Application and Prospects of Exogenous Enzyme Preparations in Ruminants: Postprint

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Abstract

Ruminants can convert roughage that cannot be utilized by humans into high-quality protein—meat and milk, which is of great significance to agriculture and human society. However, this conversion efficiency is closely related to the digestibility of plant cell walls; currently, the relatively low digestibility of plant-based feedstocks affects the conversion efficiency. Supplementing exogenous enzyme preparations in ruminant diets has the potential to improve plant fiber digestion, thereby enhancing feed utilization efficiency. This article summarizes the current application status, modes of action, and future development directions of exogenous enzyme preparations, noting that while positive results have been reported for their application in ruminants, there are also reports of insignificant effects, which may be related to factors such as enzyme profile, supplementation level, supplementation method, substrate type, and the production level of the host animal. It also elaborates that exogenous enzyme preparations function in different environments—pre-feeding, within the rumen, and post-rumen—but the detailed mechanisms require further in-depth investigation. In summary, despite certain issues remaining in the application of enzyme preparations, enzyme preparations aimed at improving feed digestibility will continue to be an important direction in the development of feed additives for ruminants.

Full Text

Application and Outlook of Exogenous Enzyme Preparations in Ruminants

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Abstract: Ruminants play a crucial role in agriculture and human society by converting forage that humans cannot utilize directly into high-quality protein in the form of meat and milk. However, this conversion efficiency is closely related to the digestibility of plant cell walls, which remains low for current plant materials, thereby limiting overall conversion efficiency. Supplementing ruminant diets with exogenous enzyme preparations shows potential for improving plant fiber digestion and thus enhancing feed utilization efficiency. This review summarizes the current application status, modes of action, and future development directions of exogenous enzymes. While positive results have been reported for exogenous enzyme applications in ruminants, inconsistent effects have also been documented, likely related to factors such as enzyme profile, supplementation level, application method, substrate type, and host productivity level. The review also elaborates on how exogenous enzymes function in different environments—before feeding, within the rumen, and post-rumen—though detailed mechanisms require further investigation. In conclusion, despite existing challenges, enzyme preparations targeting improved feed digestibility will remain an important direction for ruminant additive development.

Keywords: exogenous enzyme preparations; ruminants; rumen; plant cell wall

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The growing global population and sustained economic development have increased demand for high-quality meat and dairy products, placing greater demands on ruminants' ability to utilize forage. Forage has always been an important component of ruminant diets due to economic considerations and the need to maintain rumen health, yet its digestibility in the rumen does not exceed 50%, and whole-tract digestibility remains below 65%[1-2], thus limiting full forage utilization. In recent years, enzyme preparations for ruminants have been a hot research topic. Despite variability in application effects, supplementing ruminant diets with exogenous enzymes is widely recognized as an effective and economical method to improve feed utilization efficiency[3]. Numerous scholars have conducted research on exogenous enzyme applications in ruminants[4-5], primarily focusing on the effects of exogenous cellulase on fiber digestibility, nutrient utilization, and production performance. Studies have shown improvements in dry matter and fiber digestibility[6], increased milk yield[7-8], and enhanced daily weight gain[9], though some reports indicate negligible effects[10]. Given that enzyme preparations represent an important future direction for ruminant additives, this review aims to summarize recent application effects and potential action pathways of exogenous enzymes in ruminants, and to propose research directions and methodologies for further improving forage utilization efficiency and promoting sustainable development of ruminant production.

1. Enzyme Sources in Preparations

Enzyme preparations currently used in animal production are primarily derived from fermentation products of fungi (such as *Aspergillus oryzae*, *Trichoderma* species, and *Trichoderma reesei*) and bacteria (*Bacillus* species, *Lactobacillus acidophilus*)[2]. Feed-grade enzymes are mainly produced through microbial fermentation via inoculation and culture medium growth. Although microbial sources and species are limited, different enzyme types may be produced due to strain selection, fermentation substrates, and culture conditions[11].

Generally, degrading plant cell wall structural carbohydrates requires a complex enzyme profile[12]. Commercial enzyme preparations typically consider cellulase and xylanase as primary components, with protease, amylase, lipase, pectinase, and ferulic acid esterase also potentially included. Compound enzyme preparations offer advantages by enabling reactions across a range of substrates, though this makes it difficult to identify which specific enzyme improves digestibility. It is widely believed that single enzymes may not be suitable for specific feeds, whereas compound enzymes can accommodate various feed types[13].

