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Role of exogenous fibrolytic enzymes in ruminant feeding

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ABSTRACT

The complex crop residues and straws are main feed resource available for feeding livestock in developing countries like India. These straws mainly consist of ligno-cellulose complex, which is not very easily and fully digested. Several attempts were tried to address the issue but met with varying degree of success. One of the approaches which holds promise is the use of exogenous fibrolytic enzyme in animals that are mainly fed on crop residue based diets. The major enzymes involved in cellulose hydrolysis are endocellulase (endoglucanase, endo-ß–1, 4-glucanase, carboxymethyl cellulase or ß-1, 4-glucan glucanohydrolase; E.C. 3.2.1.4), exocellulase (exoglucanase, exo-ß-1, 4-glucanase, cellulose ß-1, 4-cellobiosidase; E.C. 3.2.1.91), and ß-glucosidase (cellobiase or glucohydrolase, E.C. 3.2.1.21). Many of these enzymes are being used worldwide in numerous industrial applications such as food industry in addition to animal feed industry. Use of the exogenous enzymes in cattle improved the digestibility of feeds, growth rate and milk production although results obtained were inconsistent. This review gives an insight into various exogenous fibrolytic enzymes involved in cell wall digestion, probable mode of action, and their level of inclusion as productivity enhancer in ruminant feeding.

Key words: Biotechnology, Exogenous enzyme, Nutrition, Ruminants

The bulk of the livestock farmers in the tropical regions, mostly resource poor small-holders, are unable to afford good quality feeds due to the high feed cost, leading to sub-optimal productivity of their animals. The bulk of feeds available for ruminants in these regions are the crop residues. In some regions, there is deficiency of crop residues during certain seasons, in some, a constant shortage may exist, while in other regions crop residues are available in abundance but are largely wasted. Thus, straws worth millions of rupees are burnt in the fields in these places after the grain harvest. Apart from the wastage of a potential feed, the burning of straws causes environmental pollution and degradation of soil fertility. A common problem facing ruminant animal production in our country is the lack of constant supply of good quality forages, and its digestibility. Several methods tried to improve digestibility and utilization of crop residues were met with limited success. One of the alternate methods of improving digestibility of crop residues could be use of exogenous fibrolytic enzymes in the feeding of ruminants.

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In animal feed biotechnology, enzymes are added in monogastric feeds to eliminate anti-nutritional factors, improve the nutritional value of the feeds by degrading cereal components or to supplement the enzymes lacking in the animals digestive system (Galante *et al*.1998). Cellulases, hemicellulases and even pectinases are used in ruminant feed biotechnology to improve feed utilization, production of milk or meat and to improve the digestibility of certain feed components.

Over the years, significant improvements in forage cell wall digestibility have been achieved through forage breeding programmes and agronomic advances. Despite these improvements, forage digestibility continues to limit the intake of available energy by ruminants, and correspondingly, contributes to excessive nutrient excretion by livestock. The use of exogenous fibrolytic enzymes holds promise as a means of increasing forage utilization and improving the productive efficiency of ruminants. This article reviews the research work on the selection of enzymes for use in ruminant diets, the animal responses to feed enzymes and the potential sources that contribute to the variability in response, and the mechanisms by which exogenous fibrolytic enzymes improve nutrient utilization.

Enzymes involved in cell wall digestion

Feeding straws mainly consist of carbohydrate in the form of lignocellulose, and its digestion is accomplished through the enzymatic action of the rumen microbes.

Cellulose and hemicellulose, the major structural polysaccharides in plants (Van Soest 1994) are converted to soluble sugars by enzymes collectively referred to as cellulases and hemicellulases. The major enzymes involved in cellulose hydrolysis are endocellulase (endoglucanase, endo-ß-1, 4-glucanase, carboxymethyl cellulase or ß-1, 4-glucan glucanohydrolase; E.C. 3.2.1.4), exocellulase (exoglucanase, exo-ß-1, 4-glucanase, cellulose ß-1, 4-cellobiosidase; E.C. 3.2.1.91), and ß-glucosidase (cellobiase or glucohydrolase, E.C. 3.2.1.21). In general, endoglucanases hydrolyze cellulose chains at random to produce cellulose oligomers of varying degrees of polymerization; exoglucanase hydrolyze the cellulose chain from the non-reducing end, producing cellobiose, and ß-glucosidases, hydrolyze short-chain cellulose oligomers and cellobiose to glucose (Beauchemin *et al.* 2003). The main enzymes involved in degrading the core polymer of xylan to soluble sugars are xylanases (EC 3.2.1.8) and ß-1, 4 xylosidase (3.2.1.37) (Bhat and Hazlewood 2001). The xylanases include endoxylanases, which yield xylo-oligomers and ß-1, 4-xylosidases, which in turn yields xylose. Other hemicellulase enzymes involved primarily in digestion of side chains include ß-mannosidase (3.2.1.25), α-L-arabinofuranosidase (3.2.1.55), α-D-glucuronidase (3.2.1.139), α-D-galactosidase (3.2.1.22), acetyl xylan esterases (3.1.1.72), and ferulic acid esterase (3.1.1.73) (White *et al.* 1993, Bhat and Hazlewood 2001).

