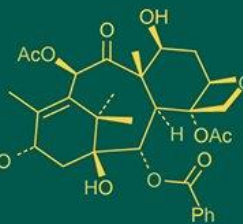
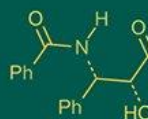


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Optimization of small ruminant production with fibrolytic enzymes: A review

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Abstract

Reshaping the small ruminant sector for a sustainable future involves optimizing production systems to be both economically viable and ecofriendly. This includes adopting sustainable practices like integrated crop-livestock systems, rotational grazing and agroforestry, along with improving animal feed and breeding management. Small ruminants provide a sustainable livelihood to many of the farmers in rural areas as they don't need special investment for establishment. Fibrous feeds form the staple feed for small ruminants and these are to be digested optimally for economic reasons. Fibrolytic enzymes added externally (Exogenous Fibrolytic Enzymes: EFE) along with the feed is a potential alternate to obtain maximum production with fibrous feeds. One advantage of using fibrolytic enzymes is that they are produced from the bacteria which grow well on low quality roughages and crop residues. The potentiality of these fibrolytic enzymes differ in their activities depending upon their source of production from bacteria. The fibrolytic enzymes was reported to act in 3 ways by improving feed conversion efficiency, by releasing the entrapped nutrients from the cell wall portions of diets and by releasing the oligosaccharides which act as a feed resource for beneficial microflora. The mode of application of these fibrolytic enzymes also plays a major role in improving the production. Some authors reported that EFE applied in solubilized form and fed to the lambs after incubation, recorded higher growth rate and improved nutrient digestibility of certain nutrients. They are said to improve the digestibility of nutrients, improves the dry matter intake in addition to improving the feed conversion ratio. When enzyme cocktail containing amylase, protease, cellulase and xylanase were supplemented to goats, it influences the rumen microbial populations. Fibrolytic enzymes lower the acetate to propionate ratio which is associated with improved feed efficiency and energy utilization, as propionate is a more efficient precursor for glucose synthesis in ruminants. However, the action of these enzymes depends on many factors like source of supply of the enzymes (bacterial/fungal), type of substrate, mode of application of EFE (solid/solubilized), type and stability of the enzyme, p H, temperature and 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. It is concluded in the present study that supplementation of fibrolytic enzymes becomes a potential source of nutrient utilization, improving the productivity, alter the rumen fermentation delivering the maximum energy out output from the feed stuffs to the livestock.

Keywords: Fibrolytic enzymes, performance, cell bound nutrients, application mode

Introduction

Small ruminant rearing plays an important role in livelihood of small, marginal farmers especially in hilly regions of India, where crop farming is difficult. Sheep in India are mostly maintained on natural vegetation. There are about 40 recognized breeds of sheep in India. The present sheep population in India is about 55 million. In spite of wool and meat, sheep skins and manure are also obtained. Hence to obtain more wool from sheep, care should be taken regarding their balanced feeding on scientific lines.

The role of small ruminant production in achieving sustainable and resilient food systems in low-and middle-income countries (LMICs) due to many factors is neither fully understood nor incorporated into current agro ecological practices. The small ruminant production should be integrated into the current agro ecological conditions for which eight principles have to be considered viz: input reduction, animal health, soil health, biodiversity, recycling, synergy, economic diversification and co-creation of knowledge. It is important to note that all these practices cannot be implemented in isolation and should be integrated taking into account the social, cultural and economic and other related factors.

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In tropical countries like India, ruminant production systems are based on fibrous rich crop residues and more of agricultural by-products which is important for salivation, rumen buffering and efficient production of rumen end products. This process also controls the rumen acidosis. It was reported (Van Soest, 1994) ^[25] that to a maximum of 65% fibrous material in the feed stuffs are efficiently digested and the rest of the portion to be utilized for optimum production. Forage digestibility can be improved by simple physical methods like soaking to other methods like supplementation of enzymes, treatment with fungi like *Pleurotus* which can degrade lignin.

Plants do not have a skeleton to support themselves and so the cells are bound together by bonds. Plant cell walls are composed of cellulose (35-50%), hemicellulose (20-35%) and lignin (10-15%) and lignification varies as the age of the plant advances (Paloheimo *et al.*, 2010) ^[14]. Vertebrates lack the ability to hydrolyse the beta ^[1-4] glycosidic bond of plant cellulose due to the lack of the enzyme *cellulase*. Thus, ruminants must completely depend on the microbial flora, present in the rumen or hindgut, to digest cellulose

Furthermore, the use of exogenous fibrolytic enzymes are considered a sustainable strategy to improve the degradability of dietary fibre because these enzymes are produced by micro-organisms, which can grow on low-quality substrates and agro-industrial wastes (Sakita *et al.*, 2020; Jabri *et al.*, 2022) ^[6, 8]. Another benefit of using these enzymes is that since they are produced from the micro organisms they can well adjust to the ruminal conditions and we can expect a better synergism between the rumen microbes and the enzymes.

