

Role of Exogenous Fibrolytic Enzymes in Ruminant Nutrition - A Review

Abstract

Dairy production system in developing countries mainly depends on forage plants and crop residues as major portion of the Ruminants diet. The majority of the dry matter in forage crops is made up of fibre whose digestibility is limited in rumen ecosystem. Use of exogenous fibrolytic enzymes (EFE) is gaining popularity in recent days as they overcome the limitations of other methodologies which are used to improve the digestibility of fibre. Due to microbial enzyme activity, ruminants are able to break down fibrous feedstuff, but structural polysaccharides like cellulose, hemicellulose, and lignin will only be partially broken down. The primary purpose of these enzymes is to provide as many nutrients as possible from the indigestible, potentially digestible, and digestible portions of the cell wall. EFE employed in ruminant diets can be divided into three primary categories based on the specific substrates on which their enzyme activity can take place: fibrolytic, amylolytic, and proteolytic. Enzymes can be applied in liquid or granular form to hay, silage, concentrate, TMR, supplement or premix. Even though positive results were obtained, up to date animal responses to EFE supplements have varied greatly due to enzymatic handling, dosage, diet constituents, time and method of applications. This renders need for further dedicated research efforts for the broad generalization of exogenous enzyme usage in ruminant nutrition. The goal of this study is to give a concise summary of the current state of knowledge about EFE usage in ruminant diets and to discuss potential future research areas.

Keywords: Exogenous fibrolytic enzymes, Nutrient Digestibility, growth performance, Ruminants

Introduction

India now has 467.6 MT of dry fodder and 590.4 MT of green fodder available. However, the demand for green and dry fodder is 851.3 and 530.5 MT, respectively, resulting in a net shortfall of 30.65% and 11.85% for the year 2020. (Vision-2050, ICAR-IGFRI, Jhansi, 2015; Gupta *et al.*, 2019). Forage will almost always be a part of the diet of ruminants due to both economic considerations and the maintenance of rumen health (Krause *et al.*, 2003). The primary source of forages for ruminants in developing nations like India are fiber-

rich forages like paddy/wheat straw, sorghum stover, maize stover, or other fibrous crop leftovers. However, forage cell wall digestibility ultimately limits nutrient availability as rumen conditions are frequently unfavourable for fibre breakdown (Meale *et al.*, 2014). On a dry matter basis, cellulose, hemicellulose, and lignin each make up around 35–50%, 20–35%, and 10%–25% of the plant cell wall, respectively (Sticklen, 2008). Even under ideal circumstances, the digestibility of fibre fraction in the digestive system of ruminants only reaches the range of 65–70%. Therefore, in order to satisfy the current demand for milk and meat, attention must be paid to improving digestibility (Meale *et al.*, 2014).

The limitations of adoption of numerous processing methodologies which are developed to improve the digestion in the ruminants led to the use of exogenous fibrolytic enzymes (EFE) as a biological treatment method and their use is advantageous because unlike physical and chemical treatments they are not expensive and corrosive and/or hazardous (Reddy *et al.*, 2016). Therefore, supplementation of EFE is now discussed widely by animal nutritionists (McAllister *et al.*, 2003; Iannaccone *et al.*, 2022). EFE are added to concentrate mixtures, hay, silages, total mixed rations (TMR), supplements, or premixes in granular or liquid form, which increases the availability of various nutrients in the cell wall. The EFE when added to fibrous feed, produce small amounts of oligosaccharide, and, therefore, will degrade both soluble and insoluble fiber. This causes in increasing the amorphous nature of the fiber and reducing the time for the attachment of fibrolytic bacteria, thus EFE not only improving fiber digestibility but also the ability of microbiome to degrade fiber (Wang *et al.*, 2001). Several researchers observed increase in nutrient utilization pattern (Gado *et al.*, 2009; Azzaz *et al.*, 2021; Kholif *et al.*, 2022), animal performance in terms of weight gain (Lourenco *et al.*, 2020, Abid *et al.*, 2020) and milk production (Lungaria *et al.*, 2019; Holtshausen *et al.*, 2011) due to EFE supplementation. However, either a negative or no effect on animal performance and digestibility were also reported in few studies (Hassan and Almaamory, 2019; Tewoldebrhan *et al.*, 2017).