The activity of fibre-degrading enzyme are mostly determined by measuring the rate of release of reducing sugars from pure substrates, with enzyme units expressed as the quantity of reducing sugars released per unit of time per unit of enzyme. Reducing sugars, which include monosaccharides and free sugar ends in oligosaccharides, can be measured colorimetrically using the Nelson/Somogyi copper method (Somogyi 1952) or the dinitrosalicyclic acid method (Miller 1959). The most commonly used substrate for measuring cellulase activity is carboxymethyl cellulose, which measures endo-ß–1, 4-glucanase activity (Wood and Bhat 1988). Exoglucanase activity can be measured using crystalline cellulose preparations, such as Avicel. ß-glucosidase activity is determined by measuring the release of glucose from cellobiose or the release of p-nitrophenol from p-nitrophenyl-ß-D-glucoside (Bhat and Hazlewood 2001). Xylanase activity is the most commonly measured by determining the release of reducing sugars from prepared xylan, such as oat spelt or birchwood xylan. Xylanases are specific for the internal ß-1, 4 linkages within the xylan backbone, and are generally considered endoxylanases (Bhat and Hazlewood 2001). Recently, the genes encoding the feruloyl esterase (FAE) and exoglucanase from different sources were cloned and expressed, and the enzyme activities were estimated by using different substrates (Chandrasekharaiah *et al.* 2011, 2012a, 2012b).

Enzyme levels

There are many reports available where exogenous enzymes were used and the results varies, which may be because of the types of feed, types of experimental animals, fodder, feed additives, stress level, etc. Some of the variability connected with the use of exogenous enzyme products in ruminant diets is due to supplementation with insufficient or excessive enzyme activity. *In vivo* responses to enzyme addition are typically nonlinear (Beauchemin *et al.* 1995, Kung *et al.* 2000), and it is possible to over-supplement. These studies demonstrated that high levels of enzyme addition can be less effective than low levels, and the optimal level of enzyme supplementation may depend on the diet. Lack of response to low levels of enzyme addition may indicate an insufficient supply of enzyme activity, however, the rationale for reduced efficacy of added enzymes at high levels of incorporation is not clear. Nsereko *et al.* (2002) reported a quadratic response in total bacterial numbers in ruminal fluid of dairy cow with increasing levels of an enzyme product from *Trichoderma longibrachiatum*. When excess enzyme was applied, the beneficial disruption of the feed surface structure may have diminished because the excess exogenous enzyme attached to feed may have restricted microbial attachment and limited digestion of feed. Kung *et al.* (2000) offered forage (60% corn silage and 40% lucerne hay; on DM basis) treated with increasing levels (0, 1, 2.5 mUkg of TMR) of an enzyme product (3,500 carboxymethyl cellulase (CMCase) and 16,000 xylanase units / kg of forage dry matter) to cows. Cows fed the low level of enzyme tended to produce more milk (39.5 kg/d) than those fed the control diet (37.0 kg/d) or those fed the high level of enzyme (36.2 kg/d). Applying fibrolytic exogenous enzymes in a liquid form on to feeds prior to consumption can have a positive effect on animal performance (Rode *et al.* 1999, Kung *et al.* 2000). Lewis *et al.* (1996) observed an increase in total tract NDF digestibility when an enzyme solution was applied to dry hay prior to feeding, but there was no difference between applying the enzyme immediately before feeding and a 24 h incubation period. Similar results were reported by Colombatto *et al.* (2000).

