The use of exogenous enzymes in ruminant diets

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Summary

Enzymes have been used successfully for many years to improve the nutritive value of diets used in the intensive monogastric livestock industries. This success has not occurred in the ruminant species reflecting their more complex digestive system and the greater concomitant challenge to nutritional science. Nevertheless, from defining nutritional objectives for ruminants and coupling this with detailed knowledge of possible target substrates, and the activity of enzymes, promising research directions can be postulated. Initial targets are likely to be the fibre (polysaccharides) components of feeds, and some experimental data have already been gathered indicating some potential for glycanase enzymes. Other feed components such as starch and plant toxins may also be suitable targets, particularly when certain feeding regimens are considered.

Introduction

Almost since it was first realised that the digestive processes of all animals involved the breakdown of the macro-nutrients by endogenous enzymes, animal nutritionists have attempted to enhance the process by application of exogenous enzymes. This has been used most successfully in monogastrics and, particularly, in broiler chickens. The science behind the use of enzymes in broiler chickens is well-reviewed (Annison and Choct, 199 1; Annison, 1993). The reasons for the success of the application of exogenous enzymes in the broiler industry are:

- advanced analytical techniques have been applied defming target substrates in the diets and identifying required activities of enzymes;
- the relatively simple digestive tract of the chicken is particularly sensitive to nutritional perturbations which can subsequently be overcome by enzymes; and
- the suitability of chickens as inexpensive laboratory animals allows large scale, statistically well-designed experiments to elucidate the chemistry, biochemistry and physiology behind the nutritional observations. Subsequent product development is also greatly enhanced by the ease of experimentation with chickens.

In light of this, the use of enzymes to improve the quality of ruminants diets can be seen to be a far greater challenge. Although the same analytical techniques can be applied to ruminant feeds, the digestive tract of ruminants is far more complex than that of chickens. Much is known, of course, about the rumen, but nevertheless attempts to modify its function with enzymes have been much less spectacular than seen in broiler chickens.

Notwithstanding the difficulties in using enzymes in ruminant diets stemming from the complexity of the rumen, and the expense of using sheep or cattle as experimental animals, opportunities for use of exogenous enzymes as feed additives do exist. These are founded on two observations:

- the digestibility of organic matter in ruminants rarely approaches 100% and is usually much less; and
- new feed raw materials, many of low quality, are being promoted for ruminants and enzymes may assist in their breakdown in the rumen or elsewhere in the digestive tract.

The use of enzymes in ruminants has recently been extensively reviewed (Beauchemin and Rode, 1996; Hristov *et al.* 1996). This paper looks past the recent developments to some potential uses of enzymes in the future.

Applications of enzymes-silage vs. diets

Enzymes may find application in ruminant nutrition in two ways:

- as silage-improving agents; or
- as feed additives expected to act immediately prior to ingestion or at some point during passage through the gastro-intestinal tract of the animal.

In this paper only the application of enzymes in feeds will be discussed. The use of enzymes as silage–improving agents holds great potential but it could be argued that this is more of a feed technology or biochemical engineering problem and less attention needs to be paid to the physiology, biochemistry and nutrition of the ruminant. The constraints on developing successful enzyme uses are quite different for the two situations.

Challenges for nutritionists using enzymes in ruminant diets

In looking ahead at possible directions in which enzymes for use in ruminants should be developed, it is important to consider the basic function of the rumen and nutritional requirement of the ruminant. The biggest challenge for nutritionists is to supply adequate amounts of protein and energy to the animal, and it is in meeting this demand that enzyme technology is likely to yield the most profitable results. The provision of micronutrients is also important, of course, but enzyme technology is unlikely to play a role in this area in the short term. The rumen has two important roles:

- to release energy from carbohydrate sources which would otherwise be unavailable to the animal; and
- to fix non-protein nitrogen in a form which can be utilised by the animal, *i.* e. as microbial protein.

These functions are carried out by the rumen microflora and fauna which consist of bacteria, fungi and protozoa. The microflora produce a vast array of enzymes which 'process' the feed material. The complexity of this system cannot be overstated. The organisms function synergistically and competitively and, due to their great diversity, can adapt to a wide variety of feeds converting them to energy substrates and proteins suitable for the host animal. Many of these feedstuffs are virtually indigestible in simple-stomach animals. Thus ruminants represent a remarkable means of transforming feedstuffs into products suitable for human use (i.e. meat, milk and fibres).

