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Use of exogenous fibrolytic enzymes as feed additive in ruminants: A review

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Abstract

The use of exogenous enzyme in ruminants is still the matter of debate among the researchers and the facts associated with enzyme feeding need to unravel. Ruminants were fed on low quality roughage having limited digestibility. Over period of interventions different approaches were developed to enhance digestibility by chemical, physical and biological methods. Exogenous enzymes were used to degrade of complex fibrous feed material and improve nutrient utilisation and production responses. Although ruminants have capability to degrade fibrous feedstuff due to microbial enzyme activity, but the structural polysaccharides such as cellulose, hemicellulose and lignin will be degraded up to some extent. Exogenous enzymes were used for specific substrate degradation, improve the efficiency of feed utilisation as well as to accelerate the decomposition and reduce the wastage production. Substantially exogenous enzyme improves nutritive value of feed can eliminate common anti nutritional factors present in unconventional feed resources. The information regarding source of enzyme, their activity and doses for animal feeding is reviewed. An attempt is made to explain mode of action and understanding functioning of exogenous enzymes in animal feeding system. This study was aim to redefine application of exogenous fibrolytic enzyme in animals feeding and related response on growth performance and milk production. Whether the use exogenous enzyme will profitable in dairy business and what will be the return of investment.

Keywords: Exogenous fibrolytic enzymes, milk production, growth performance, animals

Introduction

Indian farmer have scare or limited resources for fodder crops, dairy animals are mostly fed on poor quality fodder, native grasses and concentrate. Quality of roughage mainly attribute by digestibility and nutritive value. At present the country faces the deficit of 35.6% of green fodder, 10.95% of dry crop residue and 44% of concentrate feed ingredients (Vision 2050, IGFRI). Dairy farming is in transition stage where sustainable production and profitability are big issues, higher authorities in dairy farming and animal husbandry need to be answer these challenges very effectively. Feeding high-producing dairy on poor quality fodder abide difficult for dairy farmers and nutritionists. With the progression of time different physical, chemical and biological method were introduced for improving fibre digestibility. Urea (Saadullah *et al.*, 1981) [32], ammonia and NaOH (Wanapat *et al.*, 1985) [39] method were most promising among them but they require skilled persons and produces huge DM losses (Lynch *et al.*, 2014) [22]. On other side chances of secondary infections with biological method prohibits the practical acceptability by dairy farmers. Exogenous fibrolytic enzyme preparations can be used to improve fibre digestibility of poor quality roughage. Exogenous fibrolytic enzymes, have different nutritive and non-nutrient roles, practically most acceptable, easy to handle and provide scope to formulate diet with cheaper feed ingredients. The use of exogenous fibrolytic enzyme (EFE) additives for ruminants was first examined in the 1960s, as reviewed by Beauchemin and Rode (1996). Enzyme products for ruminant diets are of fungal (mostly *Trichsoderma longibrachiatum*, *Aspergillus niger* and *A. oryzae*); bacterial (*Bacillus* spp., Pendleton, 2000) or rumen bacterial (Gado *et al.*, 2009) [12] origin. The improved performance of cattle (Bhasker *et al.*, 2012; Tewoldebrhan *et al.*, 2017) [7, 35] buffaloes (Gaafar *et al.*, 2010) [11], lambs (Salem *et al.*, 2012) [33] has been reported and suggesting potential of exogenous enzymes use. Although use of enzymes in ruminant's diet is like to be expensive expenditure but the industry associate with livestock feeding need to explore and recognise there return benefits.

Befits of using exogenous fibrolytic enzymes

According to Sheppy (2001) there are four main reasons for using enzymes in animal feed:

1. To break down anti-nutritional factors;
2. To increase the availability of starches, proteins and minerals enclosed within fiber-rich cell walls
3. To break down specific chemical bounds in raw materials which are not usually broken down by the animals' own enzymes
4. To supplement the enzymes produced young animals.