As said earlier, exogenous enzymes are concentrated enzyme extracts obtained either by fungal (*Aspergillus oryzae*) or bacterial (*Bacillus subtilis*) fermentation. Exogenous enzymes mainly act by a process called catalysis due to their high degree of affinity for a particular substrate and site of action (Gurung, Ray, & Rai, 2013) ^[7], transforming macromolecules into simpler substances. For example exogenous enzymes, such as cellulases act on cellulose, xylanases on xylose and proteases on cell bound proteins and so these enzymes are specific in their action on substrates.

Exogenous Fibrolytic Enzymes (EFE) are generally considered as feed additives which improve the nutrient utilization in the host and improves the rumen fermentation and microbial protein synthesis. (Thammiah *et al.*, 2017) ^[22]. 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 (Suryanarayana, 2020) ^[17]. These fibrolytic enzymes not only digest the fibre particles of the feed particles but also releases the cell bound proteins entrapped in the cell wall layers (Suryanarayana and Ramana, 2016; Tirado-González *et al.*, 2021) ^[20, 13]. 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. The most common types of fibrolytic enzymes that break down the structural carbohydrates in the rumen are xylanase, β -glucanase and β -mannanase for hemicellulose

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) ^[24] digestibility and rumen fermentation in beef steers and this could be due to the associated effects of the se mixed enzymes. The effectiveness of supplementing ruminant diets with exogenous enzymes has been highly variable based on the factors mentioned here under in the text.

The activity of the EFE on a feed depends on various factors like source of supply of the enzymes (bacterial/fungal), type of substrate, mode of application of EFE (solid/solubilized), type and stability of the enzyme, p H, temperature and 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 and so the contradicted results were reported by various authors. Marquez *et al.*, (2009) ^[13] has stated that the activities of xylanase and cellulase produced from *Trametes* species is 5 and 7 times and 10 and 8 times more active as compared to these produced from *Aspergillus* and *Pleurotus* species, respectively.

Ruminants consume diets containing relatively high amounts of forage, which contains cell wall fractions containing complex compounds such as β -mannans (Roque *et al.* 2019) ^[16]. They are typically known for their structural resistance to solubility, leading to high viscosity in various feeds and become anti-nutritional properties in animal diets. The digestion of plant cell walls in fibrous feeds by ruminants is done mainly because of the enzymes produced by ruminal bacteria, protozoa, and fungi.

The fibre matrix and nutrients entrapped in fibre usually bypass digestion limiting the nutrient digestion and absorption (Sauvant *et al.*, 2004) ^[19]. They also reduce protein digestion by entrapping amino acids which make them unavailable for the digestion and absorption which are otherwise called as *cell bound proteins*. One advantage of this release of these nutrients is that the soluble sugars present in the cell bound nutrients act as an energy source for the fibrolytic bacterial present in the rumen and thus their population multiples giving a scope for more fibre attack.

According to Sujani and Seresinhe (2015) ^[26], research on the use of exogenous enzymes in ruminant feed has taken place since the 1960 s. Due to the high cost of production and less awareness on their use there has been little development in this area. With technological advances since last decade, it has become possible to reduce the cost of production of exogenous enzymes, increasing the amount of these products on the market, and calling researchers' attention to further studies on the form of use (e.g., in concentrate, TMR), form of application of these EFE and their inclusion levels.

Supplementation of exogenous enzymes helps to improve the digestibility and ruminal degradability of fibre, starch and protein, animal performance and reduce feed costs. The benefits of supplementation on intake, digestibility, efficient rumen fermentation and performance are contradictory because the benefits seen with EFE are either from fibrolytic, amylolytic or proteolytic enzymes and so it was hypothesized that the positive results are due to the cumulative effects of all the EFE put together (Beauchemin and Holtshausen, 2011) ^[1]

In cows, it has been reported that the use of EFE improves the energy status by reducing plasma concentrations of β

hydroxybutyrate, indicating reduction in the mobilization of fat from adipose tissue during lactation (Dean *et al.*, 2013)^[4]. There is evidence that exogenous enzymes increase microbial protein synthesis, an indicator that the bacterial population of the rumen is increased (Elwakeel *et al* 2007)^[5].