Feng *et al.* (1996) showed the effect of exogenous fibrolytic enzymes on dry matter (DM) and neutral detergent fibre (NDF) through *in vitro* technique and reported improved DM and NDF digestibility. Beauchemin *et al.* (2000) reported increased DM and NDF digestibility. In a further study, *in vivo* data indicated enhanced intake, digestibility, particulate passage and ruminal degradability when fibrolytic enzymes were added to dry grass instantaneously before feeding (Feng *et al.* 1996). Several studies showed that the addition of such enzymes to grass or alfalfa before ensiling reduced the concentration of plant structural carbohydrates compared with untreated silages (Jacobs and McAllan 1991, Stokes 1992).

Krause *et al.* (1998) used concentrated enzyme mixture containing mainly cellulase and xylanase, dissolved in water (100 g/L) and then added to barley during tempering. The barley was tempered by adding 15% water and leaving it overnight. The grain was then rolled and dried. This process improved ADF digestibility by 14% in barley straw and by 55% barley and silage based diets. Feng *et al.* (1996) applied an enzyme solution containing cellulase and xylanase directly to grass and observed no effect when added to fresh or wilted forage; however, when it was applied to dried grass, enzymes increased OM and fibre digestibility. Muwalla *et al.* (2007) used a fungal cellulase derived from *Trichoderma* spp. The enzyme contained 200 unit/mg cellulase activity at pH 7.0 and applied @ 150 g/ton of forage consumed. Enzyme requirements for 1 ton of the rations were mixed in 500 g of ground corn, mixed with the micronutrient part of the ration and added to the concentrate part at mixing, but no significant effect was observed. Jafari *et al.* (2005) used enzyme (exoglucanase, endoglucanase and xylanase) in liquid form with activity 1,437, 788, and 7,479 µmol/ min/ ml respectively. Concentrate enzymes were diluted and sprayed on the wheat straw 1h before feeding. Wheat straw was mixed in the alfalfa hay with different levels i.e., 0, 10, 20 and 30%. Replacing 20, and 30% treated wheat straw with exogenous fibre digesting enzyme (EFDE) for alfalfa hay caused numerical increase ($P<0.20$) of wool diameter to 34.40 and 34.89 µm, respectively, when compared with control (30.49 µm). The wool growth depends on the energy and protein supply. These enzymes facilitated the degradation of the cell wall bound proteins increasing the absorption of amino acid. They also found that ionophores, direct fed microbial and exogenous degrading enzymes improved digestibility of poor quality (fibrous) forages in ruminant livestock.

Probably, improvement of wool characteristics with treating wheat straw by EFDE resulted in an increase in ADF, NDF and CP digestibility and feed intake (Jafari *et al.* 2004a). Changes in wool growth and characteristics are associated with quality and relative mixture of absorbed amino acids (Ferguson *et al.* 1967, Reis and Colebrook 1972), even at the same dry matter intake (DMI) level. According to McAllister *et al.* (2001), EDFE seems to have some proteolytic activities as they facilitate degradation of cell wall bound proteins. Also cellulase increased protein degradation of forages *in vitro* by making proteins more available to proteolytic enzymes (Kohn and Allen 1992). There is some evidence that adding feed enzymes to the diets directly, increased the numbers of non-fibrolytic, as well as fibrolytic bacteria in the rumen (Nsereko *et al.* 2002). The stimulation on wool growth rate resulting from treating wheat straw with EFDE can be explained by an increased growth of reticuloruminal micro-organisms and microbial flow to the small intestine, hence a greater absorption of amino acids and supply of metabolizable protein to the animal.

Eun *et al.* (2007) reported improvements in NDF and ADF degradation by 9.6% and 25.6%, respectively, when lucerne hay was treated with exogenous enzymes containing endoglucanase and xylanase. Rodriguez *et al.* (2007) reported that in lambs, exogenous fibrolytic enzymes increased the soluble fraction of DM and ruminal *in situ* disappearance rate of DM and neutral detergent fibre (NDF).

Effect of different exogenous enzymes and their levels on growth and milk production