Sources of Enzymes

There are numbers of enzyme preparation available in international market for livestock feed primarily the culture and extraction done from only four bacterial (*Bacillus subtilis, Lactobacillus acidophilus, L. plantarum,* and *Streptococcus faecium,* spp.) and three fungal (*Aspergillus oryzae, Trichoderma reesei,* and *Saccharomyces cerevisiae*) species (Muirhead, 1996) [27]. Food and Drug Administration authority regulating and monitoring this list of organism used as enzyme source and prohibited to add a new organism (Pendleton, 1996) [29]. The source enzyme will be subjected for series of trials and studies on successful testing their commercialization started. Enzymes are naturally occurring biocatalysts produced by living cells to bring about specific biochemical reactions. Enzyme are catabolic product of living organism produced in combinations enzyme none of these commercial products are preparations of single enzymes; secondary enzyme activities such as amylases, proteases, or pectinases are invariably present. Degradation of cellulose and hemicellulose alone requires a number of enzymes and differences in the relative proportions and activities of these individual enzymes impacts the efficacy of cell wall degradation by the marketed products. Even within a single microbial species, the types and activity of enzymes produced can vary widely depending on the strain selected and the growth substrate and culture conditions employed (Considine and Coughlan, 1989; Gashe, 1992) [9, 13]. It is observed the combination and diversity of enzyme activities present in commercially available enzyme preparations is advantageous, in that a wide variety of substrates can be targeted by a single product, but it presents problems in terms of quality control and extrapolation of research findings among different preparations. For ruminants, enzyme products are usually standardized by blending crude enzyme extracts to obtain specified levels of one or two defined enzyme activities, such as xylanase and/or cellulase. These products are not currently standardized for secondary activities. In fact, these activities, which may well be affecting the overall effectiveness of a given product, are seldom even measured.

Types of enzymes

On the basis of site of action carbohydrate breaking enzymes mainly categorised as endoglucanases and exoglucanases (Zhang and Lynd, 2004) [43]. For degradation of protein fraction, amylolytic and proteolytic (Eun and Beauchemin, 2005; Vera *et al.,* 2012) [10, 37] enzymes can also be applied. Fibrolytic enzymes can be cellulose hydrolysing or hemicellulose hydrolysing. Cellulose hydrolysed by endoglucanase, cellobiohydrolase, β-glucosidase, while hemicellulose is hydrolysed by endoxylanases, β-1,4-xylosidases. Some other enzymes like acetyl xylan esterase, ferulic acid esterase, α-D-glucoronidase, α-L-arabinofuranosidase also have some fibrolytic activity. Beside cellulolytic and hemi cellulolytic enzymes some new generation of exogenous enzymes are frequently used in animal diet. One of them like lignolytic enzyme, white-rot basidiomycetes are main source for producing (Maganhotto *et al.,* 2005) [23] peroxidase and phenoloxidase enzymes known as lignin-modifying enzymes (LME's) catalyse lignin through mineralization. Other are oxidase enzyme such as Laccases (benzenediol: oxygen oxidoreductase) copper-containing enzymes catalyze the oxidation process and hydrolyse of various substrates. Laccase enzymes have unique properties they act on nearly similar catalytic site enenthough produced from different species of fungi, and high stability for heat. These exogenous fibrolytic enzymes have been shown to be a promising way to improve feed conversion efficiency (FCE).Among hemicellulose degrading enzyme β-Mannanase is an important polysaccharide β-mannan degrading enzyme.

Mode of action of exogenous enzyme

Exogenous enzymes could exert a number of effects, both on the gastrointestinal microflora and on the ruminant animal itself. It is highly probable, therefore, that physiological responses to exogenous enzymes are multi-factorial in origin. Beauchemin *et al.,* 2005 [4] has summarised mode of action 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 hydrolases of ruminal microorganisms.

a) Preconsumption effects: There is ample evidence that exogenous enzymes can release reducing sugars from feedstuffs prior to consumption (Beauchemin and Rode, 1995) [5]. Release of sugars from feeds arises at least partially from the solubilization of NDF and ADF prior to consumption (Lynch *et al.,* 2014) [22]. However, the degree of sugar release depends on both the type of feed and enzyme used Enzyme substrate solubilisation phenomenon, witnessed by McAllister *et al.,* 2010 [25] through In-vitro experiment where fibrous feed were subjected to exogenous enzyme at higher concentration, increment in number of digestive pits on plant cell wall were observed under electron microscopy.