To optimise the performance of ruminants, nutritional strategies with the following objectives are required:

- maximised fermentation of carbohydrates which are unable to be digested and absorbed in the small intestine;
- minimised fermentation of carbohydrate which is digested and absorbed in the small intestine;
- maximum synthesis of microbial protein from non-protein nitrogen; and
- minimal breakdown of dietary protein.

Each of these objectives will be discussed below in terms of how they may be affected by the application of enzymes.

Rumen carbohydrate fermentation

As with monogastrics, carbohydrates in ruminants can be classified into two types- those, which are digested and absorbed in the small intestine (available carbohydrates), and those which are not. Available carbohydrates include the monosaccharides glucose, fructose and galactose, disaccharides such as lactose, the maltose series and starches. Unlike monogastrics, ruminants appear not to have a sucrase function in their small intestine (Harmon, 1993). The unavailable carbohydrates are essentially the other carbohydrates in plant feedstuffs. They are the non-starch polysaccharides (NSPs) and also the oligosaccharides such as those of the raffinose series and fructo–oligosaccharides. These unavailable carbohydrates are found in the 'fibre' component of feedstuffs.

Unlike the nutritionist, the rumen microflora do not distinguish between these two types of carbohydrates. Both types may be fermented in the rumen leading to the common metabolic products- primarily short chain fatty acids (SCFAs) and methane and heat or the fermentation intermediates used for synthesis of microbial polymers including protein. The SCFAs are absorbed directly from the rumen and can be used for both catabolic and anabolic (ie. gluconeogenesis) processes. The process of fermentation results in significant losses of energy in the form of methane, and heat (and occasionally hydrogen). Thus when dietary glucose, for example, by-passes the rumen and is absorbed in the small intestine, the efficiency of energy utilisation is increased by around 30%.

The rumen microflora degrade and ferment NSPs very efficiently. Whilst there is great diversity in the types of NSP found in feedstuffs there are also many carbohydrases produced by the rumen microflora to degrade them (Table 1).

The list of enzymes in Table 1 is by no means complete and for each type many different individual enzymes with slightly different optimal activities, binding sites and products have been identified.

For the degradation of NSPs to constituent sugars, which is the prerequisite for fermentation, many polysaccharidases acting in concert are required. For

Table 1 Types of, carbohydrates-degrading enzymes found in the rumen.

Cellulase	α–L–arabinofuranosidase
Endoglucanase	Ferulic acid esterase
Exoglucanase	βD–glucuronidase
β–glucosidase	Amylase
Xylanase	Arabinase
Xylosidase	Laminarinase
Acetyl xylan esterase	Lichenase
Acetyl esterase	Pectinase

[1] *From Cheng et al.* (1989).

example, the breakdown of arabinoxylans (structural polysaccharides found in cell walls of forages and in the endosperm of cereals) requires a range of enzymes working sequentially. Essentially, enzymes that remove the side chains of arabinose, acetyl groups, ferulic acid and glucuronic acid act first, followed by xylanases which can cleave the xylan main chain (Figure 1). The breakdown of cellulose also requires a series of enzymes which includes endo–1, 4–β–D–glucanases, 1, 4–β–D–glucan cellobiohydrolases and β–glucosidases.

The breakdown of NSPs to fermentable sugars is therefore a complex system of co-operation between microorganisms and their enzymes. To match, or better this, with the application of exogenous enzymes as feed additives is a tall order. Nevertheless, digestion of fibrous material is rarely complete in the rumen, and examining the factors that may limit the breakdown of the NSPs provides direction for future enzyme research.

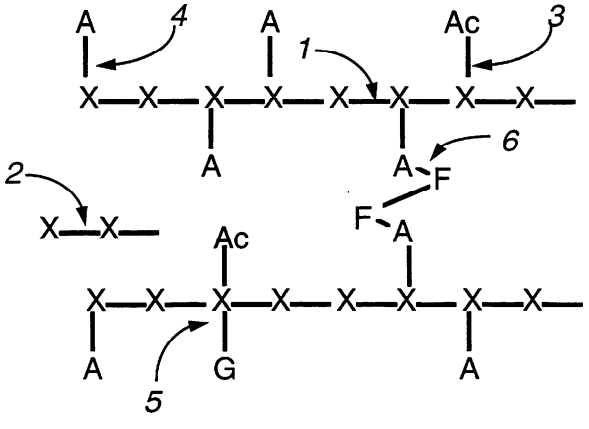

Figure 1 The structure of arabinoxylans of cereals and grasses and the enzymes necessary to break it down in the rumen. A – arabinose, X – xylose, G – glucuronic acid, Ac – acetate, F – ferulic acid. 1 – xylanase, 2 – xylobiase, 3 – acetyl esterase, 4 – arabinofuranosidase, 5 – glucuronidase, 6 – ferulic acid esterase.