Substrate conditions affect the action of exogenous enzymes. Exogenous enzymes act more effectively in wet feed than in dry feed because water facilitates the dissemination of the enzymes and is essential for hydrolyzing the polymers to release the monomers (Suryanarayana and Ramana, 2016)^[20]

An important point to be remembered on the use of exogenous enzymes in ruminant feeding is the potential to reduce the grain level in the ration reducing costs as these enzymes are aimed to improve the fibre digestion in TMR. Economic losses will be more if the investment in enzymes is not compensated by an increase in production and lowers the cost of production because if the diet does not contain enough fibre, it limits the effect of the enzyme. (Mendoza *et al* 2013)^[10]

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 act as a feed resource for beneficial microflora and when these populations increase the pathogenic bacteria are suppressed. 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 and to achieve this the use of EFE is required. (Suryanarayana, 2020)^[17]

Mode of application of EFE

Fibrolitic enzymes cause partial solubility of cell wall components and reduce sugars, which act as a ready source of energy for microbes (Mc Allister *et al.*, 2001). During the release of soluble sugars, the rumen bacteria increase the thickness of glycocalyx barrier, thereby improves the adhesion among the bacteria and between the bacteria and substrate. Many researchers have shown positive effects of incubating probiotic and EFE with substrate (Suryanarayana and Ramana, 2016; Lopez-Aguirre *et al.* 2016)^[17, 20].

Smerjai Bureenok *et al* (2024) in their study with goats fed with *Leucaena* silage supplemented with EFE cocktail have reported an increased DMI with an enhanced NDF digestibility and they justified the reason as the enzymes can break down hemicellulose-cellulose, making cellulose more accessible. In addition, a greater NDF digestibility results in a more rapid disappearance of NDF from the rumen, thereby reducing the physical fill of the rumen, and therefore allowing a greater voluntary feed intake. They also reported an increased CP digestibility if the enzymes cocktail contains proteases, as xylanases and cellulases act in combination with proteases to attack nitrogen-containing compounds in cell walls. Enhancing the dose of EFE in feed is also not advisable as low levels of exogenous enzymes stimulate the adhesion of rumen microbes to substrate, while high levels of exogenous enzymes may compete with the enzymes produced by the rumen population for binding sites on feed hindering the effective fibre digestion (Daniel *et al.*, 2026)

Rumen Microbes

When enzyme cocktail containing amylase, protease, cellulase and xylanase were supplemented to goats, it

influences the rumen microbial populations (Takizawa *et al*, 2022)^[23]. *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Euryarchaeota* were found to be the dominant phyla of the rumen microbiome of the goats. (Takizawa *et al.*, 2022)^[23].

VFA Production

EFE also influences the volatile fatty acid ratio and was reported by Sutton *et al* (2019)^[18] in dairy cows as a significant change in the acetate-to-propionate ratio. This ratio is an important indicator of the balance between fibre and starch fermentation in the rumen. A lower acetate to propionate ratio is often associated with improved feed efficiency and energy utilization, as propionate is a more efficient precursor for glucose synthesis. Acetate is primarily produced by the fermentation of fibrous carbohydrates, whereas propionate is mainly derived from the fermentation of non-fibrous carbohydrates or soluble carbohydrates. (Kim *et al*, 2021)^[23]

On growth rate

Supplementation of exogenous fibrolytic enzymes (cellulase and xylanase @ 8000 and 16,000 IU/kg TMR DM; and 12,000 and 24,000 IU/kg TMR DM) increased voluntary intake (DM, OM, and CP) and availability of different nutrients (TDN, DCP, digestible NDF, digestible TCHO, DE, and ME) in Black Bengal kids. But, the blood metabolites (glucose, albumin, globulin) and serum enzymes (ALT and AST) remained unaffected (Manoj Kumar *et al.*, 2023) due to EFE supplementation. The growth rate and feed conversion efficiency were improved

It was reported (Pradeep Kumar Reddy *et al.*, 2018) that EFE added to TMR can promote the growth by increasing nutrient utilization and reducing the cost of production. EFE applied in solubilized form and fed to the lambs after incubation recorded higher growth rate and improved nutrient digestibility of certain nutrients. The authors say that the EFE applied in solubilized form was effective in nutrient utilization and performance in ram lambs

Conclusions

Enzymes called cellulases and xylanases release soluble sugars by dissolving the bonds in cellulose and hemicellulose (Tirado-González *et al.*, 2021)^[13]. These enzymes break down the components of cell walls to provide substrates that benefit particular populations of rumen microbes (Salem *et al.*, 2013)^[24]. Improvements in ruminal fermentation, fiber and DM degradability, and microbial protein synthesis can be achieved by supplementing EFE, which can improve the energy output from fibrous feeds. But the positive effects of these enzymes are in consistent due to various factors mention in the text.

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