Enzyme activity is defined as the amount of enzyme required to catalyze the conversion of one unit of substrate to product under specific conditions of time, temperature, and pH[13]. For carbohydrate enzymes, activity is typically measured by glucose production. Enzyme activity assays require strict control of variables such as temperature, pH, ionic strength, substrate concentration, and substrate type, often using chemically synthesized substrates measured through staining and spectral characteristics. Currently, many enzyme activity assays lack national standards, with many companies using their own enterprise standards. Therefore, enzyme quality should be evaluated based on application effects rather than activity alone. Furthermore, activity assay methods cannot fully simulate digestive tract conditions, and the substrates used are not typical ruminant diets, which may affect the application of exogenous enzymes as ruminant feed additives.

Biological analytical methods are recommended for ruminants, including two-step methods, gas production methods, and nylon bag techniques. These involve adding feed substrates (such as grains, hay, silage, or straw) to culture tubes with rumen microorganisms and enzymes to study substrate degradation. Using the nylon bag method, effective degradation rates of dry matter, neutral detergent fiber (NDF), and acid detergent fiber (ADF) can be calculated by recording nutrient disappearance at different time points. This approach allows screening of appropriate enzyme profiles based on digestion effects. However, in vitro methods cannot fully simulate in vivo conditions[14] and cannot account for individual differences in microbial populations between animals, creating uncertainty when extrapolating results to whole-animal effects. Additionally, in vitro methods cannot explain the effects of exogenous enzymes on physiological functions such as feed passage rate and post-rumen nutrient digestibility. Therefore, only animal production trials can provide the most accurate assessment of exogenous enzyme effects on ruminant feed utilization.

3. Effects of Exogenous Enzymes on Ruminant Production Performance

3.1. Dairy Cattle

Numerous studies have investigated exogenous enzyme applications in dairy cows, primarily examining effects on milk yield and composition[5,15]. Most products are based on cellulase or xylanase, with fewer protease and amylase products. Due to inconsistent definitions and measurement methods of enzyme activity, different trials cannot be compared on an activity basis. Even with consistent assay methods, product standardization is difficult because many products are compound enzymes that cannot be standardized simultaneously. Therefore, products should be compared based on application effects rather than enzyme activity alone.

Some studies have found that exogenous enzymes can improve milk composition. Beauchemin et al.[15] reported that supplementing dairy cows with a complex of β-glucanase, xylanase, and endoglucanase increased milk protein content by 2%. Similarly, Sutton et al.[16], Eun et al.[17], and Li et al.[18] reported that exogenous enzyme supplementation increased milk protein or fat content. Other studies have shown increased milk production[18]; Yang et al.[19] found that adding 2 g/kg of a cellulase and xylanase complex to dairy cow hay increased milk yield by 1.9 kg/d, possibly due to a 12% improvement in dietary dry matter digestibility. Additionally, enzyme supplementation has been shown to improve health status, with dietary addition of propylene glycol and cellulase ameliorating postpartum negative energy balance in dairy cows, particularly at 0.5% propylene glycol supplementation[20].

Nevertheless, effects of exogenous enzymes in dairy diets show considerable variability. Klingerman et al.[21] found that amylase did not improve milk yield or composition in diets based on alfalfa hay, alfalfa haylage, corn silage, mixed legume hay, and corn silage. Peters et al.[22] reported no increase in milk production, and Arriola et al.[9] found no improvement in milk quality. These results indicate that enzyme type, substrate type, and dosage significantly affect application outcomes, necessitating further research to improve application reliability.

Recent studies suggest conducting in vitro simulation trials before animal experiments. Hu et al.[23] screened a cellulase-based complex that improved milk yield in lactating cows, while Holtshausen et al.[24] evaluated five cellulase supplementation levels in vivo, finding that 11.3% supplementation increased corrected milk yield. Similarly, Arriola et al.[9] conducted in vitro screening before feeding trials, finding that enzymes increased milk yield in low-concentrate diets but not high-concentrate diets. Therefore, careful research on dosage and application method is needed before adding exogenous enzymes to dairy diets.