NSP Degradation-limiting factors as possible enzyme targets

Assuming a competent microflora and an absence of extrinsic factors which may constrain or redirect their activity, the rate and extent of NSP degradation is determined broadly by two factors:

- the molecular structure and 'order' of polysaccharides in the feed material; and
- the presence of inhibiting structures (such as lignin).

Polysaccharide structure

Cellulose, the main NSP in forage occurs as a highly ordered insoluble crystalline structure which is very resistant to enzymic attack. In the rumen, the diverse microflora employ a wide range of cellulase complexes, some with as many as five distinct enzyme activities. Enzymic attack necessarily involves attachment of microflora to the feed (Cheng ***et al.*** 1989) which allows the microbial cell surface associated cellulase complexes to come into close contact with the substrate. Adhesive forces are very large at the micro level, and this probably results in a localised disruption of the ordered cellulose structure and depolymerisation proceeds.

The cellulase complexes are found mainly associated with the cell walls of bacteria and other microflora and very little free cellulase activity is found in the rumen liquor. This would suggest that any application of exogenous enzymes to provide fibrolytic activity may require application of live celluloytic organisms. This option of using 'probiotic' organisms to deliver enzymic activity is certainly feasible, but falls outside the scope of this paper.

An encouraging development is that a highly active cell–free cellulase complex has been isolated from the rumen fungus *Neocalleomastix frontalis* (Wood ***et al.*** 1986). Production of the cellulase complex as a feed additive may be possible. Another promising observation is that, although the thermophilic bacterium ***Clostridium thermocellum*** possesses 15 unique endoglucanases, only one of these, (in association with a non-catalytic protein, that might have a role in bacterial cell adhesion to cellulose) is necessary for the hydrolysis of a crystalline cellulose (Wu ***et al.*** 1988). Clearly this type of enzyme/protein combination may also provide opportunities for cellulolytic exogenous enzyme development.

As far as other NSPs are concerned, they are thought to be present as less ordered structures than cellulose. They tend to be more soluble and certainly more fermentable, but there is evidence that subtle changes in their structures can affect their digestibility. Li ***et al.*** 1992 examined the chemical composition and nutritive value of the annual legume *Trifolium subterraneum* **cv.** Junee and *Trifolium resupinatum*, **cv** Kyambro. In spite of the concentrations of most

Table 2. *Composition of dry matter (DM) of mature annual legumes Trifolium subterraneum cv. Junee and Trifolium resupinatum, cv Kyambro and the in vivo digestibilities of the some components.*[1]

	Junee		Kyambro	
	g/kg DM	Digestibility	g/kg DM	Digestibility
Hemicellulose	114	65.6	115	46.9
Cellulose	294		287	
Lignin	98.2		88.9	
Arabinose	31.4	74.9	30.9	72.6
Xylose	49.9	52.2	50.5	36.7
Mannose	8	85.8	19.3	45.8
Galactose	21.7	77.3	32.2	53.3

[1] From Li *et al.* 1992

components being similar, including the sugars arabinose and xylose, the digestibility of xylose was lower in Kyambro than Junee (Table 2). One interpretation of these findings is that the structure of the arabinoxylans in the Kyambro is different- ***i.e., the*** pattern of substitution of the arabinose sugars along the xylan backbone in Kyambro may be less vulnerable to arabinase and xylanase attack. Another possibility is that the higher levels of galactose and mannose containing polysaccharides in the Kyarnbro may protect the arabinoxylan polysaccharide. Observations such as these suggest a role for specific highly active arabinoxylanases or galactomannases as feed additives for the animals given these types of forage.

