

# Use of Exogenous Fibrolytic Enzymes to Improve Feed Utilization by Ruminants<sup>1,2</sup>

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**ABSTRACT:** Research has demonstrated that supplementing dairy cow and feedlot cattle diets with fiber-degrading enzymes has significant potential to improve feed utilization and animal performance. Ruminant feed enzyme additives, primarily xylanases and cellulases, are concentrated extracts resulting from bacterial or fungal fermentations that have specific enzymatic activities. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fiber digestion resulting in increased digestible energy intake. Animal responses are greatest when fiber digestion is compromised and when energy is the first-limiting nutrient in the diet. When viewed across a variety of enzyme products and experimental conditions, the response to feed enzymes by ruminants has been variable. This variation can be attributed to experimental conditions in which energy is not the limiting nutrient, as well as to the activities and characteristics of the enzymes supplied, under- or over-supplementation of enzyme activity, and inappro-

priate method of providing the enzyme product to the animal. A limited number of ruminant enzyme products are now commercially available, and this list of products is expected to grow. However, random addition of enzymes to diets without consideration for specific situations and substrate targets will only discourage or delay on-farm adoption of enzyme technology. Although much progress has been made in advancing enzyme technology for ruminants, considerable research is still required to reduce the variability of response. With increasing consumer concern about the use of growth promoters and antibiotics in livestock production, and the magnitude of increased animal performance obtainable using feed enzymes, there is no doubt that these products will play an increasingly important role in the future. This paper reviews the research on enzyme selection, the animal responses to feed enzymes, and the mechanisms by which these products improve nutrient utilization.

Key Words: Cellulase, Cellulose Digestion, Digestion, Enzymes, Fiber, Ruminants

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## Introduction

Over the years, significant improvements in forage cell wall digestibility have been achieved through forage breeding programs 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 increas-

ing forage utilization and improving the productive efficiency of ruminants.

Recent studies have shown that adding exogenous fibrolytic enzymes to ruminant diets increases 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 ADG (Beauchemin et al., 1995; 1997; 1999b; Iwaasa et al., 1997; McAllister et al., 1999) in some cases. These increases in animal performance are due to increases in feed digestion. Numerous studies have 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). However, not all studies report improved animal performance due to the use of exogenous enzymes (Higginbotham et al., 1996; Pritchard et al., 1996; ZoBell et al., 2000), and viewed across a

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variety of enzyme products and experimental conditions the response to feed enzymes by ruminants has been variable. This article reviews the research 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.

### *Feed Enzymes for Ruminants*

Commercial enzymes used in the livestock feed industry are products of microbial fermentation. Feed enzymes are produced by a batch fermentation process, beginning with a seed culture and growth media (Cowan, 1994). Once the fermentation is complete, the enzyme protein is separated from the fermentation residues and source organism. Although the source organisms are, in many cases, similar among enzyme products, the types and activity of enzymes produced can vary widely depending on the strain selected and the growth substrate and culture conditions used (Considine and Coughlan, 1989; Gashe, 1992; Lee et al., 1998).

Compared to the fermentation extract, these enzyme products are relatively concentrated and purified, containing specific, controlled enzyme activities. They usually do not contain live cells. Enzyme products for ruminant diets are of fungal (mostly *Trichoderma longibrachiatum*, *Aspergillus niger*, *A. oryzae*) and bacterial (mostly *Bacillus* spp.) origin (Pendleton, 2000). Furthermore, most of the commercially available enzyme products that have been evaluated as ruminant feed additives are produced for nonfeed applications; cellulases and xylanases are used extensively in the food, pulp and paper, textile, fuel, and chemical industries (Bhat and Hazlewood, 2001). Several fibrolytic enzyme products evaluated as feed additives in ruminant diets were originally developed as silage additives (Feng et al., 1996).

In addition to these relatively pure sources of enzymes, crude fermentation products and some nonbacterial direct-fed microbials (DFM) are also marketed, at least partly or implicitly, based on their residual enzymic content (Muirhead, 2001). In this case, the enzymes, as well as the entire medium, are recovered complete with metabolites and fermentation substances. Most nonbacterial DFM consist of *A. oryzae* fermentation extract, *Saccharomyces cerevisiae* cultures, or both (Martin, 2000). In comparison to concentrated feed enzyme products, these crude products contain relatively little (<5%) enzyme activity. There is no minimal level of enzyme activity required for products to be registered as feed enzymes, which adds tremendous confusion in the marketplace. Consequently, it can be difficult to distinguish commercially between "true" enzyme products and products with trace levels of activity. The scope of this paper is limited to concen-

trated fermentation products that have specific, controlled enzyme activities.