Ruminant responses to exogenous enzymes are likely to be greatest in conditions where energy is the first limiting nutrient in the diet. The growing cattle and milch animals require high levels of available energy to meet the demands of milk or meat production. The present enzyme technology is not very much helpful for ruminants fed on maintenance ration, but the greatest responses will be observed when ruminants were fed for maximal productivity. Similarly, the response to exogenous enzymes is greater for dairy cows in early lactation than for those in later lactation (Nussio *et al.* 1997, Schingoethe *et al.* 1999). Recent studies showed that addition of exogenous fibrolytic enzymes to ruminant diets increased milk production (Nussio *et al.* 1997, Lewis *et al.* 1999, Rode *et al.* 1999, Schingoethe *et al.* 1999, Kung *et al.* 2000, Yang *et al.* 2000) and average daily gain (ADG) (Beauchemin *et al.* 1995, 1997, 1999b, Iwaasa *et al.* 1997, McAllister *et al.* 1999) in some cases. These improvements in animal performance are due to increase in feed digestion. Tricarico and Dawson (1999) found that the addition of xylanase and cellulase enzyme preparations directly into feed improved the *in vitro* rumen digestion. The nitrogen and NDF digestibility was increased by 23 and 5% with the supplementation of a rumen stable fibrolytic enzyme (Zinn and Salinas 1999). Numerous studies reported increased digestion of DM and fiber measured *in situ*, *in vitro* (Nakashima *et al*. 1988, Feng *et al*. 1996, Hristov *et al.* 1996, Yang *et al.* 1999, Colombatto 2000, Colombatto *et al.* 2002c), or *in vivo* (Feng *et al.* 1996, Krause *et al.* 1998, Rode *et al.* 1999, Yang *et al.* 1999, Beauchemin *et al.* 2000, Kung *et al.* 2000).

The significant increase in weight (6.8 to 24%) was observed when the cattle was fed (ground maize, oat silage, maize silage or lucern hay) with an enzyme cocktail containing amylolytic, proteolytic and cellulolytic enzymes (Krause 2003).

Several studies in dairy cattle discussed, the responses to exogenous fibrolytic enzymes. Kung *et al*. (2000) treated the forage portion of the total mixed ration with cellulose and xylanase and found that pH was an important factor for the enzyme activity and suggested that these enzymes could be used to improve the milk production. Lewis *et al*. (1999) treated forage with a commercially available cellulase/xylanase mixture supplying the enzyme at 1.65 mL/kg (cellulase at 23,300 units/mL, xylanase at 5,800 IU/mL) of total mixed ration (TMR), on DM basis and observed that cows in early lactation produced 6.3 kg/d (16%) more milk. However, higher and lower levels of the same enzyme product were less effective. Rode *et al*. (1999) applied an enzyme product, which mainly consisted of xylanase and cellulase to the concentrate portion of a diet (supplying 1.3 g/kg of TMR on a DM basis) and observed a 3.6 kg/d or 10% increase in milk production for cows in early lactation. Yang *et al*. (2000) added a commercially available enzyme mixture, which contained relatively high xylanase and low cellulase to the concentrate, and cows in early lactation produced 2 kg/d or 5.9% more milk. However, there was no response when the same enzyme was added to the TMR. Thus from these studies, it is clear that exogenous enzymes can be effective for ruminants, but it is important to determine the conditions that are most likely to give positive responses like the dose, type of diet and physiological conditions. Beauchemin *et al*. (2000) also reported the improvement in the production of milk yield of dairy cows using commercially available fibrolytic enzymes (beta-glucanase, xylanase, and endocellulase) in ruminant ration.

Inconsistent results were also observed with regards to the effect of enzyme supplementation on milk yield. Chen and Stokes (1992) treated alfalfa hay and observed a significant increase in milk yield in early lactation cows whereas, Fredeen and McQueen (1993) observed a decrease in milk yield from grass hay treated with enzyme (5.56 kg of a cellulase and glucose oxidase mixture, 2.19 kg of an amylase mixture and lactobacillus inoculant in 60 L water). However, Lewis *et al*. (1995) reported that cows consuming hay treated with the enzyme mixture (2:1 ratio of cellulase and xylanase diluted in water and sprayed on a combination of alfalfa hay and silage and whole cottonseed immediately before mixing with a concentrate based on barley) produced more milk even though they lost more body weight. The effects of enzyme treatments such as alfazyme and cornzyme on the nutritive value of hay crop silage and corn silage were evaluated with Jersey and Holstein cows (Chen and Stokes 1992) and results indicated that cornzyme did not affect DM intake in these cows but significantly reduced milk yield by 5.1% whereas alfazyme improved both DM intake (3.2%) and milk yield by 6.2%.

Mode of action of exogenous fibrolytic enzyme in ruminants

Rumen is having very high fibre digesting capability but still it is not very easy to explain why enzyme treatment of grain or forage before feeding improves feed utilization (Beauchemin *et al*. 2003). The mode of action of exogenous enzymes on improving digestion of plant cell wall is complex. Exogenous enzymes in the rumen are generally more stable than previously thought (Hristov *et al*. 1998, Morgavi *et al*. 2000b, 2001), particularly when applied to feed prior to ingestion.

