

Review Article

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A Review on Newer Trends in the Use of Exogenous Fibrolytic Enzymes Use in the Ruminants

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ABSTRACT

Continuous efforts are being made to utilize the fibre feeds to the maximum extent in ruminants which form a staple feed in tropical countries. Though only about 65% of the fibre is effectively digested in the rumen, need to supplement certain exogenous fibrolytic enzymes (EFE) has become mandatory. The main principle with which EFE is added is to specifically target the increased bacterial multiplication of certain species which enhances rumen degradation of cellulose which constitutes the major part of the forages. Effectiveness of enzymes in animal nutrition depends on (i) type, (ii) source, (iii) level of supplemented enzymes, (iv) the type of diet fed, (v) animal health and (vi) animal productivity. Exogenous enzymes are classified into cellulolytic, amylolytic, proteolytic and others based on the type of substrate on which they act. The mode of application of EFE, incubation with the substrate is also crucial. Solubilized enzymes incubated with the substrate is more effective since the enzymes starts acting on the substrate forming a stable complex resistant to endogenous enzymes even before entering into the rumen. Supplementation of White-rot fungi in recent times improved the digestibility of the so called tough lignocellulosic bonded substrates fed to ruminants. Supplementation of EFE to silage can hydrolyze plant cell walls into fermentable substrates through enhanced homolactic fermentation and thus improves digestion. It was reported that EFE works in three ways - 1) improved feed conversion efficiency 2) release of entrapped nutrients from the cell wall portions of diets and 3) release of oligosaccharides which support beneficial microflora while suppressing pathogenic bacteria. It was known that the microbial mass in the rumen meets 80 to 90% energy and 60 to 70% protein needs in sheep for which efficient utilization of the fibrous crop residues is mandatory for which the use of EFE is required. However, animal responses to the enzyme supplementation are inconsistent and are possibly be attributed to four main factors: enzyme characteristics (differences in enzyme preparations, enzyme activity, units of activity added, pH and temperature effect on activity), forage (type, maturity), animal (species, age) and management (diet, mode of enzyme application, application rate, interaction time of enzymes applied to feed). It can be concluded that enzyme action is species specific, substrate specific, dosage specific, application mode specific, incubation period specific, source specific and hence long term trials are further suggested for not having consistent results and there is a great need to develop certain of the enzymes to more thermo stable and can resist the rumen pH and temperature. Since the any forage has three different digestible fractions viz- digestible, potentially digestible, and indigestible and the action of EFE which may be of any origin depends upon the extent of the digestibility of the potentially digestible fraction and this determines the magnitude of the response of the EFE towards forages.

Keywords

EFE, Forages,
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Introduction

Over the years, many methodologies have been brought to light in order to efficiently utilize the forages in ruminants by the use of different chemicals such as sodium hydroxide, hydrogen peroxide, anhydrous ammonia and urea. Owing a threat to the animals in the long use of these methods at farmers level, these methods were not popularized. As an alternate to these chemicals, Exogenous Fibrolytic Enzyme (EFE) supplementation was found to an effective way to improve the energy availability for ruminants. Use of EFE in the ruminant diet has been reported to improve the fiber digestibility and their use was popularized because of the positive effect on animal health and performance.

In tropical countries like India, ruminant production systems are based on crop residues and more of agricultural by-products rich in fibrous material which is important for salivation, rumen buffering and efficient production of rumen end products. However, to a maximum of 65 % fibrous material in the feed stuffs are efficiently digested (Van Soest, 1994) and the rest of the portion needs to be utilized to the full to follow economy. Forage digestibility has been improved by several biotechnological products like fibrolytic enzymes, ionophores, direct fed microbial products and certain fungi like *Pleurotus* which can degrade lignin. In recent times, addition of EFE to the feed stuff has shown promising results by hydrolysis of plant cell walls (Beauchemin *et al.*, 2004).

