Scope of exogenous enzymes in enhancing ruminant productivity

Abstract

Exogenous enzymes added to animal feeds hold immense scope for enhancing livestock productivity. They improve not only the utilization of the lignocellulosic biomass, but also have had a positive impact on the quality of the environment through reduced output of excreta and pollutants such as phosphate and nitrogen including ammonia. Most of the exogenous enzyme research in sheep and cattle has been restricted to cellulases and xylanases and have demonstrated varied responses, although lignolytic enzymes have proven to be highly beneficial, their use is still in its infancy. Also many problems related to product formulation, under- or over-supplementation, appropriate method and form of providing the enzyme, and the level of productivity of the animal need to be addressed before their full potential are exploited. With increasing consumer concern about the use of growth promoters and antibiotics in ruminant production, and the magnitude of increased animal performance obtainable using exogenous enzymes, these products could play a vital role in enhancing ruminant production.

Keywords: exogenous enzymes, laccase, manganese peroxidase, lignin peroxidase, delignification, ruminants

Introduction

There has been increasing interest in exploiting low quality straws for ruminant feeding in many Asian countries, because the cost of good quality forages is often high and forage availability is limited. These crop residues are often referred to as ‘lignocellulosics’ as they are rich in cellulose which is bound with a biopolymer lignin. Cellulose, hemicellulose, and lignin are the main constituents of lignocellulosic materials.1-3 Cellulose is a linear polymer of glucose linked through a -1,4 linkages arranged into microcrystalline structures, very difficult to hydrolyze. Hemicellulose is a heteropolysaccharide made up of hexoses, pentoses and glucuronic acid. Hemicellulose is comparatively more soluble than cellulose and is branched. Xylan is the most common hemicellulose component of grass and wood and when complexed with substances like lignin and cellulose the hydrolysis is complicated.4 Lignin is highly irregular and insoluble polymer made up of phenylpropanoid subunits, viz. p-coumaroyl, coniferyl, and sinapyl alcohols. These three phenyl moieties differ in the hydroxy and methoxy substituents and are called p-hydroxyphenyl (H-type), guaiacyl (G-type) and syringyl (S-type) units. Unlike cellulose or hemicellulose, no chains containing repeating subunits are present, thereby making the enzymatic hydrolysis of this polymer extremely difficult. Rumen microbiota (bacteria, protozoa and fungi), even with their hydrolytic enzymes, are not competent enough to break these bonds efficiently.5

The efficient conversion of the energy-rich carbohydrates (cellulose, hemicellulose) in lignocellulosic biomass into accessible sugars is a challenging technically as these materials naturally evolved to resist degradation. This is due to the complex fibrous structures of the materials that have constructed physical barriers to the accessibility of these carbohydrates for enzymatic breakdown. Increasing accessibility to the cellulose/hemicellulose requires degradation of lignin. Numerous efforts have been made to improve the feeding value of straws using pretreatments in order to upgrade their digestibility but commercial application of these pretreatments is limited due to cost and potential environmental hazards.6 For degradation of lignin, a wide range pretreatment methods including the use of dilute acid, steam explosion, ammonia fiber explosion, lime and organo solvent pretreatments have been employed to improve enzymatic saccharification but, these methods produce undesirable by-products which inhibit downstream processes. Furthermore, the traditional pretreatments are energy and resource (water) intensive, and cause losses of carbohydrates.

Biological treatment of such crop residues using white rot fungi (WRF) can break the ligno-cellulose complexes, liberating free cellulose and thus enhancing their feeding value for ruminants.7 Biologically treated roughages have higher digestibility for most of the nutrients (both cell walls and cell solubles) with an increase in crude protein content as compared to untreated material, besides ensuring more fermentable substrates in the rumen.8 As biological pretreatment suffers from low efficiency, long residence times, considerable loss of the carbohydrates and high enzyme costs, it would be beneficial if the accessibility of enzymes to the underlying cellulose in lignocellulosic biomass could be enhanced. Bioconversion with fungal enzymes is safe with low environmental impact. This employs micro-organisms, mainly white and soft rot fungi, actinomycetes, and bacteria which degrade lignin under mild conditions.