While in vitro methods can help screen appropriate dosages and predict performance, enzyme activity may change over time, and many commercial products have undergone limited research under specific conditions, so consistent effects across all dairy cows cannot be guaranteed.

3.2. Beef Cattle

Although exogenous enzymes were reported to improve daily weight gain in beef cattle in the 1960s, enzyme application in ruminants has progressed slowly, primarily due to high cost and inconsistent effects[25]. However, optimized fermentation processes have significantly improved enzymatic properties and reduced production costs, providing a more practical foundation for enzyme application. Chen et al.[26] found that cellulase-based complex enzymes significantly improved daily weight gain in Charolais cattle, though effects remained variable. Beauchemin et al.[27] reported that supplementing steers fed alfalfa hay or timothy hay with xylanase and cellulase products increased daily weight gain by 30% and 36%, respectively, due to improved dry matter digestibility, but showed no effect when feeding barley silage. They also found that forage type significantly affected optimal dosage: 0.25–1.0 L/t DM for alfalfa hay versus 4 L/t DM for timothy hay. Thus, enzyme effects result from interactions between enzyme type, dosage, and substrate.

Beauchemin et al.[28] subsequently found that the same enzyme formulation improved feed conversion efficiency by 11% in steers fed high-concentrate barley-based diets but had no effect on corn-based high-concentrate diets. McAllister et al.[29] reported similar complex enzyme supplementation increased steer daily weight gain by 10% and significantly improved ADF digestibility. These studies indicate that xylanase- and cellulase-based complex enzymes can improve daily weight gain in beef cattle fed barley grain or forage-based diets, but show limited effects in corn-based or barley silage-based operations.

Amylase application also shows variability. Tricarico et al.[30] reported that an *Aspergillus oryzae*-derived amylase improved beef cattle daily weight gain in a quadratic manner, effective in diets containing ground corn, high-moisture corn, and corn silage, but ineffective in diets with alfalfa hay, cottonseed hulls, and steam-flaked corn. The enzyme became ineffective when feed intake was restricted. DiLorenzo et al.[31] also observed no effect of amylase at 600 U/kg DM in diets with dry-rolled or steam-flaked corn. This variability suggests the need for further research on effects of different amylase strains on common beef feedstuffs. Additionally, the limited amylase response may relate to grain processing already maximizing starch degradation in the rumen, leaving little room for exogenous enzyme effects.

Some studies have found no significant effects of exogenous enzymes in beef cattle. Vera et al.[32] reported that protease supplementation increased dry matter intake by 14.8% but accelerated rumen passage rate and reduced NDF digestibility by 4.1%, resulting in no increase in final body weight. ZoBell et al.[33] found no effect on daily weight gain or feed conversion efficiency when adding xylanase- and endoglucanase-based enzymes (5,580 and 15,880 U/kg DM) to barley-based diets with a 65:35 forage-to-concentrate ratio. However, McAllister et al.[29] found that adding 0.5 L liquid enzyme during the growing phase and 3.5 L during finishing improved intake and daily weight gain in cattle fed barley silage. Balci et al.[34] reported improved daily weight gain and feed conversion efficiency when adding 60 g of modified cellulase and xylanase to corn and barley diets. These results demonstrate that enzyme selection and substrate type critically affect enzyme efficacy.

Application timing also influences results. Lewis et al.[35] applied cellulase to hay and barley-based diets, examining enzyme treatment time and dietary concentrate-to-forage ratios, finding that adding enzymes 24 hours before feeding or at feeding time improved dry matter, NDF, and ADF digestibility without affecting dry matter intake. Similarly, Krueger et al.[36] studied different application times of complex enzymes on bermudagrass hay digestion, finding that adding enzymes after hay chopping improved intake but did not affect final body weight, daily weight gain, or feed conversion efficiency. Some studies show efficacy when adding enzymes to total mixed rations[29], while others demonstrate effects when adding to concentrates[34]; both methods currently improve animal performance, and the optimal approach remains undetermined. Overall, pre-feeding application of cellulase- and xylanase-based enzymes shows promising results, with increased enzyme-substrate contact time improving efficacy, though amylase and protease show limited effects in ruminants.