Sources of Fibrolytic Enzymes

Currently several enzyme preparations are available commercially in the market for livestock feeding. These enzymatic activities derived mainly from four bacterial (*Bacillus subtilis*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus faecium*) and three fungal (*Aspergillus oryzae*, *Saccharomyces cerevisiae*, and *Trichoderma reesei*) species (McAllister *et al.*, 2001; Taye and Etefa, 2020). Major methods of enzyme extraction are Solid State Fermentation and Submerged Fermentation which are further combined with

numerous biotechnological aspects (Sujani and Seresinhe, 2015). Enzymes are nothing but naturally occurring biocatalysts synthesised from living cells to accomplish specific biochemical reactions. These enzymes are catabolic products produced in associations with other enzymes by living organisms, none of these commercial enzyme products contains a single enzyme, invariably secondary activities of enzymes such as amylases, pectinases, or proteases are present (Mendoza *et al.*, 2014). To breakdown complex cell wall matrix consisting structural carbohydrates (cellulose & hemicellulose), proteins, phenolics and water a wide variety of enzymes are needed (Caffall and Mohnen, 2009; Morgavi *et al.*, 2012). Even though microorganisms from which enzymes are produced constitutes a small group, types and activities of enzymes vary widely based on strain selected, culture conditions employed and growth substrate used (Meale *et al.*, 2014; Reddy *et al.*, 2016).

Methods of Application of Enzymes

Various authors concluded that enzymes can be applied in liquid or granular form to hay, silage, concentrate, TMR, supplement or premix. Due to high moisture content, EFE show enhanced effectiveness when applied to wet feeds (such as silages) as compared to dry feeds because water helps in the dissemination of enzymes and is required for the hydrolysis of complex fibre polymers to release simple monomers. Apart from this, silage pH values are usually at, or around, the optimal pH for many fungal derived enzymes (Beauchemin *et al.*, 2003). However, in practice effect is more, if enzymes are applied in liquid form to dry forage when compared to wet forage (Reddy *et al.*, 2016). In contrast to this, Nsereko *et al.* (2000) reported that hemicellulolytic activity reduced when enzymes were applied to silage; which may be due to the presence of characteristics of fermented feed which reduce the β -(1-4)-xylanase activity up to 50%. But the cellulolytic activity of enzymes was unaltered.

Feng *et al.* (1996) treated grass directly with enzyme solution and got no result when treated to fresh or wilted forage. But increased digestibility of DM and fibre was observed when applied to dried grass. In contrast to this, Yang *et al.* (1999) reported, no difference between treating enzyme product to dry fodder alone or to both concentrate and dry fodder. Hristov *et al.* (2008) observed that infusion of EFE intra-ruminally @ lower doses (10 g/cow/day) had no significant effect on nutrient utilization and rumen fermentation pattern. In contrast, Giraldo *et al.* (2008) observed enhanced fibrolytic activity in ruminal fluid when sheep were administered EFE @ 12 g/d intra-ruminally. These variable responses by fibrolytic enzymes across experiments may be due to differences in the enzyme's activity, substrate specificity, internal rumen environment and mode of application (Yang *et al.*, 2022)

Modes of Action of Exogenous Fibrolytic Enzymes (EFE)

The important determinants of feed intake and animal performance in ruminants are fibre content and digestibility. Usage of enzyme-based diets with fibrolytic activities can effectively help ruminants to digest more fibre, hence increasing nutrients digestibility (Mousa *et al.*, 2022). Possible mode of action of EFE can be explained through their effect on feed before consumption or through improvement of digestion within the rumen and/or their impact at post ruminal digestive tract. Effect of EFE on feed before consumption may be simple to complex like release of soluble carbohydrate, release or removal of structural barriers of feed which restrict microbial digestion in the rumen (Beauchemin *et al.*, 2003; Carrillo-Díaz *et al.*, 2022). In the rumen, EFE may act directly on feed or indirectly may work synergistically with rumen microbes. In the lower digestive tract, EFE may remain active which may enhance post ruminal fibre digestion or may indirectly reduce the viscosity of digesta which may further enhance the absorption of nutrients. Enzyme activity may also persist in the excreta, thereby take part in increasing the rate of decomposition of feed.