Ruminant enzyme additives are concentrated fermentation products comprised primarily of hemicellulases, cellulases and ligininas (essentially the peroxidases comprised of lignin peroxidase and manganese peroxidase and the laccases) resulting from bacterial or fungal fermentations having specific enzymatic activities. Use of exogenous fiber-degrading enzymes is a potential means of increasing the nutritive value of these straws, as enzyme costs are expected to decline in the future with developments in fermentation technology and alternative enzyme production systems.9 Supplementing ruminant diets with fiber-degrading enzymes has been shown to improve feed utilization and animal performance.9,10 The use of plant cell wall degrading enzymes as direct fed supplements in ruminant diets has
stimulated considerable research effort in recent years. However, results have been inconsistent with many factors appearing to contribute to this variability. The enzyme products most commonly used in animal nutrition are generally mixtures of proteins containing several enzymatic activities. However, most enzyme products are poorly defined, which does little to improve our understanding of their possible modes of action in ruminants.

Enzymes involved in fiber digestion

Plant biomass, consists primarily of lignin, cellulose and hemicelluloses and constitutes the major end product of photosynthetically fixed carbon and thus the major focus of most enzyme-related research for ruminants has been on the enzymes which degrade the plant cell wall. Lignin is a phenylpropanoid polymer synthesized from the phenolic precursors coniferyl, syringyl, and p-coumaryl alcohols and the linkages in it are not subject to enzymatic hydrolysis. This unique structure requiring depolymerization by extracellular oxidative mechanisms accounts for the recalcitrance of lignin toward degradation by most microorganisms. A number of extracellular enzymes capable of cellulolytic, hemicellulolytic and lignolytic activities have been reported. Several of these enzymes may act synergistically in producing high reducing sugars. Most commercially available exogenous fiber-degrading enzyme products consist of cellulases and xylanases, as produced for non-feed applications.

Cellulolytic and hemicellulolytic enzymes: Cellulose and hemicelluloses are major structural polysaccharides are converted to soluble sugars by cellulases and hemicellulases. The types of cellulases and hemicellulases differ substantially among commercial enzyme products depending on the microbial source and substrate utilized for production, and these differences in the purity and specific activities make up the cellulase complex. The major enzymes involved in complete hydrolysis of cellulose are endo-cellulase (endoglucanase, endo-β-1,4-glucanase; E.C. 3.2.1.4), exo-cellulase (exoglucanase, exo-β-1,4-glucanase, cellulase β-1,4-celllobiosidase; E.C. 3.2.1.91), and β-glucosidase (celllobiose or glucohydrolase, E.C. 3.2.1.21). The endoglucanases hydrolyze cellulose chains at random to produce cellulose oligomers and the redox potential of the Mn peroxidase system is lower than reactive and complex with chelating organic acid, as oxalate or malate for ruminants, having been used on a wide range of crop residues. These enzymes have elicited enormous delignification potential primarily by peroxidase and phenoloxidase enzymes known as lignin-modifying enzymes (LME’s). They produce highly reactive radicals which oxidize both the phenolic and non-phenolic lignin components which are of great significance to the agricultural community (Figure 1). Laccases (benzenediol: oxygen oxidoreductase EC 1.10.3.2) belong to multicomponent oxidase family and these copper-containing enzymes catalyze the oxidation of various substrates with the simultaneous reduction of molecular oxygen to water. The catalytic site of laccase is quite conserved among different species of fungi, but the rest of the enzyme structure shows high diversity. Fungal laccases are mostly inducible, extracellular, monomeric glycoproteins with carbohydrate contents of 10-20% which may contribute to the high stability of laccases. Laccases are usually the first ligninolytic enzymes secreted to the surrounding media by the fungus that normally oxidizes only those lignin model compounds with a free phenolic group, forming phenoxy radicals as the mediators that are a group of low molecular-weight organic compounds all fungi that have been examined so far produce more than one isof orm of laccase. Lignin peroxidases (EC 1.11.1.14) belong to the family of oxidoreductases were first described in the basidiomycete Phanerochaete chrysosporium (Burdsall) and the enzyme has been recorded for several species of white-rot basidiomycetes. Lignin peroxidases (LiP) is an extracellular hemeprotein, dependent of H2O2, with an unusually high redox potential and low optimum pH and is capable of oxidizing a variety of reducing substrates including polymeric substrates. It has the distinction of being able to oxidize methoxylated aromatic rings without a free phenolic group, generating cation radicals that can react further by a variety of pathways, including ring opening, demethylation, and phenol dimerization and in contrast with laccases does not require mediators to degrade high redox-potential compounds but it needs hydrogen peroxide to initiate the catalysis.