The possible modes of action of exogenous enzyme could be as follows:

- Application of enzymes to feed enhances the binding of the enzyme with the substrate, which increases the resistance of the enzymes to proteolysis and prolongs their residence time within the rumen. In the rumen, the close association between digestive bacteria and feed particle concentrates digestive enzymes to their specific substrates.
- Applying enzymes to feed also provides a slow release mechanism for enzymes in the rumen (Beauchemin *et al*. 1999a). Hence, the greater the amount of the diet treated with enzymes, the greater the chances that enzymes endure in the rumen. Without this stable feed enzyme complex, the enzymes are solubilized in ruminal fluid and flow rapidly from the rumen. There is evidence for preconsumptive effects of exogenous enzymes causing the release of soluble carbohydrates (Hristov *et al*. 1996), and in some cases, partial solubilization of NDF and ADF (Gwayumba and Christensen 1997, Krause *et al*. 1998).
- It is evident that applying enzymes to feed causes structural changes to occur, thereby making feed more amenable to degradation (Nsereko *et al*. 2000). Cell wall hydrolysis in the rumen proceeds in an erosive manner (White *et al*. 1993), and it is well recognized that a major constraint to digestion is the limited colonization and penetration of cellulolytic microbes and their hydrolytic enzymes onto the exposed surfaces of feed particles.
- It is most likely that the major portion of the positive production responses resulting from the use of enzyme additives is due to ruminal effects. Adding exogenous enzymes to the diet increases the hydrolytic capacity of the rumen mainly due to increased bacterial attachment (Yang *et al*. 1999, Morgavi *et al*. 2000c, Wang *et al*. 2001), stimulation of rumen microbial populations (Wang *et al*. 2001, Nsereko *et al*. 2002), and synergistic effects with hydrolases of ruminal microorganisms (Morgavi *et al*. 2000a). Increased hydrolytic capacity of the rumen can also lead to an increase in digestibility of the nonfibre carbohydrate fraction, in addition to increasing digestibility of the fibre components of a diet, which explains why fibrolytic enzymes can be effective in high concentrate diets.
- Exogenous enzymes seem to continue to exist in the small intestine for a time sufficient to have an effect on target substrates (Morgavi *et al*. 2001).
- Hydrolytic removal of arabinose units from the xylan polymer may increase rate of cellulose degradation (Chamberlain and Robertson 1992). The response to xylanolytic based enzyme preparation supports the suggestion that xylose: arabinose ratio may be an important factor affecting cell wall degradability.

SUMMARY

The use of exogenous fibrolytic enzymes as a promising technology has prospective for improving forage utilization, fibre digestion and production in ruminants. Though inconsistent results were reported from different studies, it is palpable from many studies that exogenous enzyme applications are valuable in enhancing fibre digestion and animal production provided that suitable environment, temperature, ruminal pH and target substrate are maintained, and that activities of enzymes are well defined. Effects of exogenous enzyme application on ruminant feeds appear to vary with the physical and chemical composition of the targeted substrate, but information on exogenous enzyme requirements in the rumen is partial. Information

regarding the production and preparations of exogenous enzymes in ruminant feeds is also limited. Information regarding the choice of exogenous enzymes, enzyme composition, applica-tion method and level, stability, target substrate and storage is needed for better defined effects of exogenous enzymes and to be considered for use in conditions at farm level.

The improvement in cell wall digestion and the efficiency of feed utilization by ruminants can be achieved by adding exogenous fibrolytic enzymes to ruminant diets. Optimistic responses in milk production and growth rate were observed in cattle fed on exogenous enzyme products, although results were inconsistent. More studies need to be conducted to understand the mode of action of these products so that on-farm efficacy of ruminant enzyme technology can be assured. With increasing consumer concern about the use of growth promoters and antibiotics in ruminant production, and the enormity of increased animal performance obtainable using feed enzymes, these products could play a significant and promising role in future ruminant production systems, particularly in the tropics where agricultural by products and crop residues form the major feed resources for livestock.

REFERENCE

Beauchemin K A, Colombatto D, Morgavi D P and Yang W Z. 2003. Use of exogenous fibrolytic enzymes to improve feed Utilization by ruminants. *Journal of Animal Science* **81**: E37–E47.

Beauchemin K A, Jones S D M, Rode L M and Sewalt V J H. 1997. Effects of fibrolytic enzyme in corn or barley diets on performance and carcass characteristics of feedlot cattle. *Canadian Journal of Animal Science* **77**: 645–53.