1. Pre-Consumption Effects

Treating EFE to feeds before consumption releases reducing sugars (Hristov *et al.*, 1996) which arises partially from solubilisation of ADF and NDF (Krause *et al.*, 1998; Lynch *et al.*, 2014). Furthermore, it increases carbohydrate availability in the rumen (Yang *et al.*, 2000) and also improves growth and attachment of rumen microbes (Forsberg *et al.*, 2000). The rate of release of sugar depends on type of feed and enzyme complex used (Singh *et al.*, 2018). McAllister *et al.* (2010) observed the enzyme-substrate solubilisation phenomenon in an *invitro* study where they observed an increase in digestive pits number under electron microscope when enzymes were applied to fibrous feed.

2. Ruminal Effects

Several studies showed that EFE can increase the degradation of fibre by rumen microbes *in vitro* (Dong *et al.*, 1999) and *in situ* (Lewis *et al.*, 1996). McAllister *et al.* (2001) reported that EFE may hydrolyse feed directly in the rumen or due to synergism with ruminal microorganisms digestion of feed may increase. EFE are shown to be stable in ruminal fluid for continuous hydrolysis of feed (Hristov *et al.*, 1998 and Wallace *et al.*, 2001). Researchers have observed that *Aspergillus oryzae* extracts can increase the ruminal bacteria number (Newbold *et al.*, 1992) and increase the rate of soluble sugars release from hay by working synergistically with extracts from rumen microbes (Newbold, 1995). Another advantage of

supplementing EFE in ruminants is that they increase numbers of glucose and cellobiose utilizing bacteria and their attachment indirectly in the rumen (Nsereko *et al.*, 2002). Few researches have observed change in rumen microbial phylotypes and feed efficiency. Beauchemin *et al.* (2004) reported that effect of EFE reduced in sub rumen conditions ($\text{pH} \leq 5.9$) produced due to high fermentable diet as compared to optimum rumen pH conditions. The optimum pH for most of the fibrolytic enzymes derived from rumen microbes is above 6.2. In contrast, Muzakhar *et al.* (1998) reported that optimum pH for enzymes derived from aerobic fungi (*Trichoderma longibrachiatum*) ranged from 4 to 6.

3. Post Ruminant Effects

EFE not only enhance fibrolytic activity within the rumen but also increases the same in the small intestine (Hristov *et al.*, 1999). When exogenous enzymes are treated to concentrate premix and wet feeds, they appeared to survive for enough period of time with sufficient impact on substrate particles in the small intestine (Morgavi *et al.*, 2001). Hristov *et al.* (1996) concluded that enhanced activity of xylanase in the small intestine is related to decline in viscosity of intestinal contents. Increasing the portion of grain in the diet increases the viscosity of duodenal digesta (Mir *et al.*, 1998), but because of supplementing exogenous enzymes, viscosity will be reduced which improves the absorption of nutrients in the small intestine of cattle received higher grain diets. Certain authors pointed there was increase in the duodenal flow of organic matter, nitrogen and non-ammonia nitrogen noticed upon fibrolytic enzymes supplementation (Alvarez *et al.*, 2020). Furthermore, EFE works synergistically even with large intestinal microbes (Beauchemin *et al.*, 2004).

Use of Exogenous Fibrolytic Enzymes in Ruminants

Initially, EFE were used only in the diets of poultry and pigs in order to degrade the pericarp of grains that covers the endosperm. The use of fibrolytic enzymes was not practiced in ruminants in earlier days, because it was thought that these are destroyed rapidly by rumen proteases, and also because rumen microbes are capable of degrading fibrous parts of feed (Beauchemin *et al.*, 2004). Researches on EFE in ruminants were started in the 1960's (Burroughs *et al.*, 1960). They observed variable results in the ruminants and said that it was not profitable to use enzymes in ruminant's diet because the production of enzymes was costly during those days. Recently fermentation costs were reduced, along with it, preparation of more active and better defined enzymes initiated which lead researchers to re-examine enzymes role in the ruminant production system (McAllister *et al.*, 1999).

It has been observed that adding fibrolytic enzymes during ensiling process can increase the nutritional value of feeds, particularly low nutritive value agricultural by-products (Abid *et al.*, 2022). Milk composition and net economic returns were enhanced in lactating cows fed with slow-release nitrogen and exogenous fibrolytic enzyme (Sharma *et al.*, 2021).