Manganese peroxidases (EC 1.11.1.13) belong to the family of oxidoreductases and MnP secreted from Phanerochaete chrysosporium was found as another lignin degrading is distributed in almost all white-rot fungi. Manganese peroxidases (MnP) seem to be more widespread among white rot fungi than lignin peroxidase. Manganese peroxidase (MnP) oxides Mn3+ to Mn4+, which oxides phenolic structures to phenoxy radicals. The product Mn4+ is highly reactive and complex with chelating organic acid, as oxalate or malate and the redox potential of the Mn peroxidase system is lower than that of lignin peroxidase and it has shown capacity for preferable oxidize in vitro phenolic substrates. Among others enzymes Versatile peroxidases (VP’s) are hybrids of lignin peroxidase and manganese peroxidase with a bi functional characteristic. They have high affinity for Mn3+, hydroquinones and dyes and oxidise veratryl alcohol, dimethoxybenzene and lignin dimmers. It has the ability to oxidise both Mn2+ and aromatic compounds. Aroyl-alcohol oxidase (EC 1.1.3.7), glyoxal oxidase (EC 1.1.3.3), and various carbohydrate oxidases (EC 1.1.3.4, 9, 10) are also involved in natural lignocellulose degradation. These enzymes, belonging to LDA1–6 families, can generate H2O2 from O2, with concomitant oxidation of aromatic alcohol, glyoxal, or reducing carbohydrates. Various genus of white-rot fungi like Pleurotus, Phlebia, Phanerochaete, Trametes and a few others have unique ability to produce extra cellular lignocellulolytic enzymes including laccase, lignin peroxidase and Mn peroxidase. These enzymes have elicted enormous delignification potential for ruminants, having been used on a wide range of crop residues like wheat, paddy, barley, mustard straws and alfalfa and grass hays. However, the production of these enzymes, or more

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likely mixtures of enzymes, must also be considered in order to develop viable enzymatic lignocellulosic deconstruction technologies.

Figure 1 Schematic representation of degradation of lignin by lignolytic enzymes of white rot fungi.

**Enzyme feed additives for ruminants**

Commercial enzyme preparations comprised primarily of hemicellulases and cellulases currently being used in the livestock feed industry are products of microbial fermentation (bacterial- mostly *Bacillus* spp, or fungal viz. *Trichoderma longibrachiatum*, *Aspergillus niger*, *A. Oryzae*). Lignolytic enzymes of the white rot fungi exclusively meant for ruminant feeding are yet to be commercialized. The types and activity of enzymes produced varies depending on the microbial strain used for fermentation, substrate employed and the culture conditions used. Compared to the harvested media, these enzyme products are relatively concentrated, purified and with high specific activities. A number of the enzymes that have been evaluated in feeding experiments with ruminants were originally meant for non feed applications. The cellulases and xylanases used extensively in food, pulp and paper, textile, fuel, and chemical industries have been employed for ruminant feeds. The fibrolytic enzyme products evaluated as feed additives in ruminant diets also were originally developed as silage additives. Crude fermentation products and some nonbacterial direct-fed microbials (DFM) are also being marketed based on their residual enzyme content. In this case the enzymes, as well as the entire medium, are recovered along with the metabolites and fermentation products. Many nonbacterial DFM consist of *A. Oryzae* fermentation extract, *Saccharomyces cerevisiae* cultures, or both. In comparison to concentrated feed enzyme products, these products contain relatively low enzyme activity but are definitely economically feasible.

Twenty-two commercial enzyme products were examined for biochemical characteristics and for *in vitro* ruminal degradation of alfalfa hay and corn silages. Enzyme treatment alone or in combination with other treatments can increase the degradability of cereal straw by the rumen microorganisms. In addition, using fibrolytic enzymes in ruminant feed have shown improvements in the average daily gain of steers, fleece weight and wool production of lambs and in milk yield of dairy cows. Some studies, using fibrolytic enzymes alone could not significantly increase the degradability of rice straw because the ability of these enzymes to break down the esterified bonds within lignin-carbohydrate complexes may be limited. However, when using in combination with other pre-treatments they could increase degradability and *in vitro* fermentation characteristics, as shown with xylanase or cellulase in combination with ammonia, with cellulase from *Penicillium funiculosum* in combination with steam pre-treatment, and with multi-enzymes (xylanase, β-glucanase, carboxymethylcellulase and amylase) in combination with NaOH. The use of combinations of fibrolytic enzyme with these pre-treatments is expected to have a synergistic effect on the nutritive improvement of straw. An amylolytic enzyme complex produced by fungus *Aspergillus awamori* and a commercial product containing multi-enzyme complex, yeast and MOS evaluated in confined beef cattle were not able to improve animal performance. Although, application of enzymes has proven to increase the feed value of poor quality feedstuffs, its use by smallholder farmers is, for the time being, economically unattractive. Especially, the use of lignin-degrading enzymes, originating from white rot fungi and which selectively target lignin seems a promising development.