### *Enzyme Activities Involved in Cell Wall Digestion*

The focus of most enzyme-related research for ruminants has been on plant cell wall degrading enzymes. 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 types of cellulases and hemicellulases can differ substantially among commercial enzyme products, and differences in the relative proportions and activities of these individual enzymes may have an impact on the efficacy of cell wall degradation by these products. In addition to fiber-degrading enzymes, these products also have secondary enzyme activities, including amylases, proteases, and pectinases.

Cellulose is hydrolyzed through a complex process involving cellulases, and numerous specific enzymes contribute to cellulase activity. The major enzymes involved in cellulose hydrolysis are endoglucanase (endoglucanase, endo- $\beta$ -1,4-glucanase, carboxymethyl cellulase or  $\beta$ -1,4-glucan glucanohydrolase; E.C. 3.2.1.4), exocellulase (exoglucanase, exo- $\beta$ -1,4-glucanase, cellulose  $\beta$ -1,4-cellobiosidase; E.C. 3.2.1.91), and  $\beta$ -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; exoglucanases hydrolyze the cellulose chain from the nonreducing end, producing cellobiose, and  $\beta$ -glucosidases hydrolyze short-chain cellulose oligomers and cellobiose to glucose.

The main enzymes involved in degrading the xylan core polymer to soluble sugars are xylanases (EC 3.2.1.8) and  $\beta$ -1,4 xylosidase (3.2.1.37) (Bhat and Hazlewood, 2001). The xylanases include endoxylanases, which yield xylooligomers and  $\beta$ -1,4-xylosidases, which in turn yield xylose. Other hemicellulase enzymes involved primarily in the digestion of side chains include  $\beta$ -mannosidase (3.2.1.25),  $\alpha$ -L-arabinofuranosidase (3.2.1.55),  $\alpha$ -D-glucuronidase (3.2.1.139),  $\alpha$ -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).

Fiber-degrading enzyme activities are generally 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 dinitrosalicylic acid method (Miller, 1959).

The most commonly used substrate for measuring cellulase activity is carboxymethyl cellulose, which

measures endo- $\beta$ -1,4-glucanase activity (Wood and Bhat, 1988). Exoglucanase activity can be measured using crystalline cellulose preparations, such as Avicel.  $\beta$ -glucosidase activity is determined by measuring the release of glucose from cellobiose, or the release of *p*-nitrophenol from *p*-nitrophenyl- $\beta$ -D-glucoside (Bhat and Hazlewood, 2001).

Xylanase activity is 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  $\beta$ -1,4 linkages within the xylan backbone, and are generally considered endoxylanases (Bhat and Hazlewood, 2001). Endoxylanases can be considered to be debranching or nondebranching based on their ability to release arabinose in addition to hydrolyzing the main chain of xylan.  $\beta$ -xylosidase activity can be determined by using *p*-nitrophenyl derivatives.

Enzyme activity measurements must be conducted under conditions closely defined with respect to temperature, pH, ionic strength, substrate concentration, and substrate type, since all of these factors will affect the activity of an enzyme. Enzyme activities of commercial enzyme products are typically measured at the manufacturers' recommended optima. A temperature of approximately 60°C and a pH between 4 and 5 are the optimal conditions for most commercial enzymes (Coughlan, 1985). However, the optimal temperature and pH for assessing enzyme activity are not representative of the conditions in the rumen, which are closer to a pH of 6.0 to 6.7 and 39°C (Van Soest, 1994). Thus, the activities quoted for commercial enzyme products are considerably higher than for those that would be measured at a pH and temperature similar to that of the rumen. Furthermore, because the conditions of the assays and method of expressing enzyme activity vary among manufacturers, it is difficult to compare enzyme products or predict the efficacy of the product in ruminant diets.

Further details of the enzymology of cellulases and xylanases are provided by Ghose (1987), Wood and Bhat (1988), Bhat and Hazlewood (2001), and McCleary (2001).

