation and composition. J. Dairy Sci. 74:4284-4296.

Lewis, G. E., C. W. Hunt, W. K. Sanchez, R. Treacher, G. T. Pritchard and P. Feng. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J. Anim. Sci. 74:3020-3028.

McAllister, T. A., S. J. Oosting, J. D. Popp, Z. Mir, L. J. Yank, A. N. Hristov, R. J. Treacher and K-J. Cheng. 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. 79:353-360.

Morgavi, D. P., K. A. Beauchemin, V. L. Nsereko, L. M. Rode, T. A. McAllister, A. D. Iwaasa, Y. Wang and W. Z. Yang. 2001. Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms and gastrointestinal proteases. J. Anim. Sci. 79:1621-1630.

Nsereko, V. L., D. P. Morgavi, K. A. Beauchemin and L. M. Rode. 2000. Inhibition of ruminant feed enzyme polysaccharidase activities by extracts from silages. Can. J. Anim. Sci. 80:523-526.

Nussio, L. G., J. T. Huber, C. B. Theurer, C. B. Nussio, J. Santos, M. Tarazon, R. O. Lima-Filho, B. Riggs, M. Lamoreaux and R. J. Treacher. 1997. Influence of a cellulase/xylanase complex(C/X) on lactational performance of dairy cows fed alfalfa hay based diets. J. Dairy Sci. 80(Suppl. 1):220(Abstr.)

Ørskove, E. R. and I. McDonal. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

Ridla, M. and S. Uchida. 1999. Comparative study on the effects of combined treatments of lactic acid bacteria and cellulases on the cell wall compositions and the digestibility of Rhodesgrass (Chloris gayana Kunth.) and Italian Ryegrass (Lolium multiflorum Lam.) silages. Asian-Aust. J. Anim. Sci. 12(4):531-536.

Rode, L. M., W. Z.Yang and K. A. Beauchemin. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82:2121-2126.

Rust, J. W., N. L. Jacobson, A. D. McGillard and D. K. Hotchkiss. 1965. Supplementation of dairy calf diets with enzymes. II. Effect on nutrient utilization and on composition of rumen fluid. J. Anim. Sci. 24:156-160.

SAS. 1990. SAS Users Guide, Version 8, SAS Institute Inc., Cary, NC.

Stokes, M. R. and S. Zheng. 1995. The use of carbohydrase enzymes as feed additives for early lactation cows. Proceedings of the 23rd Conference on Rumen Function, Chicago, Illinois, p. 34(Abstr.)

Treacher, R., T. A. McAllister, J. D. Popp, Z. Mir, P. Mir and K-J. Chen. 1997. Effect of exogenous cellulases and xylanases on feed utilization and growth performance of feedlot steers. Can. J. Anim. Sci. 77:541(Abstr.)

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods of dietary fibre neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wallace, R. J., S. J. A. Wallace, N. McKain, V. L. Nsereko and G. F. Hartnell. 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms *in vitro*. J. Anim. Sci. 79:1905-1916.

Wang, Y., T. A. McAllister, L. M. Rode, K. A. Beauchemin, D. P. Morgavi, V. L. Nsereko, A. D. Iwaasa and W. Yang. 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen Simulation Technique (Rusitec). Br. J. Nutr. 85:325-332.

Yang, W. Z., K. A. Beauchemin and L. M. Rode. 1999. Effects of enzyme feed additives on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci. 82:391-403.

Yang, W. Z., K. A. Beauchemin and L. M. Rode. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J. Dairy Sci. 83:2512-2520.