The presence of substituent groups on polysaccharide structures may inhibit the activity of carbohydrolases. Common non-carbohydrate substituents are acetyl groups and ferulic acid groups such as those found on arabinoxylans. Both of these have been identified as possible inhibitors of the degradation of forage in the rumen (Richards, 1976). Both groups are attached through ester linkages; thus the development of potent esterases as feed additives may enhance the degradation of feed materials, particularly the hemicellulose portion.

NSP-protecting structures as enzyme substrates

Lignin is the major component of feedstuffs directly inhibiting the degradation of polysaccharides in the rumen. It is overcome during the colonisation of feed particles by the rumen microflora, particularly the protozoa and fungi. Lignin is a three dimensional network built up from phenylpropane units. The precursors are coniferyl, sinapyl and p-coumaryl alcohols. Lignin may protect the NSPs of plant feedstuffs by creating a physical barrier, by creating a hydrophobic micro-environment close to the substrate, or by direct covalent linkage to the substrate polysaccharides (Theander, 1988).

Lignin is degraded to some extent by the action of rumen microflora, primarily the fungi. The lignin does not, however, appear to be utilised by the rumen microflora to any extent: it is dissolved and can be recovered from the rumen liquor as lignin–polysaccharide complexes (Orpin, 1988).

A characteristic feature of low quality roughages, such as straw, is the refractory nature of their highly lignified cell walls. Lignin degradation is very slow and acceleration of this process in the rumen may be beneficial in increasing both the rate and extent of release of fermentable carbohydrate. A single lignase produced by the soft-rot fungus ***Phanerochaete*** *chrysosporium* is capable of causing a high degree of lignin depolymerisation (Tien and Kirk, 1983). The enzyme acts like a peroxidase, causing a free-radical-mediated cleavage of carbon-carbon bonds, which requires hydrogen peroxide or oxygen. The requirement for such oxidative conditions may limit the usefulness of this enzyme as a feed additive for ruminants as most of the rumen contents are highly anaerobic. Nevertheless, some oxygen does find its way into the rumen from the epithelial contact surfaces and this may be enough to allow this enzyme to operate. It is also possible that, with the application of recombinant-DNA technology, the lignase gene may be modified to allow the enzyme to operate in more anaerobic conditions.

The cuticula layer of forage materials also represents a considerable barrier to the invasion of rumen microorganisms. Microscopic examination of the feed particles taken from the rumen shows the cuticle to be virtually devoid of attached microorganisms. Cutin is a polyester of Cl6 and Cl8 hydroxy and hydroxy–epoxy fatty acids. The ester linkage is known to be hydrolysed by some pathogenic bacteria and some aerobic bacteria but, there are no reports of rumen bacteria exhibiting cutinase activity (Cheng ***et al.*** 1989). Cutinase therefore represents an opportunity as a feed enzyme additive for cattle fed low quality forage.

Enzymic enhancement of starch digestion

The major component of cereal grains is starch, a polymer of glucose. The digestion of starch in the rumen

is usually high, although the rate of digestion can vary considerably between cereal types. Maize starch, for example is digested slowly, as the starch granules are surrounded by a protein matrix which acts a barrier to amylase enzymes. This has been demonstrated with ***in vitro*** rumen fluid incubation experiments where starch from maize degrades more slowly than wheat starch (Opatpatanakit ***et al.*** 1994; Figure 2). Rapid fermentation of starch in the rumen is not considered desirable as it can lead to acidosis, bloat and liver abscess (Rowe and Pethick, 1994). The use of diets high in cereals is associated with a depression of digestion of fibre from forage components. This is thought to be due primarily to an inhibition of the cellulolytic microflora by the low pH that accompanies rapid fermentation of the cereal starch. Clearly there is no role for amylases that would exacerbate this problem and enhance a process that, as far as the host animal is concerned, is energetically unfavourable. Under these circumstances, however, exogenous cellulases and hemicellases, particularly those active at low pH, may be of value.

The studies of Rowe and Pethick (1994) have highlighted another problem. When starch in cereals such as maize and sorghum escapes digestion in the rumen with it may also pass through the small intestine to be fermented in the large bowel. Extensive fermentation in this organ may cause severe scouring. Thus acidosis may also occur in the hindgut in the ruminant. Rowe and Pethick, 1994 counsel the use of antibiotic feed additives such as virginiamycin to control this problem, but the judicious use of amylase enzymes to enhance the small intestinal breakdown of starch and absorption of glucose may also be a solution. The challenge here, of course, is to get the enzyme past the rumen to the site of preferred activity, namely the small intestine. Encapsulating technology has already been developed for the delivery of methionine and lysine to the small intestine, and it should be possible to adapt it to enzyme delivery.