**Activity and level of enzyme employed as feed additive**

The discrepancies in results in the use of exogenous enzyme products in ruminant diets could possibly be due to supplementation with either insufficient or excessive enzyme activity. *In vivo* responses
to enzyme addition are mostly nonlinear and it is always possible to over-supplement. Although enzyme activity units are important for quality control these activity units bear little very little or no relationship to the efficacy of the product as a ruminant feed additive. 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 indicates an insufficient supply of enzyme activity. When excess enzyme is applied, the beneficial disruption of the feed surface structure is diminished because the excess exogenous enzyme attached to feed may restrict microbial attachment and limit digestion of feed. However as on date there is no minimal level of enzyme activity prescribed for products to be registered as feed enzymes. In evaluating the effect of different doses of three exogenous lignolytic enzymes - laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) obtained from immobilized Pleurotus flabellatus, Poria placenta and Coriolus versicolor (Polystictus versicolor) on the nutritional profile and in vitro digestibility of ragi straw, enzyme treatment at a ratio of 2:5, rather than supplementation was observed to be more beneficial in all the fungi tested.39

**Enzyme specificity**

The array of enzyme activities required to improve fiber digestion varies depending on the composition of the feed. Enzyme specificity towards each feed poses a major constraint in formulating new ruminant feed enzyme products as most commercial ruminant diets contain a mixture of several forages and concentrates. Thus to obtain maximum benefit, a number of different enzymes need to be used in a typical ruminant diet. The most feasible approach would be to use an enzyme that is not ideal for all diets, but is relatively suitable for most feeds. This principle has been adopted in the development of enzyme products for ruminants. Because of the relatively high cost of feed enzymes compared to other technologies, livestock producers expect an equally high response in animal productivity and in so in future a more targeted approach where in feed enzyme products are formulated for various types of feeds would be necessary. This “designer enzyme” type approach is definitely complex but is the only way to ensure the feasibility of the feed enzyme technology for ruminant nutrition.

**Mode of enzyme administration**

Application of enzymes in a liquid form onto feeds prior to consumption has been reported to have a positive effect on animal performance while infusion of enzymes (oral dosing) into the rumen has not been very effective. Enzyme treatment comprised of spraying enzymes to feed enhances the binding with substrate, which increases the specific substrates. The greater the proportion of the diet treated within the rumen. In the rumen, the close association between digestive bacteria and feed particles concentrates digestive enzymes close to their specific substrates. The greater the proportion of the diet treated with enzymes, the greater the chances that enzymes will endure the rumen. Without this stable feed-enzyme complex, the enzymes are prone to solubilization in the ruminal fluid and flow out rapidly from the rumen. It is likely that a major portion of the positive production responses resulting from the application 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, stimulation of rumen microbial populations and synergistic effects with hydrolyses of ruminal microorganisms. The net effect gives rise to 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 and explains why lignolytic enzymes are effective when added to the concentrate portion of a diet.
Conclusion
Though the ruminant gut is equipped with a sophisticated microbial community for attacking lignocellulosic substrates, their digestion in the rumen is still very low. Positive responses in growth rate and milk production obtained in cattle fed exogenous enzymes, have been inconsistent. Some of the variation could 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 animal. Research emphasis should be directed to understand the mode of action of these products to obtain superior on farm efficacy in performance. Investigations to quantify the mode of action of these enzymes would enhance the digestion of ‘lignocellulosic biomass’ resulting in production of nutritionally improved feeds and feed ingredients for ruminants. Adoption of the ideal product formulations, their application methods and quantities, would yield favorable and economically viable results; Application of lignolytic enzymes as feed supplements holds immense promise for the ruminant feed industry towards enhancing productivity.

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Conflict of interest
Author declares that there is no conflict of interest.

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