b) Synergistic action: Synergism in microbial enzyme activity was observed, their potency of fiber solubilisation in rumen and intestine were supported by exogenous fibrolytic enzyme. Several studies with EFE have made mention of the increase of microbial activities in the rumen, which resulted in an enhancement of animal performance traits. Despite the increase in feed digestibility and subsequent production traits, the relationship between the improvement in forage utilization and enzymatic activities is yet to be explained in ruminant systems (Eun *et al.,* 2005) [10]. The extent of cross-linking by *p*-coumaryl and feruloyl groups to arabinoxylans has been identified as one factor that limits the digestion of plant cell walls. *Aspergillus oryzae* has been shown to produce an *esterase* capable of breaking the ester bridges form between ferulic and *p*-coumaric acids form and arbinoxylan.

c) Post ruminal effect: Exogenous enzymes not only heighten fibrolytic activity in the rumen, but also increase fibrolytic activity in the small intestine (Hristov *et al.,* 1999) [16]. Increased xylanase activity in the small intestine is associated with a decline in intestinal viscosity (Hristov *et al.,* 1999) [16]. Because viscosity of duodenal digesta increases with increasing levels of grain in the diet (Mir *et al.,* 1998) [26], enzyme-mediated reductions in viscosity could improve nutrient absorption in the small intestine of cattle fed grain diets. Reduced intestinal viscosity was associated with 1.2% and 1.5% increases in total tract digestibility of DM when enzymes were applied to the feed or infused into the abomasum.

Production response of exogenous fibrolytic enzyme

a) Lactating animals: The effect of exogenous enzymes on milk production in dairy cows was first examined in the mid 1990s (Lewis *et al.,* 1999) [19]. Beauchemin *et al.* (1998) [6] used lactating and cannulated Holstein cows to investigate the effects of fibrolytic enzymes supplementation on ruminal fermentation, nutrients digestion in the rumen and in intestine, and milk production. Two grains (barley and hull-less barley) were combined with and without enzymes achieved the increase (4 kg d^{-1}) in milk production. Dietary addition of fibrolytic enzymes either to forages or concentrate portion increased milk production from 5- 16% (Lewis *et al.,* 1999; Gado *et al.,* 2009) [19-12] no milk response was reported in others (Bernard *et al.,* 2010). Exogenous fibrolytic enzyme supplementation enhances the rate of sugar release from fibres resulting increased TVFA concentration, decreased rumen pH. Rumen liquor protein and nitrogen concentration was at optimum concentration by supplementing exogenous fibrolytic enzyme (240mg/kg TMR) which indicated better utilization of carbohydrate and protein in nonpregnant Gir and crossbred dairy cows (Lunagariya *et al.,* 2017) [21]. Mohamed *et al.,* 2013 [27] supplemented exogenous fibrolytic enzymes (Fibrozyme, *Alltech inc* company, USA) for 12 weeks in 120 multiparous Holstein dairy cows at early lactation in total mixed ration (TMR) reported improved milk yield (41.0 vs. 39.5 kg/cow/d), fat corrected milk, energy corrected milk, SNF and feed efficiency. Lopuszanska-Rusek and Bilik (2011) [20] repoeted enhanced milk production with xylanase-esterase supplementation and a tendency of improving DMI and milk production with xylanase and cellulase enzyme supplementation. The series of studies has been conducted to enumerate whether the exogenous enzyme can with concentrate or forage or TMR. In context to this the meta regression study was conducted first time to summary the studies and tried to drawn some conclusion from research conducted with exogenous enzyme in diet over a period of time. Tewoldebrhan *et al.*, 2017 [36] supplemented 3 different dose β-mannanase: 0% dry matter (DM; control), 0.1% of DM (low supplement, LS), and 0.2% of DM (high supplement, HS) feed conversion for 1 kg milk production was higher for 0.1% of DM feed dose of β-mannanase and milk quality was improved as Somatic cell count (SCC) was lower compared with cows fed control diets. Most recently Romero *et al.,* 2016 the used fibrinolytic enzyme in total mixed ration of *in vitro* assays and identified the most potent hydrolytic enzyme they documented that use of exogenous enzyme in Bermuda grass based TMR increase the milk production @ 1 ml per kg TMR Dry matter. Nonetheless Peter *et al.,* 2015 experiment with use of exogenous enzyme @ 3.8 and 3.9 ml per kg total mixed ration in early lactation did not get any positive effect on milk production and performance. Arriola *et al.,* 2017 [1] given some conclusion by meta-analysis of 36 observations 17 experiments in 15 studies used different enzymes with different rate of application in concentrate or forage or total mixed ration. There premier findings were exogenous fibrinolytic enzyme improve overall dry matter intake feed efficiency, increase total track digestibility and neutral detergent fibre digestibility while fat corrected milk and protein was increased upto small amount. In different type of exogenous fibrolytic enzyme, Cellular xylanase were most effective for improving of milk yield and milk composition. Whereas use of exogenous fibrolytic enzymes in TMR Ration was most effective then concentrate or forage diet.