Effect on Feed Intake

Large ruminants

Exogenous fibrolytic enzymes can affect the degradability of dry matter, fibre hydrolysis, gas production, and milk yield depending on the type of ruminant (large & small) and quality, proportion of forage (legumes or grasses), and the number of ingredients in the diet (Tirado-González *et al.*, 2021). Beauchemin *et al.* (2000) observed that supplementation of enzyme product (Natugrain) having activities of mainly xylanase, β -glucanase and endocellulase to lactating dairy cows @1.22, and 3.67 litre of enzyme product/tonne of TMR increased the DMI (kg/d) by 7.5 and 5.2% in both low and high level supplemented groups, respectively. Apart from this, intake of OM, NDF and ADF were also similar to DM intake. Similarly, DM intake (18.2 vs. 16.1 kg/day) and OM intake (16.4 vs. 14.1 kg/day) increased positively due to supplementation of enzyme ZADO[®] but the NDF intake (7.4 vs. 7.1 kg/day) was not altered in Brown Swiss cows (Gado *et al.*, 2009). Romero *et al.* (2016) reported that supplementation of Xylanase plus @ 1 mL/kg DM of TMR substantially increased ($P < 0.001$) intake of DM, OM and CP in dairy cows. Lungaria *et al.* (2019) supplemented EFE (Roxozyme GT[®]) @ 240 mg/kg TMR as this dose revealed optimum in an *in vitro* study (Lunagariya *et al.*, 2017a) to lactating HF crossbred cows. The result showed numerical improvement ($P > 0.05$) in DM and nutrients (CP, DCP and TDN) intake.

In contrary, supplementation of a EFE (ZADOs) @ 40 g/hd/d to crossbred Baladi Friesian steers showed no effect on DM intake (Salem *et al.*, 2013). Furthermore, Vicini *et al.* (2003) concluded that exogenous fibrolytic enzyme supplementation does not affect feed intake and body weight gain during the experimental period. Similarly, supplementation of EFE with sugar beat pulp @ 0.2% and 0.4% W/W to buffalo male calves did not affect dry matter intake ($P > 0.05$) (Kady *et al.*, 2006). Opposite to this, Lourenco *et al.* (2020) supplemented endo-1,4- β -xylanase enzyme @ 13,800 fungal xylanase units/kg DM of creep feed to beef cattle calves. In one research station, the result showed that intake of DM

reduced substantially in enzyme feed as compared to plain creep feed. But in another research station, only a trend for decreased intake of DM was observed.

Small ruminants

Sheikh *et al.* (2017) observed that feeding of complete feed to Corriedale Sheep prepared from urea molasses treated paddy straw (T1) and exogenous enzyme (9 g/kg DM) plus urea molasses treated paddy straw (T2) significantly ($P < 0.01$) increased the DM intake (g/day) in T1 and T2 compared to control group (745.77 ± 12.39). 11.69% and 2.46% reduction in feed cost was observed in T2 and T1 group compared to T0. Abid *et al.* (2020) also obtained similar results in lambs due to EFE supplementation. Similarly, significantly higher DM intake on the basis of metabolic body weight was observed due to supplementation of EFE to Patanwadi sheep @ 0.025% of TMR (Patel, 2012). But there was non-significant difference in the final body weight of both the groups ($P > 0.05$). Similarly, addition of EFE (6.23 unit protease and 78 unit cellulose/g) to the wheat straw based ration of Barkey lambs showed higher intake of DM (0.908 Vs. 0.860 kg/d), TDN (0.788 Vs. 0.708 kg/d), CP (155.6 Vs. 153.4 kg/d) and DCP (120.0 Vs. 110.1 kg/d) in enzyme supplemented group compared to control (El-Bordeny *et al.*, 2017).

In contrast to this, Pinos-Rodriguez *et al.* (2008) confirmed in an *in situ* and *in vivo* study that EFE (Fibrozyme) has no significant ($P > 0.05$) effect on feed intake of ruminally cannulated Rambouillet lambs which were fed TMR with different forage:concentrate (F:C) ratios. Similar results were obtained in lambs by Sakita *et al.* (2022). Furthermore, Bueno *et al.* (2013) supplemented EFE (Fibrozyme) to lambs @ 0, 5 and 10 g enzyme per kg of forage respectively along with the basal diet and observed no significant difference ($P > 0.05$) in average daily gain and feed conversion ratio; the only linear effect was observed ($P = 0.04$) with respect to feed intake.