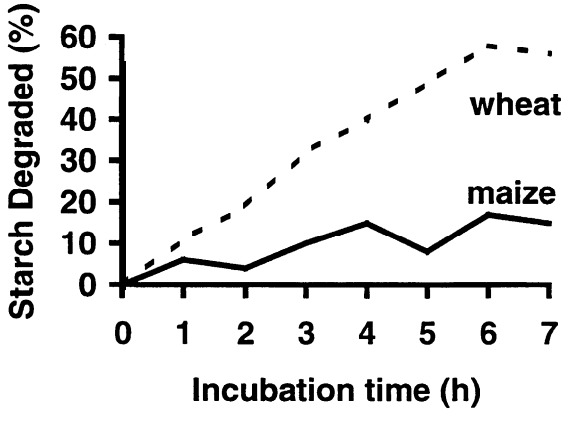

Figure 2 Degradation of wheat and maize starch during incubation in rumen fluid (after Opatpatanakit *et al.* 1994).

Protein digestion in the ruminant

A major concern for ruminant nutritionists is that on low quality forage diets the release of energy from carbohydrates by fermentation is so slow that the microflora breakdown dietary protein to use it as an energy source. The only solution to this problem is to speed up the carbohydrate fermentative processes, and possible enzyme strategies to achieve this have been described above. Clearly, the use of exogenous proteases to increase the rate of degradation of feed proteins in the rumen would be inappropriate. The small intestine is the preferred site for the breakdown of proteins from the diet and is also the site for rumen microbial protein digestion. As small intestinal digestibility of dietary and microbial protein is only in the region of 60 to 70% (Harmon, 1993) an opportunity may exist here for the use of exogenous proteases as feed additives. Again, as described above, how to deliver the enzyme to the site of activity is a concern but this may be achieved with the rumen by-pass encapsulating technology.

The ruminant as a monogastric

When young ruminants are suckling, milk by-passes the rumen through the oesophageal groove and passes to the abomasum and, hence, to the small intestine where digestion occurs. The reflex responsible for this usually diminishes into adult life, but can be maintained indefinitely if animals continue to be given some of their daily ration in liquid form (Orskov, 1983). One can envisage feeding regimes being developed in the feedlot situations where feed concentrates high in cereal and protein are fed in the liquid form so that they by-pass the rumen. Lower quality forage may also be fed which would be digested in the rumen.

In this situation the NSPs from the cereal ***(i.e.*** wheat, barley, rye) may display anti-nutritive activity as has been described in poultry and pigs. These would be appropriate targets for the exogenous enzymes. As ruminants seem to be sensitive to rapid fermentation in the large bowel if starch is digested incompletely, exogenous amylases may also prove to be beneficial.

Naturally occurring toxins

Many plant feedstuffs, particularly those eaten by grazing ruminants, contain toxins which inflict stock losses. The tropical legume ***Leucaena leucocephala*** has caused problems because Australian sheep are sensitive to poisoning by the compound mimosine, which this plant produces, and its degradation product hydroxypyridone. To combat this problem, microorganisms responsible for the resistance of Indonesian goats to mimosine were transferred to ruminants in Australia.

Further work has examined the possibility of using microorganisms to detoxify fluoroacetate which occurs naturally in the tissues of many plants such as ***Acacia,***

Table 3 Positive effects of fibrolytic enzymes in corn silage fed to cattle.[1]

	Enzyme level			
	0	15x	22.5x	30x
ADG[2], kg/day	1.06	1.07	1.12	1.23
% of Control	100	101	106	116
DM[2] Intake, kg/day	6.6	6.3	6.3	6.2
% of Control	100	95	95	94
FCR[2], kg DM/kg gain	6.22	5.88	5.63	5.05
% of Control	100	95	91	81

[1] From Beauchemin *et al.* (1996).
[2] ADG, average daily gain; DM, dry matter; FCR, feed conversion ratio.