b) Growing animals: First time ground ear corn, oat silage, corn silage or alfalfa 10 hay treated with an enzyme cocktail containing amylolytic, proteolytic and cellulolytic activities 11 (Agrozyme®**,** Merck Sharp and Dohme Research Laboratories), in cattle reported in improvement in body weight and feed efficiencies (Malik and Bandla 2010). Similarly Lynch *et al.,* (2014) [22] also given fungal enzyme (Enzyme 19AP®, Rohm and Hass Co.) additive in alfalfa hay-based diet and found no improvement in the ADG or feed efficiency in calves. Although the earlier studies were not sufficient to explain proper dose with activity of enzyme the way of application and the response of animals. There after researcher used the enzyme preparation for different feed types (Beauchemin *et al.*, 1995; Beauchemin *et al.*, 1998) [5, 6], application levels, enzyme products (Pritchard *et al.*, 1996) [31] and enzyme application methods (Beauchemin *et al.,* 1998; Yang *et al.,* 2000) [6, 42] have been compared under controlled conditions. The particular enzyme response may dependent of application of different levels (0.25 to 4.0 L $tonne^{-1}$) of a mixture of xylanase and cellulase products (Xylanase B, Biovance Technologies Inc., Omaha, NE) and cellulase (Spezyme CP®, Genencor, Rochester, NY) increased ADG of steers fed alfalfa hay or timothy hay cubes by 30 and 36%, respectively, and type of feed used like same enzyme preparation had no effect on ADG when applied to barley silage (Beauchemin *et al.,* 1995) [5]. Application of a different mix of fungal enzyme preparations (Cellulase A, Xylanase B, Finnfeeds International Ltd. Marlborough, UK) at rates up to 5.0 L $tonne^{-1}$, however, increased the final weight and ADG of feedlot cattle given diets based on alfalfa silage (Pritchard *et al.*, 1996) [31] or barley silage (McAllister *et al.*, 2010) [25]. The researcher recommended the use of exogenous fibrolytic enzymes @ 1.5 g mixture/kg DM improved the growth rate in Murrah buffalo calves (Thakur *et al.,* 2010) [37]. Exogenous fibrolitic enzyme may improve the efficiency of feed utilisation by improving fibre digestibility and rumen activity.

c) Economics of the exogenous fibrolytic enzymes in diet

There are some studies accounted that supplementing exogenous fibrolytic enzyme were become profitable for dairy business. In a study lactating cow supplemented with Hostazym Dairy 5 gm/Day/Animal, cost around Rs. 2.25 per animal per day, results 4% increase in milk fat and SNF and return of investment was 1:4.25. Mohamed *et al.,* 2013 [27] reported supplementing exogenous fibrolytic enzymes @ rate of 15 g/cow/d for 12 week to early lactating dairy cows achieved higher net profit by 0.93US$ per cow than control group.