Beauchemin K A, Yang W Z and Rode L M. 1999a. Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. *Journal of Dairy Science* **82**: 378–90.

Beauchemin K A, Rode L M and Karren D. 1999b. Use of feed enzymes in feedlot finishing diets. *Canadian Journal of Animal Science* **79**: 243–46.

Beauchemin K A, Rode L M and Sewalt V J H. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. *Canadian Journal of Animal Science* **75**: 641–44.

Beauchemin K A , Rode L M , Maekawa M , Morgavi D P and Kanpen R. 2000. Evaluation of non-starch polysaccharidase feed enzyme in dairy cows diets. *Journal of Dairy Science* **83**: 543–53.

Beg Q K , Kapoor M, Mahajan L and Hoondal G S. 2001. Microbial xylanases and their industrial applications: a review. *Applied Microbiology and Biotechnology* **56**: 326–38.

Bhat M K and Hazlewood G P. 2001. Enzymology and other characteristics of cellulases and xylanases. *Enzymes in Farm Animal Nutrition*. Page 11. (Eds) Bedford M and Partridge G. CABI Publishing, Oxon, UK.

Bhat M K. 2000. Cellulases and related enzymes in biotechnology. *Biotechnology Advances* **18**: 355–83.

Chamberlain D G and Roberston S. 1992. The effects of addition of various enzyme mixtures on the fermentation of perennial ryegrass silage and on its nutritional value for milk production in dairy cows. *Animal Feed Science and Technology* **37**: 257–64.

Chandrasekharaiah M, Thulasi A, Vijayarani K, Prasanna K D, Santosh S S, Palanivel C, Lyju J V and Sampath K T 2012a. Expression and biochemical characterization of two novel feruloyl esterases derived from fecal samples of *Rusa unicolor* and *Equus burchelli*. *Gene* **500** (1) 134–39.

Chandrasekharaiah M, Thulasi A, Bagath M, Prasanna K D, Santosh S S, Palanivel C, Lyju J V and Sampath K T. 2012b. Identification of cellulase genes from the metagenomes of *Equus burchelli* fecal samples and functional characterization of a novel bifunctional cellulolytic enzyme. *Applied Biochemistry and Biotechnology* **167**(1): 132–41.

Chandrasekharaiah M, Thulasi A, Bagath M, Prasanna K D, Santosh S S, Palanivel C, Lyju J V and Sampath K T. 2011. Molecular cloning, expression and characterization of a novel feruloyl esterase enzyme from the symbionts of termite (*Coptotermes formosanus*) gut. *Biochemistry and Molecular Biology Reports* **44** (1): 52–57.

Chen J and Stokes M R. 1992. Effects of enzyme treatments on the preservation and nutritive value of hay crop silage and corn silage. *Journal of Dairy Science* **75**: 272.

Colombatto D. 2000. 'Use of enzymes to improve fibre utilization in ruminants. A biochemical and *in vitro* rumen degradation assessment.' Ph.D. Diss., University of Reading, UK.

Colombatto D, Mould F L, Bhat M K and Owen E. 2002c. The effect of fibrolytic enzyme application on rate and extent of alfalfa stem fermentation, assessed *in vitro*. *Annual Proceedings of British Society of Animal Science*. Pp 209.Penicuik, U.K.

Eun J S and Beauchemin K A. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using *in vitro* fermentation characteristics. *Animal Feed Science and Technology* **132**: 298–15.

Feng P, Hunt C W, Pritchard G T and Julien W E. 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. *Journal of Animal Science* **74**: 1349–57.

Ferguson K A, Hemsley J A and Reis P J. 1967. Nutrition and wool growth. The effect of protecting dietary protein from microbial degradation in the rumen. *Australian Journal of Science* **30**: 215–17.

Fredeen A H and McQueen R E. 1993. Effect of enzyme additives on quality of alfalfa/grass silage and dairy cow performance. *Canadian Journal of Animal Science* **73**: 581–91.

Galante Y M, De Conti A and Monteverdi R. 1998. Application of *Trichoderma* enzymes in food and feed industries. *Trichoderma and Gliocladium* – Enzymes, biological control and commercial applications. Vol. 2, pp. 327–42. (Eds) Harman G F and Kubicek C P. Taylor and Francis, London.

Grassin C and Fauquembergue P. 1996a. Fruit juices. *Industrial Enzymology.* 2nd edn, pp. 226. (Eds) Godfrey T and West S. Macmillan, UK.