### *Animal Responses to Feed Enzymes*

The use of exogenous fiber-degrading enzyme additives for ruminants was first examined in the 1960s, as reviewed by Beauchemin and Rode (1996). Many of these early enzyme products were poorly characterized, animal responses were variable, and little to no effort was made to design these products specifically for ruminants. In the last decade, researchers have reexamined the potential use of exogenous enzymes for ruminants due to higher feed costs, lower costs of enzyme production, and the availability of more active and better defined enzyme preparations. However, descriptions of the enzyme products in most research papers are still generic at best. The formulation of

enzyme products for ruminants has evolved over time, but it is difficult to follow these changes based on the information provided in the published literature. For example, there is no way of knowing whether an enzyme product from a particular enzyme supplier is the same from study to study. Typically, the enzyme products evaluated for ruminants are blends of various cellulases and xylanases that were originally produced and marketed for other uses. Thus, the components of a ruminant enzyme product can change over time due to improvements in production and strain selections of the enzymes driven by other markets. Also, ruminant enzyme products have been reformulated as new information became available.

### *Beef Cattle*

Several recent studies have examined the use of exogenous enzyme products in high-forage diets fed to growing cattle (Beauchemin et al., 1995; Michal et al., 1996; Pritchard et al., 1996; McAllister et al., 1999; Wang et al., 1999; ZoBell et al., 2000). There is evidence that adding fibrolytic enzymes to forage diets can improve fiber digestibility (Beauchemin et al., 1995; Feng et al., 1996), but whether increased digestibility improves performance of cattle may depend on the physiological status of the cattle and the conditions of the experiment.

The results of adding fibrolytic enzymes to high-grain diets have been surprisingly more consistent than those for high-forage diets. Applying an enzyme product (Xylanase B, Biovance Technologies Inc., Omaha, NE) to a 95% barley-based diet improved feed efficiency by 6 to 12%, depending upon the level of enzyme addition (Beauchemin et al., 1997; 1999b; Iwaasa et al., 1997) (Table 1). Increased feed efficiency was due to an increase in diet digestibility (Iwaasa et al., 1997). Similarly, Krause et al. (1998) reported a 28% increase in ADF digestibility using a similar enzyme product added to a high-concentrate diet. Using another enzyme product (Finnfeeds Int. Ltd., Marlborough, U.K.), McAllister et al. (1999) reported that treating both the forage (ryegrass silage; 30% of the diet) and grain (barley, 70% of the diet) portions of the diet with 3.5 L/t of DM increased ADG by 10% (DM basis). However, ZoBell et al. (2000) reported no effect when what appears to be the same enzyme product was added to a high-grain barley-based feedlot finishing diet containing 17% forage (DM basis).

Despite the potential benefits of using exogenous enzymes, the adoption of enzyme technology by the beef industry has been slow due to the relatively high cost of enzyme products compared with ionophores, antibiotics, and implants. Furthermore, there are few enzyme products commercially available to the beef industry, and most of these have not been widely evaluated under a range of dietary conditions.

**Table 1.** Effects of adding a commercial feed enzyme product to high-concentrate feedlot finishing diets consisting of barley grain, supplement, and barley silage

Item	Control	Enzyme level		Change
		1×	2×	
Beauchemin et al. (1997) <sup>a</sup>				
No. of animals	10	9		
Initial weight, kg	407	414	—	—
DMI, kg/d	9.99	9.53	—	-5%
ADG, kg/d	1.43	1.52	—	+6%
Kilograms of feed DM:	7.11 <sup>e</sup>	6.33 <sup>d</sup>	—	-11%
Kilograms of gain				
Iwaasa et al (1997) <sup>b</sup>				
No. of animals	10	10	10	
Initial weight, kg	476	479	481	—
DMI, kg/d	10.6	9.8	9.8	-8%
ADG, kg/d	2.0	2.1	2.2	+1%
Kilograms of feed DM:	5.2 <sup>g</sup>	4.9 <sup>g</sup>	4.6 <sup>f</sup>	-6 to 12%
Kilograms of gain				
DM digestibility, %	65.7 <sup>f</sup>	69.3 <sup>g</sup>	68.9 <sup>g</sup>	+5%
Beauchemin et al. (1999b) <sup>c</sup>				
No. of animals	86	101		
Initial weight, kg	385 <sup>e</sup>	360 <sup>d</sup>	—	-6.5%
DMI, kg/d	10.73	10.62	—	-1%
Weight gain, kg	172 <sup>e</sup>	188 <sup>d</sup>	—	+9%
ADG, kg/d	1.40 <sup>e</sup>	1.53 <sup>d</sup>	—	+9%
Kilograms of feed DM:	7.72	6.95	—	-11%
Kilograms of gain				

<sup>a</sup>No ionophore, no implants used.

<sup>b</sup>Ionophores and implants used.

<sup>c</sup>Cattle were vaccinated, implanted, and melangesterol acetate was provided in the supplement.

<sup>d,e</sup> $P < 0.05$ .