Effect on Rumen Fermentation Pattern

Large ruminants

Fibrolitic enzymes enhance the digestibility of dietary fibre portions by solubilizing the fractions, thus changing the dynamics of rumen fermentation (Bennett and Faciola, 2022). Arriola *et al.* (2011) observed that supplementation of EFE @ 3.4 mg/g TMR DM to HF cows decreased the ruminal pH values after 4 hours of feeding and did not have any effect on concentration of ruminal ammonia but concentration of TVFA increased significantly ($P = 0.03$: 114.5 vs 125.7 mM). Apart from this, acetate to propionate ratio reduced (3.09

vs2.87) ($P=0.04$), suggesting the improvement of energy utilization efficiency in the rumen and also amount of methane production was reduced. Similar results along with increased microbial N synthesis (220 versus 190 g/d; $P<0.05$) were obtained by Gado *et al.* (2009) in dairy cows due to EFE supplementation. Furthermore, Salem *et al.* (2013) reported that supplementation of enzyme (ZADOs) @ 40 g/hd/d to crossbred Baladi Friesian steers enhanced ($P<0.05$) production of SCFA, ammonia N concentration and also total purine derivatives ($P=0.04$) suggesting increased synthesis of microbial protein. Similarly, EFE (Fibrozyme) addition in TMRs lowered ($P<0.01$) the rumen pH and elevated ($P<0.01$) the amount of $\text{NH}_3\text{-N}$, N fractions and TVFA in buffalo bulls, 4 hour post feeding irrespective of R:C ratio (Rajamma *et al.*, 2014).

In contrary to this, study conducted by Romero *et al.* (2016) reported that supplementation of Xylanase plus to lactating dairy cows numerically decreased ($P=0.13$) the level of acetate but it did not had any effect on concentrations of ammonia-N, TVFA, butyrate, propionate, isobutyrate, valerate, isovalerate, ruminal pH, acetate:propionate (A:P) and acetate plus butyrate:propionate ratios. Furthermore, there was no significant effect ($P>0.26$) on molar proportions of individual VFA due to enzyme supplementation. Moreover, Wang *et al.* (2018) concluded that populations of *F. succinogenes* and *B. fibrisolvens* for pre and post-weaned calves and *R. flavefaciens* for post-weaned calves elevated with isobutyrate or fibrolytic enzymes addition to the diet of Holstein bull calves.

Small ruminants

Rojo *et al.* (2005) stated that supplementation of alpha-amylase produced from the fermentation of *Bacillus licheniformis* increased the rumen pH level but the total VFA level and protozoal count decreased linearly; contrarily, glucoamylase supplementation which is a fermentation product of *Aspergillus niger* increased the rumen protozoal count in Suffolk lambs. Furthermore, supplementation of cellulase degrading enzyme Asperozym at 3.08 U/kg diet DM to lactating Baladi goats showed highest value of TVFA concentration, total nitrogen, ammonia nitrogen, non-protein nitrogen, microbial protein and true protein levels which is followed by lambs fed with enzyme Tomoko® at 1.54 U/kg diet DM and control group (Kholif and Aziz, 2014). Sheikh *et al.* (2017) supplemented exogenous enzyme mix @ 9 g/kg DM to the complete feed prepared from urea molasses treated paddy straw to Corriedale sheep. The result showed the significant ($P<0.05$) higher level of TVFA, ammonia-N nitrogen and total nitrogen concentrations. While, no significant difference was observed in rumen pH, NPN, and TCA ppt. N (mg/dl).

Abid *et al.* (2020) observed that feeding olive cake which is sprayed with cellulase and xylanase mix (50:50 by volume) @ 4 (CX04) or 16 (CX16) ml per kg OC DM 12 hour before actually feeding to the lambs had no significant effect on ruminal pH which was recorded 3h post feeding (pH values between 6.89 and 6.92). It suggested, improvement in digestibility of fibre will not cause reduction in rumen pH or rise any possibility of ruminal acidosis. They observed non-significant reduction in ruminal ammonia level due to enzyme supplementation indicating slight higher synthesis of microbial protein due to increased uptake of ammonia-N by ruminal microbes. Contrarily, Patel. (2012) observed that supplementation of EFE to Patanwadi sheep @ 0.025% of TMR showed non-significant difference ($P > 0.05$) in rumen pH, TVFA level, ammonia- N and NPN levels. Furthermore, total N and protein N increased significantly in enzyme supplemented group compared to control.