Grassin C and Fauquembergue P. 1996b. Wine. Industrial enzymology. 2nd edn, pp 374–83. (Eds) Godfrey T and West S. Macmillan, UK

Gwayumba W and Christensen D A. 1997. The effect of fibrolytic enzymes on protein and carbohydrate degradation fractions in forages. *Canadian Journal of Animal Science* **77**: 541–42.

Hristov A N, Rode L M, Beauchemin K A and Wuerfel R L. 1996. Effect of a commercial enzyme preparation on barley silage *in vitro* and in sacco dry matter degradability. *Proceedings of Western Section, American Society of Animal Science.* Pages 282–84.

Hristov A N, McAllister T A, and Cheng K J. 1998. Stability of exogenous polysaccharide-degrading enzyme in the rumen. *Animal Feed Science and Technology* **76**: 161–68.

Iwaasa A D, Rode L M, Beauchemin K A and Eivemark S. 1997. Effect of fibrolytic enzymes in barley-based diets on performance of feedlot cattle and *in vitro* gas production. *Joint Rowett Institutnational De* La *Recherche Agronomique* Rumen Microbiology. Symposium. Aberdeen, Scotland, Poster 39.

Jacobs J L and McAlian A B. 1991. Enzymes as silage *additives*. Silage quality, digestion, digestibility and performance in growing cattle. *Grass Forage Science* **46**: 63–73.

Jafari A, Edriss M A, Alikhani M and Emtiazi G. 2005. Effects of treated wheat straw with exogenous fibre-degrading enzymes on wool characteristics of ewe lambs. *Pakistan Journal of Nutrition* **4**: 321–26.

Jafari A, Alikhani M and Edriss M A. 2004. The effects of substituting treated wheat straw with fibrolytic enzymes for alfalfa hay on Naieni replacement ewe lambs performances. *Proceedings of the Science of Changing Climates. University of Alberta, Edmonton, Canada*.

Kohn R A and Allen M S. 1992. Storage of fresh and ensiled forages by freezing affects fibre and crude protein fraction. *Journal* of the *Science* of *Food* and *Agriculture* **58**: 215–20.

Krause D O, Denman S E, Mackie RI, Morrison M, Rae A L, Attwood G T and McSweeney C S. 2003. Opportunities to improve fibre degradation in the rumen: microbiology, ecology, and genomics. *FEMS Microbiology Review* **27**: 663–93.

Krause M, Beauchemin K A, Rode L M, Farr B I and Nørgaard P. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. *Journal of Animal Science* **96**:1010–15.

Kung L Jr, Treacher R J, Nauman G A, Smagala A M, Endres K M and Cohen M A. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. *Journal of Dairy Science* **83**: 115–122.

Lewis G E, Hunt C W, Sanchez W K, Treacher R, Pritchard G T and Feng P. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage based diet fed to beef steers. *Journal of Animal Science* **74**: 3020–28.

Lewis G E, Sanchez W K, Hunt C W, Guy M A, Pritchard G T, Swanson B I and Treacher R J. 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. *Journal of Dairy Science* **82**: 611–17.

Lewis G E, Sanchez W K, Treacher R, Hunt CW and Pritchard GT. 1995. Effect of direct-fed fibrolytic enzymes on lactational performance of mid-lactation Holstein cows. *Proceedings, Western Section, American Society of Animal Science and Western Branch Canadian Society of Animal Science*. July 6th–8h, Lethbridge, AB. **46**: 310–13.

McAllister T A, Oosting S J, Popp J D, Mir Z, Yanke L J, Hristov A N, Treacher R J and Cheng K J. 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. *Canadian Journal of Animal Science* **79**: 353–60.

McAllister T A, Hristov A N, Beauchemin K A, Rode L M and Cheng K J. 2001. Enzymes in ruminant diets. *Enzymes in Farm Animal Nutrition.* Pp. 273–98. (Eds) Bedford M R and Partridge G G. CABI Publishing, Marlborough, Wiltshire, UK.

Miller G L. 1959. Use of dinitrosalicylic acid reagent for determinationof reducing sugar. *Anals of Chemistry* **31**: 426–28.

Morgavi D P, Beauchemin K A, Nsereko V L, Rode L M, McAllister T A, Iwaasa A D, Wang Y and Yang W Z. 2001. Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms. *Journal of Animal Science* **79**: 1621–30.