<sup>f,g</sup> $P < 0.10$ .

### Dairy Cattle

A number of studies have examined the effects of fibrolytic exogenous enzymes on digestibility and milk production in dairy cows (Stokes and Zheng, 1995; Higginbotham et al., 1996; Nussio et al., 1997; Zheng and Stokes, 1997; Beauchemin et al., 1999a, 2000; Lewis et al., 1999; Nussio et al., 1997; Rode et al., 1999; Schingoethe et al., 1999; Yang et al., 1999, 2000; Kung et al., 2000; Phipps et al., 2000a,b; Bowman, 2001; Sutton et al., 2001; K. A. Beauchemin, L. M. Rode, B. I. Farr, J. A. Shelford, J. Baah, and G. F. Hartnell, unpublished data). Across 20 studies and 41 treatments, the average increase in DMI was  $1.0 \pm 1.3$  kg/d, and the average increase in milk yield was  $1.1 \pm 1.5$  kg/d ( $3.4\% \pm 4.7$ ). Thus, when viewed across a variety of enzyme products and experimental conditions, the response is positive, but the variability is also high.

In some studies, the response to enzymes has been substantial. For example, Lewis et al. (1999) treated forage with a cellulase/xylanase mixture (FinnFeeds Int.; supplying 1 mL/kg of total mixed ration [TMR], DM basis) and observed that cows in early lactation produced 6.3 kg/d (16%) more milk (Table 2). However, higher and lower levels of the same enzyme product were less effective. Rode et al. (1999) applied an enzyme product (Promote, Biovance Technologies Inc.,

Omaha, NE) 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 (10%;  $P = 0.11$ ) increase in milk production for cows in early lactation (Table 2). Yang et al. (2000) added an enzyme mixture (Biovance Technol, Omaha, NE) to the concentrate, and cows in early lactation produced 2 kg/d (5.9%) more milk (Table 2). However, there was no response when the same enzyme was added to the TMR.

It is clear that exogenous enzymes can be effective for ruminants, but it is important to determine the conditions that are most likely to result in positive responses.

### Understanding the Variability in Animal Response

In general, results with beef cattle and dairy cows indicate a positive response to enzymes, but the results are variable. Although this variability may be viewed as an indication that feed enzyme additives are not a suitable technology for ruminants, we believe that much of the variability can be attributed to factors such as enzyme type, level of supplementation, method of enzyme application, and the energy balance of the test animals.

### Enzyme Activity

There is increasing evidence that plant cell wall-degrading enzymes stimulate fiber digestion in the

**Table 2.** Effects of supplementing diets fed to cows in early lactation with an enzyme mixture<sup>z</sup>

	Lewis et al., 1999				Rode et al., 1999		Yang et al., 2000		
	Control	Low enzyme	Medium enzyme	High enzyme	Control	Enzyme in conc.	Control	Enzyme in conc.	Enzyme in TMR
DMI, kg/d	24.4 <sup>b</sup>	26.2 <sup>a</sup>	26.2 <sup>a</sup>	26.6 <sup>a</sup>	18.7	19.0	19.4	19.8	20.4
Milk production, kg/d	39.6 <sup>b</sup>	40.8 <sup>b</sup>	45.9 <sup>a</sup>	41.2 <sup>b</sup>	35.9 <sup>f</sup>	39.5 <sup>g</sup>	35.3 <sup>b</sup>	37.4 <sup>a</sup>	35.2 <sup>b</sup>
Milk composition, %									
Fat	3.99 <sup>a</sup>	3.83 <sup>ab</sup>	4.00 <sup>a</sup>	3.75 <sup>b</sup>	3.87 <sup>a</sup>	3.37 <sup>b</sup>	3.34	3.19	3.14
Protein	2.95 <sup>a</sup>	2.87 <sup>b</sup>	2.88 <sup>b</sup>	2.85 <sup>b</sup>	3.24	3.03	3.18	3.13	3.13
Lactose	4.89 <sup>ab</sup>	4.91 <sup>ab</sup>	4.92 <sup>a</sup>	4.81 <sup>b</sup>	4.73 <sup>c</sup>	4.62 <sup>d</sup>	4.65	4.65	4.56
BW change, kg/d	NA	NA	NA	NA	-0.63	-0.60	0.15	0.04	0.14
DM digestibility, %	NA	NA	NA	NA	61.7 <sup>a</sup>	69.1 <sup>b</sup>	63.9 <sup>a</sup>	66.6 <sup>b</sup>	65.7 <sup>ab</sup>
NDF digestibility, %	NA	NA	NA	NA	42.5 <sup>a</sup>	51.0 <sup>b</sup>	42.6	44.3	45.9

<sup>a,b</sup>Means within a study differ ( $P < 0.05$ ).