Fibre is a major source of energy for ruminants (Avellaneda *et al.*, 2009) because cellulose, one of the main components, is the most abundant biopolymer on Earth (Paloheimo *et al.*, 2010). This fibre available to the animal, is of low quality and the energy present in the bound form in the fibre cell

wall is unutilized to the animal, leading to a large excretion of nutrients and incomplete use of fractions of the cell wall in the rumen due to the complex links which limit the degradation of nutritional compounds. Here comes the use of EFE produced from fungal (largely *Trichoderma longibrachiatum*, *Aspergillus niger*, and *A. oryzae*) and bacterial (*Bacillus* spp., *Penicillium funiculosum*) sources with high cellulosic and hemicellulosic activity. EFE can be incorporated in liquid or granular form with the total mixed ration, hay, silages, concentrates, supplements or premix, and increase the availability of nutrients in the cell wall.

Exogenous enzymes have been used to improve nutrient utilization in several species of livestock, particularly non-ruminants. In comparison, the use of exogenous enzymes in ruminant diets is restricted because it is hypothesized that the hydrolytic capacity of the rumen could not be enhanced by enzyme supplementation and concerns that such enzymes would be ineffective due to ruminal hydrolysis (Adesogan, 2005). Based on the work mainly in non-ruminant animals, efficacy of exogenous enzyme supplementation has been reported to work in three ways: 1) improved feed conversion efficiency, 2) release of nutrients trapped in the cell wall portions of diets and 3) release of oligosaccharides which support beneficial microflora while suppressing pathogenic bacteria (Paloheimo *et al.*, 2010).

Enzymes in cattle

Classes of exogenous enzymes

Exogenous enzymes supplemented to animal diets include amylolytic enzymes, proteolytic enzymes, β -glucanase, xylanase, Cellulase and β -mannanase. These enzymes are expected to increase the digestion and thus utilization of dietary starch, protein, β -

glucans, arabinoxylans, and mannan, respectively. Here we describe briefly amylolytic, proteolytic and fibrolytic enzymes supplemented to diets for ruminants.

Amylolytic enzymes

Amylolytic enzymes, also called as starch degrading enzymes are often fed with cereal grains such as corn and barley. These mainly act on glycosidic bonds and release more than 600 metabolizable glucose units (Pérez *et al.*, 2009) and makes a very good energy source. Rojo *et al.*, (2007) reported that the amylolytic enzyme α -amylase was more effective in increased starch digestion in ruminally cannulated beef steers. Alternatively, Tricarico *et al.*, (2008) reported modified rumen fermentation products. Tricarico *et al.*, (2005) found increased proportions of acetate and propionate in steers and lactating dairy cattle supplemented with exogenous α -amylase. However contradictory results were also reported by some workers which are attributed to several factors including enzyme dose, type of feed, host and management factors (Mendoza *et al.*, 2014).

Proteolytic enzymes

These enzymes degrade the feed proteins in the rumen and release amino acids which are rightly utilized by the rumen microbes to synthesize the microbial protein. These enzymes also release the fibre cell bound protein. Though cell bound protein accounts to only 7-11% of the total cell wall content, its contribution to the overall protein digestibility is recognized by increased organic matter rumen fermentation.

Exogenous fibrolytic enzymes

Since most of the diet for ruminants includes carbohydrates, soluble carbohydrates are of

no problem for hydrolysis but the issue is with non-starch polysaccharides in plant cell walls. Fibrolytic enzymes hydrolyze non-starch polysaccharides in plant cell walls by targeting the main constituents including cellulose and hemicellulose. Hemicelluloses also have a substantial role in maintaining cell wall structure in plants and accounts for about one fourth of the total plant biomass (Scheller and Ulvskov, 2010). The most common types of fibrolytic enzymes that break down the structural carbohydrates in the rumen are xylanase, β -glucanase and β -mannanase for hemi-cellulose and cellulase for cellulose. Many authors say that instead of adding a single fibrolytic enzyme, mixture of enzymes in the form of enzyme cocktail improved (Salem *et al.*, 2013) digestibility and rumen fermentation in beef steers. The effectiveness of supplementing ruminant diets with exogenous enzymes has been highly variable. This is likely due to variability in type of enzyme used, species, physiological status, rumen pH, gastrointestinal conditions, enzyme dosage and nutrient composition of diets (Mendoza *et al.*, 2014). Seo *et al.*, (2016) reported increased average daily gain in growing beef heifers with dietary supplementation of β -mannanase. Tewoldebrhan *et al.*, (2017) reported increased milk yield per unit of dry matter intake and milk protein yield per kilogram of crude protein intake with β -mannanase supplementation in a corn silage and alfalfa hay-based diet fed to mid-lactating Holstein dairy cows.