Morgavi D P, Beauchemin K A, Nsereko V L, Rode L M, Iwaasa A D, Yang W Z, McAllister T A and Wang Y. 2000a. Synergy between ruminal fibrolytic enzymes and enzymes from *Trichoderma Longibrachiatum. Journal of Dairy Science* **83**: 1310–21.

Morgavi D P, Newbold C J, Beever D E and Wallace R J. 2000b. Stability and stabilization of potential feed additive enzymes in rumen fluid. *Enzyme* and *Microbial Technology* **26**:171–77.

Morgavi D P, Nsereko V L, Rode L M, Beauchemin K A, McAllister T A and Wang Y. 2000c. A *Trichoderma* feed enzyme preparation enhances adhesion of *Fibrobacter succinogenes* to complex substrates but not to pure cellulose. *Proc. XXV Conf. Rumen Function.* Chicago. Page 31.

Muwalla M M, Haddad S G and Hijazeen M A. 2007. Effect of fibrolytic enzyme inclusion in high concentrate fattening diets on nutrient digestibility and growth performance of Awassi lambs. *Livestock Science* **111**: 255–58.

Nakashima Y, Orskov E R, Hotten P M, Ambo K and Takase Y. 1988. Rumen degradation of straw. Effect of polysaccharidase enzymes on degradation characteristics of ensiled rice straw. *Journal of Animal Production* **47**: 421–27.

Nsereko V L, Beauchemin K A, Morgavi D P, Rode L M, Furtado A F, McAllister T A, Iwaasa A D, Yang W Z and Wang Y. 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. *Canadian Journal of Microbiology* **48**: 14–20.

Nsereko V L, Morgavi D P, Rode L M, Beauchemin K A and McAllister T A. 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms *in vitro*. *Animal Feed Science and Technology* **88**:153–70.

Nsereko V l, Beauchemin K A, Morgavi D P, Rode L M, Furtado A F, McAllister T A, Iwaasa A D, Yang W Z and Wang Y. 2002. Effect of fibrolytic enzyme preparation from Trichoderma longibrachiatum on the rumen microbial population of dairy cows. *Canadian Journal of Microbiology* **48**: 14–20.

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

Reis P J and Colebrook WF. 1972. The utilization of abomasal supplements of protein and amino acids by sheep with special reference to wool growth. *Australian Journal of Biological Science* **25**: 1057–71.

Reis P J, Tunks D A and Munro SG. 1992. Effects of abomasal protein and energy supply on wool growth in Merino sheep. *Australian Journal of Agricultural Reseasech* **43**: 1353–56.

Rode L M, Yang W Z and Beauchemin K A. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. *Journal of Dairy Science* **82**: 2121–26.

Rodriguez J M, Mendoza P, Gonzalezc S S, Robinsond P H, Mendozae G and Lvareza G A. 2007. Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. *Animal Feed Science and*

Technology **142**: 210–19.

Schingoethe D J, Stegeman G A and Treacher R J. 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. *Journal of Dairy Science* **82**: 996–1003.

Somogyi M. 1952. Notes on sugar determination. *Journal of Biological Chemistry* **195**: 19–23.

Stokes M R and Zheng S. 1995. The use of carbohydrase enzymes as feed additives for early lactation cows. *Proceedings of 23rd Conference on Rumen Function.* Chicago. Pp 34.

Tricario J M and Dawson K A. 1999. Effects of defined xylanase and cellulase enzyme preparations on digestive processes of rumen microbial cultures. *Journal of Dairy Science* **77**: 252 (Abstr).

Van-Soest P J. 1994. *Nutritional Ecology of the Ruminant.* 2nd edn. Cornell University Press, Ithaca, NY.

Wang Y, McAllister T A, Rode L M, Beauchemin K A, Morgavi, D P, Nsereko V L, Iwaasa A D and Yang W. 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the rumen simulation technique (Rustec) *British Journal of Nutrition* **85**: 325–32.

White B A, Mackie R I and Doerner K C. 1993. Enzymatic hydrolysis of forage cell walls. *Forage Cell Wall Structure and Digestibility.* (Eds) Jung H G, Buxton D R, Hatfield R D and Ralph J. *American Society of Agronomy*, *Crop Science of Society America*, *Soil Science Society* of *America*, Madison, WI. Pp 455–84.

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

Yang W Z, Beauchemin K A and Rode L M. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *Journal of Dairy Science* **83**: 2512–20.

Zinn R A and Salinas J. 1999. Influence of Fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing diet. *Proceedings of Alltech 15th Annual Symposium. Biotechnology in the Feed Industry.* Nottingham University Press, pp. 313–19.