<sup>c,d</sup>Means within a study differ ( $P < 0.10$ ).

<sup>f,g</sup>Means within a study differ ( $P = 0.11$ ).

<sup>z</sup>Conc = concentrate, TMR = total mixed ration, NA = not available.

rumen (Feng et al., 1996; Yang et al., 1999). The response has been shown to be due to enzymatic activities (Nsereko et al., 1999), but the key enzymes involved have not been identified. Identification of the important enzyme activities would provide a rationale for designing more effective enzyme products for ruminants.

Wallace et al. (2001) used six enzyme products to examine the relationship between enzyme activities and in vitro gas production using grass and corn silage. A significant positive correlation was reported between cellulase activity and gas production from grass silage. In a companion study, it was observed that preparations relatively high in cellulase activity increased the rate of gas production from corn silage compared with the control (no added enzyme). In contrast, products with relatively high xylanase activity did not increase gas production when glucanase activity was low. Based on the results of this study, one may assume that it would be possible to improve the effectiveness of enzyme preparations by increasing cellulase activity. However, it should be noted that the levels of enzymes used in these studies were 20 to 40 times higher than the levels normally (0.5 to 2 mg/g of TMR DM) used as ruminant feed additives. The authors were unable to document any positive effects of exogenous enzymes on rate of gas production when using lower enzyme levels, which may have been a reflection of the techniques used. Caution must be applied when extrapolating from in vitro studies where relatively high levels of enzyme addition are used.

Colombatto et al. (2002a) used 23 commercial enzyme products to determine the relationship between enzyme activity and the in vitro degradation of feeds. The enzyme products were assayed for 16 different activities using pH and temperature conditions similar to those of the rumen (39°C and pH 6.0), and the level of enzyme product added per unit of feed was similar to that used in vivo (1.5 mg/g of DM). For incubations performed in buffer without ruminal fluid, there was a strong relationship between the release

of reducing sugars from alfalfa hay or corn silage and the biochemical characteristics of the enzyme products. Protein content of the enzyme product explained about 60% of the variation in reducing sugars released, indicating that concentrated products with higher protein content were most effective. This can be explained by the fact that the same amount of each enzyme product was used even though the enzyme activity varied tremendously among products.  $\beta$ -glucanase activity explained a further 24% of the variation in reducing sugars released from alfalfa hay, whereas endoglucanase, exoglucanase,  $\beta$ -glucosidase, xylanase, and amylase explained a further 37% of the variation for corn silage. Thus, for alfalfa hay, about 84% of the variation, and for corn silage, about 97% of the variation, was explained by these factors.

The same enzyme products were evaluated for their effects on in vitro DM degradation (Colombatto et al., 2002b). Five of the 23 products significantly improved the 18-h degradation of alfalfa hay, and nine of the 23 products improved the degradation of corn silage. There was a significant relationship between xylanase activity and feed digestion. However, the relationship was positive with alfalfa hay, but negative with corn silage. This negative relationship observed for corn silage is perplexing and may not be one of "cause and effect." In addition, it should be noted that whereas the correlations between xylanase activity and digestion were significant, the xylanase activity alone explained less than 30% of the variation in degradation. No other significant relationship was observed between enzyme activities and feed degradation.

Similar observations have been reported in vivo. For example, Kung et al. (2000) compared two different enzyme products with similar cellulase and xylanase activities in the diets of lactating dairy cows. Only one of the two enzyme products resulted in an increase in milk yield.

Although further study is warranted, these results indicate that it may not be possible to predict the potential of increasing cell wall digestion in the rumen

using exogenous feed enzymes based only on their biochemical characterization. This observation is not surprising because enzyme activities are measured on model substrates that do not represent the complexity of plant cell wall material. Enzyme assays are based on the initial rate of reaction with the substrate and do not relate to overall enzyme persistency. Furthermore, the conditions of the assays may not represent those of the rumen. Although enzyme activity units are important for quality control and to ensure that the customer is actually getting the product they expect, it must be recognized that these activity units bear little relationship to the efficacy of the product as a ruminant feed additive.