Gastrolobium and *Oxylobium*. In ruminants, a lethal dose of fluoroacetate is between 0.25 and 0.5 mg/kg. A bacterium *Moraxalla sp.* possesses an enzyme which hydrolyses fluoroacetate which has transferred into the rumen species *Butyrivibrio fibrisolvens* and ***Bacteriodes ruminicola*** (Gregg and Sharpe, 1989; Gregg; in this Symposium).

A major problem with introducing new detoxifying bacteria to the rumen is that they must colonise and remain present for extended periods in the rumen. In order to do this they must compete with the other rumen organisms.

An alternative approach to providing live organisms would be to simply provide to the rumen the enzymes responsible for the detoxification. In some cases this might require several different enzyme activities to ensure the final degradation products are innocuous.

Examples of promising results from the use of enzymes in ruminant diets

In recent years ruminant nutritionists have been following the direction established by their colleagues working with monogastrics. Whilst progress has been limited, some results reflect the potential for enzymes identified earlier in the text.

Forage–based diets

Beauchemin et ***al.*** (1996) make the point that experiments investigating the effects of enzymes in ruminant diets have recorded both positive and negative results since the 1960s. There are, however, several recent studies demonstrating positive effects of feed enzymes in diets given to lactating and growing cattle. A fibrolytic enzyme preparation has been shown to improve the nutritive value of an alfalfa hay/alfalfa silage/barley mixed diet (Stokes and Zhang, 1995). Dry matter intake was increased by 10.7% compared to controls while milk yield was up by 14.8%.

Lewis et ***al.*** (1995) also demonstrated effects of enzymes in cows on this type of diet. The cows were fed a diet treated with enzyme produced 1.3 kg/day more milk than cows fed control diets. The feed intake of these cattle was also increased significantly by 2 kg/day.

Alfalfa hay diets were also shown to be amenable to enzyme treatment by Beauchemin and Rode (1996).

Table 4. Performance data for cows fed forage treated with three levels of enzyme[1].

	Enzyme			
	Untreated	1.25 l/ton	2.5 l/ton	5 l/ton
Dry matter intake (kg/day)	24.3a[2]	26.2b	26.1b	26.6b
Energy corrected milk yield (kg/day)	41.1a	42.1a	48.1b	41.9a
Condition score (1–5)	2.6a	3.0b	2.6a	3.0b

[1] Sanchez *et al.* (1996).
[2] $P<0.05$

An enzyme solution containing xylanase activity sprayed on the forage resulted in an increase in average daily gain (ADG) of 13% without significant changes in dry matter (DM) intake. A second enzyme preparation containing xylanase and cellulase activities tested in the same study at three different levels increased ADG and DM intake at the lowest level, depressed ADG and DM intake at the intermediate level and increased ADG, resulting in an improved feed conversion ratio (FCR), at the highest level.

A similar enzyme preparation has also been effective at raising the nutritional value of corn-silage diet when sprayed on before feeding. In this case high levels of enzyme were required but a convincing dose–response relationship for ADG and DM intake was observed (Table 3).

Less convincing dose-responses were reported by Sanchez et *al.* 1996. For dairy cattle fed an alfalfa haylage mixed ration with three levels of addition of enzyme. The intermediate level of enzyme addition resulted in a greater improvement in production than the other levels. (Table 4). A return of the energy–corrected milk yield to levels similar to those of the controls at high levels of enzyme addition is surprising given feed dry matter intake was higher when enzyme was added to the diet. The authors suggested a partitioning of energy towards improved body condition at the higher levels of enzyme of addition but the mechanism by which this could possibly happen remains obscure.

Cereal–based diets

As with the studies of exogenous enzyme use in forage–based diets, experiments examining the effect of enzymes in cereal-based diets have given small and variable responses.

Beauchemin and Rode (1996) reviewed a number of their experiments. They postulated that barley had higher levels of fibre and lower levels of starch than corn. Thus, enzyme preparations high in xylanase and cellulase activity might be expected to improve significantly the nutritive quality of the barley compared to the effects on corn. Enzyme preparations high in xylanase and cellulase activity, as measured by reducing sugar release, are more effective ***in vitro*** with barley than with corn.

Addition of enzyme preparations high in xylanase and low in cellulase activity and the opposite ***(i.e.*** high in cellulase but low in xylanase) did not produce substantial performance responses when used with barley or corn diets and subsequently fed to feedlot cattle (Table 5). The barley was clearly shown to be better than corn as judged by ADG.