Effect on Nutrient Digestibility

Large ruminants

Exogenous fibrolytic enzyme treatment to ruminant feeds has the potential to improve forage cell wall degradability and, consequently, feed efficiency (Iannaccone *et al.*, 2022). Application of enzymes to the low-concentrate diet led to milk production on par with cows fed with untreated the high-concentrate diet (Arriola *et al.*, 2011). Salem *et al.* (2013) reported that supplementation of an enzyme (ZADOs) @ 40 g/hd/d to steers increased the digestibility of OM, CP, NDF and ADF by 11.7%, 4.7%, 21.8% and 26.7% respectively. Similarly, increased digestibility coefficients of DM, OM, NDF and ADF was observed by Beauchemin *et al.* (2000) due to supplementation of low dose of EFE (Natugrain, 1.22 L /tonne of TMR DM) in lactating dairy cows. However, higher level of enzyme (3.67 L/tonne of TMR DM) had no effect on digestion. Gado *et al.* (2009) observed that supplementation of enzyme ZADO[®] to lactating Brown swiss cows, significantly increased the digestibility of DM (663 vs. 743 g/kg in T0 and T1), OM (667 vs. 741 g/kg in T0 and T1), NDF (418 vs. 584 g/kg in T0 and T1) and ADF (401 vs. 532 g/kg in T0 and T1) in enzyme supplemented group (T1) compared to control group (T0). Similar results were observed in buffalo male calves by Kady *et al.* (2006), (Malik and Srinivas, 2010) and Marwan *et al.* (2019). Supplementation of Xylanase plus @ 1 mL/kg DM of TMR (T1) significantly ($P < 0.001$) increased the DCP intake (kg/d) in Holstein cows (Romero *et al.*, 2016). Furthermore, Supplementing the diet with in-farm produced cellulase enzymes cocktail to lactating Egyptian buffaloes had showed significantly higher CP, NDF and ADF digestibility (Azzaz *et al.*, 2021)

Contrary to this, significant decrease ($P < 0.05$) in digestibility % of DM, OM, and CP were observed by Tewoldebrhan *et al.* (2017) upon supplementation of commercial EFE (CTCZYME) @ 0.1% and 0.2% of DM of TMR in lactating multiparous Holstein cows. While, the digestibility of starch, NDF and ADF were not affected due to supplementation of β -mannanase.

Small ruminants

Sheikh *et al.* (2017) observed that feeding of complete feed to Corriedale Sheep prepared from urea molasses treated paddy straw (T2) and exogenous enzyme (9 g/kg DM) plus urea molasses treated paddy straw (T3) significantly ($P < 0.01$) increased digestibility of DM, CP, NDF, ADF and cellulose in T3 group which is followed by T2 and T1 (control). While, digestibility of NFE and hemicellulose was similar in all the groups. Treating tifton-85 hay with fibrolytic enzymes extract 24 hours before feeding to lambs resulted 12% higher ADF digestibility (Sakita *et al.*, 2022). In Ossimi lambs similar results were obtained by Mousa *et al.* (2022) due to Supplementation of Calfo Care® (Probiotics and enzymes) @ 0.5, and 1kg/ton diet DM. Similarly, Kholif *et al.* (2022) reported that feeding of date palm leaves ensiled with EFE and probiotics to Farafra ewes significantly increased digestibility of all nutrients (except NDF for probiotics treatment and EE for both enzyme and probiotics treatments). Furthermore, substantial improvement in the digestibility of all the nutrients and intake of DCP and TDN (kg/day) were observed by El-Bordeny *et al.* (2017) due to addition EFE (6.23 unit protease and 78 unit cellulose/g) to the wheat straw based ration of Barkey lambs.