### *Enzyme Level*

Some of the variability associated 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. For example, Kung et al. (2000) offered forage (60% corn silage and 40% lucerne hay; DM basis) treated with increasing levels (0, 1, 2.5 mL/kg of TMR) of an enzyme product (FinnFeeds Int.) to cows. Cows fed the low level of enzyme tended ( $P < 0.10$ ) 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). Nonlinear responses have also been reported for growing beef cattle (Beauchemin et al., 1995). In that study, ADG of cattle fed alfalfa hay increased by 24 to 30% with lower levels of added enzyme (0.25 to 1 mL/kg of DM) as a result of increased intake of digestible DM, but higher levels of enzyme (2 and 4 mL/kg of DM) were not effective. With timothy hay, a high level (4 mL/kg of DM) of exogenous enzymes increased ADG of cattle by 36% as a result of a 17% increase in ADF digestibility and a 14% increase in digestible DM intake.

These studies demonstrate 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 with increasing levels of an enzyme product from *Trichoderma longibrachiatum* added to a dairy cow diet. The authors speculated that application of a moderate level of enzyme to ruminant feeds caused some beneficial disruption of the surface structure of the feed either before or after ingestion. 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.

### *Enzyme-Feed Specificity*

The array of enzyme activities required to improve fiber digestion varies according to the composition of the feed. The principle of feed-enzyme specificity is illustrated in a study conducted in our lab (D. Colombatto, K. A. Beauchemin, and D. P. Morgavi; unpublished data) in which 26 enzyme products were used. The products were characterized biochemically, and then assessed in vitro in the presence of rumen fluid to test their ability to influence DM degradation of alfalfa hay or corn silage at 18 h of incubation. The enzyme products that effectively increased degradation were different for both forages (Table 3). Of the candidates that increased degradation of alfalfa, only enzyme E was also effective for corn silage (ranked 10th). These data clearly indicate the importance of matching the enzyme product to the forage.

Enzyme-feed specificity presents a major dilemma for formulating new ruminant feed enzyme products because most commercial ruminant diets contain several types of forages and concentrates. Therefore, to achieve maximal benefit, a number of different enzyme sources would need to be used in a typical diet. A middle approach is to use an enzyme that may not be the best on all forages, but is relatively suitable for most feeds. This generalized approach has been the one taken for the most part in the development of enzyme products for ruminants. To some extent, this approach has limited the rate of progress in terms of bringing effective products to the marketplace. On-farm efficacy of some products may not be high in all situations, contributing to the variability associated with enzyme technology. Because of the relatively high cost of feed enzymes compared to other technologies, livestock producers expect an equally high response in animal productivity. In future, the “one-size-fits-all” approach may be replaced by a more targeted approach in which feed enzyme products are formulated for various types of feeds. Although this “designer enzyme” targeted approach presents an added degree of complexity in the marketplace, it may be the best way to ensure efficacy of feed enzyme technology on the farm.

### *Method of Providing Enzyme to Animals*

Applying fibrolytic exogenous enzymes in a liquid form onto feeds prior to consumption can have a positive effect on animal performance (Rode et al., 1999; Schingoethe et al., 1999; Kung et al., 2000; Yang et al., 2000). In contrast, infusion of enzymes into the rumen has not been effective (Lewis et al., 1996; McAllister et al., 1999; Sutton et al., 2001). The close association of enzymes with feed may enable some form of preingestive attack of the enzymes upon the

**Table 3.** Top-ranked enzyme products for their ability to increase in vitro dry matter degradation of forages. Letters (A to Z) indicate enzyme products. Xylanase (Xyl) and endoglucanase activity (CMC) of each product is expressed as a percentage of the maximal activity measured across all 26 products

Rank	Alfalfa hay <sup>a</sup>			Corn silage <sup>b</sup>		
	Enzyme	Xyl/CMC	Increase in IVDMD, %	Enzyme	Xyl/CMC	Increase in IVDMD, %
1	A	83.2/2.0	10.3*	Z	88.6/15.4	10.9*
2	B	0.9/0	9.8*	J	15.1/40.2	9.1*
3	C	84.3/21.5	9.5*	N	4.3/40.1	8.7*
4	D	90.7/17.0	9.3*	Y	3.0/12.3	8.3*
5	E	34.6/6.8	8.5*	S	13.3/63.5	7.5*
6	F	62.0/68.0	7.8	P	10.0/57.1	7.3*
7	G	10.5/85.6	7.5	X	2.5/31.7	6.7*
8	H	100/6.9	6.3	Q	2.9/7.1	5.9*
9	I	16.2/100	6.3	I	16.2/100	5.9*
10	J	15.1/40.2	5.6	E	34.6/6.8	5.8*
11	K	4.5/34.0	5.5	G	10.5/85.6	5.6*

<sup>a</sup>In vitro DM digestibility (IVDMD) of alfalfa hay control was 434.9 g/kg.