Since Cellulose forms the major structural carbohydrate and also a major source of feed for ruminants, there is every need to discuss more about this. Cellulase is an enzyme produced chiefly by fungi, bacteria and protozoans and it can catalyze cellulolysis, the decomposition of cellulose and of some related polysaccharides. Cellulases break down the cellulose molecule into

monosaccharides (simple sugars) such as beta-glucose or shorter polysaccharides and oligosaccharides. Most mammals have only very limited ability to digest dietary fibres like cellulose by themselves. In many herbivorous animals such as ruminants like cattle and sheep and hindgut fermenters like horses, cellulases are produced by symbiotic bacteria.

EFE mainly consists of Xylanase, beta-glucanase and Cellulase and were evaluated for their potential to improve diet utilization and dairy cow performance and found the improvement in digestibility (Yang *et al.*, 2000) but not others (Lewis *et al.*, 1999). Supplementation has increased or tended to increase milk production in some studies (Kung *et al.*, 2002), but not in others. These discrepancies are likely due to differences in enzyme activity and composition, stage of lactation of cows, physiological stage of the animals and method of enzyme addition.

The addition of EFE can be in different forms Viz- solid and liquid forms. The EFE can also be given by direct infusion into the rumen or abomasum but the results were not prominent (Hristov *et al.*, 1998) as compared to the EFE addition by solid or liquid forms to the dietary forage (Lewis *et al.*, 1999) or concentrate (Rode *et al.*, 1999) indicating a close relation between the fibre substrate and the enzyme for fiber hydrolysis (Beauchemin *et al.*, 1999). Addition of EFE to forages are said to be more effective than adding to the concentrates. Exogenous enzymes should also be more effective when applied to high-moisture feeds such as silages than to dry feeds because of the importance of water for enzymatic cell wall hydrolysis (Beauchemin *et al.*, 1999).

Yang *et al.*, (2000) has reported that EFE application to concentrates were more effective than for TMR. It was also reported that the efficacy of EFE is positively

correlated to the composition of the diet (Bowman *et al.*, 2002). Supplementation with (EFE) is thought to enhance ruminal fermentation and to increase the degradability of forage cell walls, potentially reducing feed costs and sustaining the productive performance of ruminants; however, the underlying interactions are unknown and the effects of using EFE are highly variable.

Within the rumen, EFE hydrolyse cell wall components and produce substrates that favour the multiplication of certain specific microbes even with low-forage diets (Bedford and Cowieson 2012). In the ruminal environment, EFE can affect bacterial attachment and colonization and microbial populations (Colombatto and Mould *et al.*, 2003; Wang *et al.*, 2012), affecting *in vitro* (Srinivas *et al.*, 2008), *in situ* (Tirado-Estrada *et al.*, 2015) and *in vivo* NDF digestibility.

There are reports saying contradictory results on the growth performance on adding EFE. The reasons are attributed to many factors like method of application of EFE viz-in liquid form or in solid form, dose of enzyme, contact time of the enzyme with the substrate, type of substrate, presence of strong ligno-cellulosic bonds etc. The problem appears when fibre high in ligno-cellulose diets are fed. However, when using high ligno cellulolytic ingredients (agricultural by products), the results have been variable and inconsistent in terms of fibre digestibility (Gallardo *et al.*, 2010) because the potential of exogenous enzymes depends mainly on the dose and type of substrate. So it is possible that lower quality forages require higher doses of exogenous fibrolytic enzymes in order to show benefits such as those observed with higher quality forage (Eun *et al.*, 2008 a).

Supplementation of enzymes in solubilized form recorded higher digestibility coefficients for CF, NDF, ADF, cellulose and

hemicellulose which was due to the pre-treatment of dry feeds with enzyme in liquid form that forms stable feed-enzyme complex (Pradeep Kumar Reddy *et al.*, 2018) before entering the rumen where the action of the enzyme starts on the substrate. Suryanarayana and Ramana (2015) conducted a trial on 100 ram lambs by applying the EFE in both solubilized and dry forms and concluded that the FCR was lower ($P < 0.05$) by 21%, 26% and 13% for the solubilized enzyme supplemented group as compared to control and other groups. Improvement in ADF and NDF ($P < 0.001$) digestibility was noticed (Mc Allister *et al.*, 2000) in lambs supplemented with Cellulase and Xylanase for forage diets containing barley straw, wheat straw and alfalfa hay. Similar trend ($P < 0.05$) in digestibility of DM, OM, CF, NDF and ADF was noticed for lambs fed with TMR supplemented with Cellulase (Titi and Tabbaa, 2004). While applying the enzymes care needs to be taken to have uniform distribution of enzymes since these are added at relatively low levels. Enzyme application at ensiling is a classical example because uniform distribution throughout the forage can be ensured when enzymes are applied using properly calibrated sprayers.