In contrary, no significant difference ($P > 0.05$) in the in vivo digestibility of DM and NDF was observed due EFE (Fibrozyme) supplementation in lambs @ 0, 5 and 10 g enzyme per kg of forage respectively along with the basal diet (Bueno *et al.*, 2013). Apart from this, González-García *et al.* (2008) supplemented EFE in lactating Murciano-Granadina dairy goats and observed significantly increased (4.4%; $P < 0.05$) digestibility of DM and OM (3.6 %; $P = 0.07$) in enzyme supplemented group compared to control. However, increase in the digestibility of ADF and NDF was non-significant ($P > 0.05$).

Effect on Blood Parameters

Large ruminants

Supplemental enzymes accelerate metabolic process in response to increased apparent digestibility, optimal utilization of dietary proteins and overall increasing the nutrient

availability. Substantial improvement ($P \leq 0.05$) in total protein and albumin and non-significant reduction in triglycerides, creatinine, urea, ALT and AST concentrations was observed due to supplementation of 12 ml Zymogen liquid/100 kg body weight/head to buffalo calves (Marwan *et al.*, 2019). Apart from this, feeding of enzyme treated TMR to lactating dairy cows decreased ($P < 0.01$) concentration of BHBA, it is an indicator of enhanced mobilization of fat or maximised energy balance due to improved ketone bodies oxidation in initial stages of lactation (Dean *et al.*, 2013). Mohamed *et al.* (2013) observed supplementation of EFE @ 15 g/d/animal in Holstein dairy cows resulted insignificant decrease in serum cholesterol (242.0 vs. 193.7 mg/dL), total protein (12.8 vs. 10.4 g/dL), globulin (8.9 vs. 6.3 g/dL) and albumin/globulin ratio (0.81 vs. 0.54) as compared to dairy cows in the control group. While, glucose, triglycerides, albumin and urea were unaffected.

Contrarily, Kady *et al.* (2006) supplemented EFE @ 0.2% and 0.4% W/W with sugar beat pulp to buffalo male calves. Result showed no significant ($P > 0.05$) difference on blood total proteins, albumin, globulin, GOT, GPT, urea nitrogen and creatinine among all groups. Similarly, supplementation of EFE and live yeast cells had no effect on haemoglobin, blood glucose, serum protein, calcium and phosphorus in lactating Jersey and Jersey crossbred (Jersey×Kankrej) cows and all the measured parameters were within the normal range (Vahora and Pande, 2006).

Small ruminants

In experimental Ossimi lambs, Mousa *et al.* (2022) demonstrated that the combination of fibrolytic enzymes and probiotics enhanced the hematological and immunological variables, indicating an improved health status. Sheikh *et al.* (2017) observed that treatment of paddy straw with urea molasses and enzyme @ 9 g/kg DM to the complete feed significantly increased ($P < 0.05$) total protein and Hb (g%) level in Corriedale Sheep compared to control and urea supplemented group. Furthermore, the levels of blood glucose (mg/dl), PCV (%), serum creatinine (mg/dl) and blood urea nitrogen (mg/dl) showed no significant difference among all the three treatment groups. The increase in blood protein level could be due to greater availability of different nutrients (DCP, TDN and ME) in EFE supplemented calves. In another study, concentration of serum total protein was numerically increased ($P > 0.05$) and urea concentration reduced significantly; while the concentrations of triglyceride, globulin, albumin, creatinine, ALT, AST and alkaline phosphatase were unaffected ($P > 0.05$) due to enzyme addition (El-Bordeny *et al.*, 2017). Millamet *et al.* (2020) supplemented xylanase: glucanase combination at different ratios to the

diet of Yankasa yearling rams. The result showed increased level of PCV, erythrocytes, creatinine and decreased level of BUN in enzyme supplemented groups compared to control.

Furthermore, supplementation of EFE had no significant effect on serum metabolites, except serum cholesterol, which was higher in enzyme supplemented groups compared to control. While, no significant difference was observed in levels of liver enzymes among different treatment groups (Abid *et al.*, 2020).