<sup>b</sup>IVDMD of corn silage control was 424.0 g/kg.

\*Increase in IVDMD compared with control silage with no added enzyme ( $P < 0.05$ ).

plant fiber and/or enhance binding of the enzymes to the feed, thereby increasing the resistance of the enzymes to proteolysis in the rumen.

There is apparently little or no requirement for a reaction phase or incubation time between treatment and feeding of forages. 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. In vitro studies have reported similar results (Colombatto, 2000).

Enzymes have been applied to TMR (Higginbotham et al., 1996; Beauchemin et al., 1999; Phipps et al., 2000b; Yang et al., 2000), hay (Beauchemin et al., 1995; Lewis et al., 1996; Yang et al., 1999), ensiled forages (Beauchemin et al., 1995; Phipps et al., 2000a), concentrate (Rode et al., 1999; Phipps et al., 2000b; Yang et al., 2000), supplement (Bowman, 2001), or premix (Bowman, 2001). Exogenous enzymes may be expected to be more effective when applied to high-moisture feeds (such as silages) compared to dry feeds because of the higher moisture content. The requirement for water in the hydrolysis of soluble sugars from complex polymers is a fundamental biochemical principle. Furthermore, silage pH values are usually at, or around, the optimal pH for most fungal enzymes. However, in practice, some exogenous enzymes are more effective when applied in a liquid form to dry forage as opposed to wet forage. Feng et al. (1996) applied an enzyme solution directly to grass and observed no effect when added to fresh or wilted forage; however, when it was applied to dried grass, enzymes increased DM and fiber digestibility. Similarly, Yang et al. (2000) reported increased milk production and digestibility of the diet when enzymes were added to the concentrate portion of a dairy cow diet, but not

when they were added directly to TMR. In contrast, Phipps et al. (2000b) reported no difference between adding an enzyme product to concentrate or TMR, but the enzyme product used in that study was not effective. The reduced efficacy of exogenous enzymes applied to ensiled feeds may be due to inhibitory compounds in fermented feeds. Nsereko et al. (2000) reported the presence of compounds in whole-crop barley silage that inhibited endo-1,4- $\beta$ -xylanase activity of an enzyme product from *T. longibrachiatum* by 23 to 50%, although there was no effect on cellulase activity. Also, the application of exogenous enzymes to silages can accelerate their aerobic deterioration. The growth of the ephiphytic microbiota is stimulated by soluble sugars released by enzyme treatment, which could lead to a decrease of the silage feed value if the time elapsed between enzyme application and consumption is sufficiently long (Wang et al., 2002).

Yang et al. (1999) observed no difference between applying an enzyme product to dry forage or to both dry forage and concentrate. Others have also found that adding enzyme to concentrate to be effective (Beauchemin et al., 1997; Iwaasa et al., 1997; Rode et al., 1999; Yang et al., 2000). Bowman (2001) examined the effects of adding an enzyme product (Promote N.E.T., Agribands International, St. Louis, MO) to various proportions of a TMR fed to dairy cows. The enzyme product was added to the concentrate portion of the TMR (45% of the TMR), to the supplement (4% of the TMR), or to the premix (0.2% of TMR). Diets with enzymes delivered the same quantity of enzyme per cow daily. Total-tract digestibility of NDF was increased from 44.3 to 55.6% (25%) when enzyme was added to concentrate, but the other treatments had no effect. When the same diets were evaluated in vitro, DM degradation of the TMR at 12 h of incubation was increased by 15% when enzymes were added to the

concentrate portion, and by 17% when added to the premix portion, of the diet. At 48 h of incubation, only the treatment with enzyme added to a premix had higher DM digestibility compared to the control. Reasons for reduced response in vivo when enzymes are applied to a smaller portion of the diet are unclear. Beauchemin et al. (1999b) suggested that enzymes should be applied to a large portion of the diet to increase the chances that enzymes endure in the rumen. Adding enzyme to a small portion of the diet may allow rapid passage of enzyme from the rumen, lessening the enzyme effect in the rumen. Rapid passage of the enzyme is not an issue in vitro. Thus, in vitro batch culture assays used as a bioassay to predict the effects of exogenous enzymes on digestibility may not accurately predict variations in the in vivo response caused by method of providing the enzyme to the animal.