Supplementation of EFE while ensiling can hydrolyze plant cell walls into fermentable substrates that improve digestibility through enhanced homolactic fermentation. Reporters say that addition of fibrolytic enzymes at ensiling has improved the fermentation and nutritive value of corn silage and Bermuda grass silage.

Enzymes in sheep

In a developing country like India, where around 18 per cent of the gross domestic product (GDP) is obtained from agriculture, the livestock sector plays an important role in the national economy (4.36% of the GDP).

Among the livestock, sheep as a small ruminant has a great potential to provide gainful employment and nutrition round the year to the small, marginal and landless farmers besides providing surplus marketable products (Economic survey of India, 2008-09).

In sheep, scarcity of green fodder and high cost of conventional feed ingredients have made the researchers to improve the feeding value of poor quality crop residues. In India, due to continuous depletion of grazing lands and scarcity of feeds and fodder, farmers are forced to feed the crop residues which are poor in nutritive value owing to their low energy, low nitrogen, low minerals, high fibre and high acid insoluble ash content that are available in large quantities after harvesting the crops.

The efficiency of utilization of low quality crop residues and roughages by sheep for growth purpose could be altered by a higher rate and extent of digestion in rumen and better availability of microbial nutrients in the feed to support an efficient microbial growth, which could meet 80 to 90% energy and 60 to 70% protein needs in sheep.

To improve the consistency of the results, it is important to know the interaction within the ruminal ecosystem, the cell walls of plants and the type of EFE (Meale *et al.*, 2014).

Many researchers reported positive effects of using EFE as supplements for ruminants on dry matter and neutral detergent fibre *in vivo* digestibility (DMD and NDFD) (Arriola *et al.*, 2011) total volatile fatty acids, propionic acid proportion, acetate: propionate ratio, dry matter intake (DMI) (Chung *et al.*, 2012), milk production (Kholif and Aziz 2014) and average daily gain (ADG) (Tirado-Estrada *et al.*, 2011).

Factors on which the enzymes activity depends

The activity of the EFE on a feed depends on various factors like source of supply of the enzymes (bacterial/fungal origin), type of substrate, mode of application of EFE (solid/liquid), type and stability of the enzyme, pH, temperature and the other conditions in the rumen, dose of enzymes, presence of certain enzyme inhibitors in the gut, stability conditions of EFE and the type of livestock species (ruminants/non-ruminants). Marquez *et al.*, (2009) has stated that the activities of Xylanase and Cellulase produced from *Trametes* species is 5 & 7 times and 10 & 8 more active as compared to these produced from *Asperigillus* and *Pleurotus* species, respectively.

In conclusion, the use of EFE can be promoted in ruminant production systems by developing these at cheaper rates. In future it becomes inevitable to use these enzymes in TMR. Animal responses were inconsistent to the addition of enzymes and the reasons are multifactorial and can possibly be attributed to four main factors: enzyme characteristics (differences in enzyme preparations, enzyme activity, units of activity added, pH and temperature effect on activity), forage (type, maturity), animal (species, age) and management (diet, mode of enzyme application, application rate, interaction time of enzymes applied to feed). A thorough knowledge on the potentially degradable fibre fractions and the digestion kinetics need to be known. Addition of EFE to the fibre diets may not be a tough task but this small intervention leads to the efficient utilization of nutrients and a healthy eco balance of bacteria in the rumen. However newer fibrolytic enzymes need to be prepared that can be thermo stable and can resist a pH of 6 to 6.8 and a temperature of 39^o C simulating the rumen conditions. While supplementing

these enzymes, factors like mode of application, substrate nature, dosage, period of incubation, source of supply etc have to be taken into consideration.

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