Table 1: Studies reported improvement in parameters on use of exogenous enzyme in ruminant diet

Enzyme	Regime	Parameter	Reference
Cattle			
Fibrolytic	Added to forage	DMI, N balance, ruminal degradation, total trace digestibility, ruminal protozoa and duedonal ADF flow	Avellaneda *et al.*, 2009 [2]
Fibrolytic	Mixed with diet before feeding	BW, FI, weaning weight, milk production, total solid, fat and protein in milk	Titi and Lubbadeh 2004 [38]
Hydrolytic enzyme	@ 1 ml per kg TMR Dry matter	Increase the milk production	Romero *et al.*, 2016 [32]
Fibrolytic	0.1% Of DM	SCC and Efficiency of feed utilisation for milk production was improved	Tewoldebrhan *et al.*, 2017 [36]
Fibrolytic	240mg/kg TMR	Better utilization of carbohydrate and protein	Lunagariya *et al.*, 2017 [21]
Goat			
Fibrolytic	Supplemented with diet	Digestibilty, ruminal protozoa, milk yield and composition	Kholif and Aziz 2014 [18]
Fibrolytic	Added to TMR	DWG	Hussain *et al.*, 2014 [17]
Fibrolytic and amylolytic	Added to TMR	ADG, DMI and NH_3 N	Wahyuni *et al.*, 2012 [40]
Fibrolytic, proteolytic	Added to concentrate	Nutrient digestibility	Salem *et al.*, 2012 [34]
Fibrolytic	Added to concentrate	Digestibility of DM, OM, CP, NDF and ADF	Bala *et al.*, 2009 [3]
Buffalo			
Fibrolytic	Added to TMR	DMI, ADG and fiber digestibility	Thakur *et al.*, 2010 [37]
Fibrolytic	Added to concentrate	Digestibility of OM, NDF, ADF ADG and final BW	Malik and Bandla 2010 [24]
Fibrolytic	Added to diet	ADG, BWG, FCR, TND and Feed intake	Eun *et al.*, 2005 [10]

Exogenous enzyme additives and silage preparations

In silage preparations exogenous fiber degrading enzymes were used since days, various researchers had used enzymes in different ways. Resional behind use of EFE to degrade fibrous content of plants tissue and improves the availability of water soluble carbohydrate for microbial fermentation. Rate of lactic acid production will be becomes rapid which minimize DM losses and improves silage quality. Commercial enzyme preparation were used for ground ear corn, oat silage, corn silage or alfalfa hay treated with an enzyme cocktail containing amylolytic, proteolytic and cellulolytic activities (Agrozyme®, Merck Sharp and Dohme Research Laboratories), cattle reported average daily gain 6.8 to 24.0% more and improvement in feed efficiencies by 6.0 to 21.2%, than untreated control diets. Similar, four different enzyme preparations (Agrozyme®, Zymo-Pabst®, Rhozyme®, and Takamine®; Merck and Company, Rahway, NJ), used with diethylstilbesterol, were shown to increase gain by cattle fed a corn-alfalfa hay diet by an average of 14.0% (Nelson and Damon, 1999) [29].

Removal of antinutritional factor (ANF) by use of exogenous enzyme

Non Structural polysaccridies in feed stuff create “cage effect” for other nutrient as protein fat and minerals means its will hampers the utilisation of nutrients. The NSP (Non starch polysaccharide) fraction varies in feed with climatic condition and harvesting period. Certain fraction of NSP (mainly soluble fraction of β glucans, pentosans and pectin) store some amount of water (swelling) which contributes to increases the viscosity of digesta in gut. Such as Arabinoxylans of Rye and Wheat and Raffinose, Stachyose and Verbascose in legumes and rape seeds. Various oligosaccharides can be also broken down to glucose and galactose by α-galactosidases and then absorbed. In the case of complex NSP a number of specific enzymes are required to achieve their complete breakdown. The structural polysaccharides such as cellulose, hemicellulose and lignin will be degraded upto some extent. Cellulose are most abundant in nature, nearly indigestible by monogastric and partially digestible by ruminants (microbes). Exogenous use of enzyme can be used to enhance nutrient utilisation by specific substrate degradation.