### *Level of Animal Productivity*

Animal responses to exogenous enzymes are expected to be greatest in situations in which fiber digestion is compromised and when energy is the first-limiting nutrient in the diet. High-producing dairy cows and growing cattle require high levels of available energy to meet the demands of milk or meat production. It is not uncommon for feed intake of dairy cows to exceed four times the level of intake required for maintenance. In these commercial feeding situations, fiber digestion is often compromised due to low ruminal pH and rapid transit time through the rumen. The NRC (1989) assumes a 4% reduction in digestibility for each multiple increase in feed intake over maintenance. Thus, a dairy cow diet with a potential digestibility of 77% would have an actual digestibility of 68% when fed to high-producing dairy cows. More recently, the NRC (2001) recognized that the decrease in digestibility at high levels of intake is not constant. For high-quality diets, the decrease in digestibility is even greater than previously estimated.

Enzymes help bridge the gap between potential and actual performance of the animal. This concept is illustrated in a study in which dairy cows and sheep were fed a TMR with and without exogenous enzymes (Yang et al., 2000). When measured in dairy cows, the total-tract digestibility of the control diet was 63.9% for DM and 31.8% for ADF. As a result of lower intake as a percentage of BW, digestibility of the control diet was higher in sheep; total-tract digestion was 77.1% for DM and 49.8% for ADF. Use of an enzyme product improved digestibility of the diet when evaluated in dairy cows, but not in sheep. These results indicate that exogenous enzymes improve feed digestion when the potential digestibility of the diet is not attained because digestion is compromised. It is this "loss" in digestible energy that is captured with the use of feed enzymes.

Existing enzyme technology is not likely to benefit ruminants fed at maintenance; rather, the greatest

responses will be for ruminants 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).

### *Mode of Action: An Integrated Hypothesis*

In light of the exceptionally high starch- and fiber-digesting capacity of the rumen, it is difficult to explain why treatment of grain or forage with enzymes prior to consumption further improves feed utilization. It is clear that the mode of action for exogenous enzymes improving digestion of plant cell wall is complex. Based on our experience and the available information, we have developed a putative hypothesis that we feel accounts for the most critical factors that explain the observed increases in fiber and DM digestion.

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. 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 particles concentrates digestive enzymes close to their specific substrates. However, some ensiled feeds contain compounds that are inhibitory to xylanases (Nsereko et al., 2000); therefore, applying enzymes to dry feeds decreases the variability in response.

Applying enzymes to feed also provides a slow-release mechanism for enzymes in the rumen (Beauchemin et al., 1999a). Thus, the greater the proportion 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). Nsereko et al. (2000) demonstrated compelling evidence that applying enzymes to feed causes structural changes to occur, thereby making feed more amenable to degradation. 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). The net effect is increased enzymatic activity within the rumen, which enhances digestibility of the total diet fed. Thus, improvements in digestibility are not limited to the dietary component to which the enzymes are applied, which explains why fibrolytic enzymes can be effective when added to the concentrate portion of a diet. Increased hydrolytic capacity of the rumen can also lead to an increase in digestibility of the nonfiber carbohydrate fraction, in addition to increasing digestibility of the fiber components of a diet, which explains why fibrolytic enzymes can be effective in high-concentrate diets.

Exogenous enzymes appear to survive in the small intestine for a time sufficient to have an effect on target substrates (Morgavi et al., 2001). However, postruminal effects of exogenous enzymes on digestion are likely only a significant factor when enzymes are infused into the rumen (Hristov et al., 2000) or added to feed in a manner that allows for easy solubilization from feed and rapid passage from the rumen (Beauchemin et al., 1999). Such may be the case when enzymes are applied to wet feeds prior to feeding or when offered as a concentrated premix. Also, the relatively short retention times through the lower gastrointestinal tract means that, postruminally, enzymes are only likely to be effective against the least recalcitrant fraction of dietary fiber. Significant quantities of this easily digestible fiber will only be passing out of the rumen in abnormal circumstances such as extreme ruminal acidosis.

### Implications

Adding exogenous fibrolytic enzymes to dairy cow and feedlot cattle diets can potentially improve cell wall digestion and the efficiency of feed utilization by ruminants. Positive responses in milk production and growth rate have been observed for cattle fed some enzyme products, although results have been inconsistent. Some of the variation can be attributed to product formulation, under- or over-supplementation of enzyme activity, inappropriate method of providing the enzyme product to the animal, and the level of productivity of the test animal. Research is needed 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 magnitude of increased animal performance obtainable using feed enzymes, these products could play an important role in future ruminant production systems.

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