Effect on Growth and Feed conversion efficiency

Large ruminants

Exogenous fibrolytic enzymes (EFE) are supplements that are claimed to increase fibre degradability, accelerating ruminal fermentation kinetics and potentially lowering feed costs incurred hence maintaining ruminants productive performance (Tirado-González *et al.*, 2018). Bilik *et al.* (2009) observed that Fibrozyme™ supplementation improved nutrient utilization and feed conversion efficiency per kg production of milk compared to the control group in peri-parturient Holstein-Friesian cows. In an another study, it was observed that cows which received high enzyme ration had higher milk production efficiency compared to the control group. While, there was no significant difference in production efficiency of cows fed low enzyme diet compared to control group (Holtshausen *et al.*, 2011). Furthermore, Salem *et al.* (2013) observed that elevated feed conversion and live-weight gain by 9% and 16% respectively in Baladi Friesian steers due to enzyme (ZADOs) supplementation compared to control group. Similar results were recorded by Kady *et al.* (2006) in buffalo male calves. Exogenous fibrolytic enzymes fed to lactating dairy cows increased ($P<0.003$) milk production (41.0 vs. 39.5 kg/cow/d) and fat corrected milk ($P<0.025$) as compared to dairy cows not given any treatment (Mohamed *et al.*, 2013).

Contrarily, substantial decreased FCR ($P<0.05$) was noticed in post-weaned calves due to EFE by Wang *et al.* (2018). The gross energy, gross protein and net protein efficiency improved ($P<0.01$) by 26.52, 29.64 and 3.14% due to enzyme (Roxozyme GT ®) supplementation lactating HF crossbred cows (Lungaria *et al.*, 2019). Moreover, Marwan *et al.* (2019) observed significantly higher ($P\leq 0.05$) total gain (kg) and average daily gain (kg/h/day) in calves due to addition of 12 ml Zymogen liquid/100kg body weight/head compared to control. These results were supported by Lourenco *et al.* (2020) who supplemented EFE (endo-1, 4- β -xylanase) in a cow-calf herd.

Small ruminants

The study revealed that average daily gain (g/d) and mean final body weight (kg) of Corriedale sheep which received enzyme plus urea molasses treated paddy straw was substantially higher ($P < 0.05$) and FCR was improved (Sheikh *et al.*, 2017). Abid *et al.* (2020) observed that feeding olive cake which is sprayed with cellulase and xylanase mix (50:50 by volume) @ 4 (CX04) or 16 (CX16) ml per kg OC DM 12 hour before actually feeding to the lambs significantly increased daily weight gain in lambs of CX04 and CX16 groups by 6% and 9% respectively, as compared to enzyme untreated group. But mean body weight and feed to gain ratio was unaffected due to enzyme supplementation. Further, exogenous cellulolytic enzymes Asperozym and Tomoko improved ($P < 0.05$) milk yield in Baladi lactating goats compared to control groups (Kholif and Aziz, 2014).

Contrary to this, Patel, (2012) reported that addition of EFE @ 0.025% along with the TMR had no effect on average final body weight in Patanwadi sheep. Similarly, supplementation of EFE (Fibrozyme) @ 0, 5 and 10 g per kg of forage had no effect on ADG and FCR in lambs (Bueno *et al.* 2013). Furthermore, the observed feed per gain ratio was best in the group fed TMR with 2 g enzyme/kg DM, followed by bucks receiving 0, 4 and 6 g of enzyme/kg TMR DM (Wahyuni *et al.*, 2012).

Effect on Economics of Feeding

Lunagariya *et al.* (2019) reported that supplementation of EFE (800 IU/g endo 1,4- β glucanase, 700 IU/g 1(3),4- β glucanase and 2700 IU/g endo 1,4- β xylanase) to HF crossbred cows @ 240 mg/kg total mixed ration (TMR) resulted in 15.87% higher return over feed cost. Similarly, 0.93 US\$ higher net profit was achieved per cow due to supplementation of fibrozyme in early lactating dairy cows by Mohamed *et al.* (2013). Furthermore, Sheikh *et al.* (2017) reported that feeding of complete feed prepared from urea molasses treated paddy

Conclusions

Exogenous fibrolytic enzymes can be used as additives to achieve improved growth performance and milk production, enabling farmers to boost their net profit in the dairy sector. Still, inconsistent results are reported in ruminants mainly due to enzymatic handling, dosage, diet constituents, time and method of applications. Development of specific enzyme formulation and level of feeding for particular feed makes it complex for the producers to adopt; therefore, responses to these additives need to be broad based across a range of diet types. Need for future study is highly invited by focusing on the limitations with generalising the usage.

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UNDER PEER REVIEW