Table 2: Anti-Nutrition subtrate in feed ingredient and exogenous enzymes

Feed ingredient	Anti Nutritional substrate	Exogenous enzymes
Wheat	Arabinoxylan	Xylanases
Rye	Arabinoxylan, β glucans	Xylanases, P-glucanases
Triticale	Arabinoxylan	Xylanases
Barley	β glucans	P-glucanases
Soybean meal	Raffinose and stachyose	Oligosacchridases

Important issue for enzyme use in feed and feed processing

During feed processing, several factors can lead to the denaturation of the proteins, and these include pressure, heat, retention time and moisture level (Suwal, 2018) [35]. Higher moisture content of the biomass with enzymes may reduce its enzyme activity at high pelleting temperatures, pelleting per se involves extrusion of conditioned hot mash through a die of particular length and diameter. Beside this efficacy of enzymes is influenced by the degree of grinding and grain hardness should be taken into consideration. Feed enzymes need to be robust to stand variations in pH and temperatures.

At present most of the enzyme products in India are directly imported or the individual enzymes are imported and formulated. These enzymes attract import duty, making their usage expensive. There are very few companies producing enzymes in India due to lack of technology as well as appropriate seed organisms. Most of the multinational enzyme companies have spent years in R & D efforts for development of appropriate enzymes. India needs to look into these aspects and put up a state of art enzyme production units in the near future in order to make them economically viable.

Commercial enzyme preparations

Here are various enzyme formulations available in the market with varied activities. Although these products are standardized the method of standardization will vary from producer to producer making comparison of different feed enzyme products almost impossible. The farmer has to look for quality parameters to judge the usage. The parameters would include the type of enzymes present, their activities (unit like IU, NCU, FYT, FTU and EPU), production methodology, product form and its free flowing nature for mixability. It is difficult to convert one unit into the other since each unit has its own definition. Because the enzymes used by the feed industry are produced by different microorganisms, the enzyme characteristics as well as the composition of enzymes would be different. The manufacturer by experience and good quality control can guarantee the consistent results. However, comparison of enzyme products is difficult except by *in vivo* testing. Each microorganism produces enzymes with different optimum pH values, different optimum temperature of operation and different affinity for the substrate in feed. It has not yet been possible to develop an in-vitro method that can predict in-vivo performance.

Advances in exogenous dietary enzymes resources

Continuously advanced research work has been undertaken in area of dietary exogenous enzyme. New tool and techniques need to inculcate for developing and identifying microorganisms as source for enzymes having better activity. Enzymes which are remain active in both acidic and alkaline condition and sustain their activity at intense environmental desirable. Researchers have recently isolated, three hyperthermophilic archaea enriched cellulase enzyme has optimal activity at 109 °C, a half-life of 5 h at 100 °C, and resists denaturation in strong detergents, high-salt concentrations, and ionic liquids (Graham *et al.,* 2011) [14]. Fungi are able to survive in intense environmental conditions. They can tolerate a broad range of temperature, pH, humidity, salinity, oxygen levels and ultraviolet radiation. *Fusarium subglutinans* (MTCC 11891) was found to be eminent filamentous fungi for the production of cellulase activity, at various pH (4-9) and sustain at high temperature around 80 ˚C (thermo stable) (Sharmila D, 2014). Cellulase and xylanase activity was stored and sustained upto 60 days and 80 °C, respectively (Thankur *et al.,* 2010) [37].

Conclusion

The research work on exogenous dieatry fibrolytic enzyme still having unreval efficient to summarize the fibrozyme applied to the total mixed ration of dairy cows in early lactation has the potential to increase milk production, SNF and economic efficiency. Though reviewing the studies it can be concluded that exogenous fibrolytic enzymes may be used as feed additives in dairy farming. The most effective enzymes are cellulase and xylanase (CX), should be applied in Total mixed ration preparation instead of concentrate and roughage. The use of exogenous fibrolytic enzymes as additives may support better growth performance and enhanced milk production simultaneously; farmer can achieved higher net profit in dairy business.

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