This study highlights some of the problems faced by the experimenter when studying the effects of enzymes. Firstly, it should be noted that although barley contains high levels of xylans there are also high levels of β–glucans which contribute significantly to the fibre fraction. In fact, it is this component of the barley that has been shown to be primarily responsible for the poor nutritive value of the cereal for poultry. The β–glucan polysaccharide has subsequently been the target of β–glucanase enzymes in poultry diets with great commercial success (Annison, 1993). Xylanases, on the other hand are much less potent against barley and are ineffective against maize (corn) which contains low levels of both types of polysaccharide.

Secondly, neither cereal grain contains appreciable amounts of cellulose so one would not expect to see a great effect of cellulase enzymes in these diets. Thus, in the experiment described by Beauchemin and Rode (1996) an appreciable response to either enzyme combination on barley or corn in highly unlikely and the results confirm this. There is a possibility that side activities in the enzymes may have effects, but this underscores the importance of defining carefully the enzyme activities of preparations used in feeding studies.

Table 5. Effect of fibrolytic enzymes in corn and barley–based diets for feedlot cattle[1].

	Barley			Corn		
	Control	LX:HC[2]	HX:LC[2]	Control	LX:HC[2]	HX:LC[2]
ADG (kg/day)	1.43a[3]	1.40a	1.52a	1.33b	1.33b	1.19b
DMI (kg/day)	9.99	9.86	9.53	9.55	9.10	9.29
FCR	7.11f	7.13f	6.33e	7.26ab	6.95a	7.83b

[1] From Beauchemin and Rode (1996).
[2] LX – low xylanase, HC – high cellulase, HX – high xylanase, LC – low cellulase.
[3] Values with unlike letters are significantly different P<0.05.

The authors suggest that the FCR was significantly improved in cattle fed the barley diet with the high xylanase activity, but from the data, this is more likely to be a statistical anomaly than a true biological effect.

Other studies with cereals have yielded conflicting results. Boyles et *al.* (1992) showed that treatment of steam-flaked as well as dry-rolled sorghum with a commercial enzyme mix containing cellulytic, amylolytic and proteolytic activities resulted in improved ADG and FCR for steers. A similar preparation used by Chen et *al.* (1995) did not result in any effect on milk production or milk composition in cattle fed dry-rolled or steam flaked sorghum.

Maintaining enzyme activity in the rumen

The loss of exogenous enzyme activity from the rumen may occur through degradation by microbial proteases or through washout in the rumen liquor. There is good evidence, however, that degradation of exogenous enzymes in the rumen may not be a significant problem. Chesson (1993) has reported that extensive glycosylation of fungal enzymes protects them from proteolytic attack in monogastric animals. In addition, Annison (1992) has demonstrated that β–glucanase and xylanase activities can be detected in the terminal ileum of chickens following dietary supplementation with commercial feed enzyme preparations which indicates that they survive the proteolytic environment of the chicken digestive tract. Achieving stabilty of exogenous enzymes in the rumen therefore does not seem to be a major problem and indeed, Hristov *et al.* (1996) have demonstrated that exogenous enzyme activity is maintained over several hours in the rumen and small intestine of the dairy cow.

As long as the exogenous enzyme partitions into the aqueous phase, there must be some washout. This may not be a problem in the feedlot situation or where regular central feeding occurs. It does, however, present a problem in the grazing situation. A possible solution to this problem is to irnmobilise the enzyme within the rumen. Large, solid pellets, which remain in the rumen, have been successfully used to provide slow release of trace. This idea may be extended to providing exogenous enzymes. Immobilised enzymes, attached to inert supports, are already used extensively in some industries. It may be that highly active enzymes attached to supports that remain intact for an extended period will enhance rumen function considerably. Wide arrays of enzyme activities capable of breaking down a range of feed components could greatly enhance the efficiency of digestion and improve productivity. Interestingly, the notion borrows also from nature where, as described above, the cellulase complexes of many rumen microorganisms are associated with the cell surface.

Conclusions

The introduction of exogenous enzyme activity to improve the performance of ruminants provides considerable challenge. With the judicious application of microbiological, biochemical and nutritional science, directions for promising research and product development are becoming clear. Whilst the highly complex nature of the rumen environment presents considerable impediments to the application of simple enzyme systems it also provides opportunities for the application of ingenious biotechnologies which use the rumen to their advantage.

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