3.3. Sheep

Overall, exogenous enzyme supplementation shows limited effects in sheep. Miller et al.[37] added a commercial enzyme preparation to barley-based diets for Dorset crossbred sheep and found no significant effects on dry matter intake, daily weight gain, feed conversion, or wool production. Similarly, Flores et al.[38] reported no significant effects on milk yield, milk composition, or intake in Manchega dairy ewes. Rojo et al.[39] fed Suffolk lambs amylase from *Bacillus* species and *Aspergillus oryzae* and found no significant effects on performance.

Beyond production performance, effects on nutrient digestibility in sheep and goats have been reported. Reddish et al.[40] found that complex enzyme supplementation (4 g/head · d) did not significantly affect digestibility of dry matter, ADF, NDF, or nitrogen in lambs. Likewise, Giraldo et al.[41] added exogenous cellulase directly to the rumen of Merino lambs via fistula and found no effect on diet digestibility, possibly because direct ruminal addition reduced enzyme-feed interaction time and limited efficacy.

Nevertheless, positive results exist for small ruminants. Dai[42] found that supplementing sheep diets with cellulase combined with Tween-80 increased rumen xylanase, cellulase, and protease activities and improved feed digestibility to some extent. Bala et al.[43] added two levels of cellulase (4,000 and 8,000

U/kg) and xylanase (12,500 and 18,750 U/kg) complex to Saanen goats, finding that the high level reduced dry matter intake while increasing digestibility of dry matter, organic matter, crude protein, NDF, and ADF, thereby increasing corrected milk yield. These studies indicate limited enzyme efficacy in small ruminants, with no effects in lambs but some benefits in lactating goats and adult sheep, with outcomes influenced by enzyme type, supplementation level, and compound factors.

4. Potential Modes of Action of Exogenous Enzymes in Ruminants

Exogenous enzymes applied in ruminants mainly include carbohydrate-degrading enzymes and proteolytic enzymes, which enhance degradation of fiber and protein to improve feed digestibility. These enzymes generally improve feed utilization and animal performance through pre-feeding interactions, collaboration with rumen microorganisms, and post-rumen activities.

4.1. Pre-Feeding Action

Exogenous enzymes can increase soluble sugar content. The best effects are achieved when enzymes are added to diets before feeding, promoting soluble sugar release, though silage diets may show reduced efficacy due to certain components affecting enzyme activity[44]. Only minimal moisture is required—even the moisture content in dry hay and grains is sufficient for enzymes to degrade dietary polysaccharides[45]. Released sugars can accelerate microbial growth to some extent[15], with the degree of sugar release determined by both enzyme and substrate types. However, these released sugars represent only a small fraction of total dietary carbohydrates, so this limited energy may not substantially improve animal performance.

Adequate enzyme-feed binding reduces activity loss. Close binding between enzymes and feed allows better enzyme activity and reduces the likelihood of inactivation in the rumen. Bowman et al.[46] found that adding enzymes to crushed grain feed (45% of diet) was more effective than adding them to premixes. Therefore, to maximize enzyme efficacy, enzymes should have maximum contact with the diet and be added to major dietary components, particularly in total mixed rations.

4.2. Rumen Action

Optimizing enzyme structure improves stability. Initially, exogenous enzymes were thought to be degraded by rumen microbial enzymes, but they have been found to remain stable in the rumen[2,45]. Morgavi et al.[47] reported that four commercial enzyme preparations remained stable in rumen fluid, pepsin, and pancreatic fluid. This stability may relate to enzyme glycosylation and to improved stability from pre-feeding addition. Additionally, enzymes from certain microbial flora can resist hydrolysis by rumen fluid even without glycosylation[48]. Rumen stability is crucial for enzyme efficacy and may contribute to variability in production responses.

Enhanced rumen enzyme activity improves digestibility. Exogenous enzymes improve ruminant diet digestibility but rarely increase the extent of digestion, suggesting that enzymes producing favorable performance responses do so not by improving digestion of indigestible components but by increasing the digestibility of digestible components. Exogenous enzymes can increase total rumen enzyme activity and overall hydrolytic capacity, thereby improving digestibility of all dietary components[15]. This explains why cellulase products can simultaneously improve digestibility of both fiber and non-fiber components.

Promoting bacterial adhesion enables collaborative function. Exogenous enzymes represent only a small fraction compared to inherent rumen enzymes, making it difficult to attribute improved fiber degradation solely to direct enzyme action. Therefore, collaborative relationships between exogenous and microbial enzymes and enhanced microbial adhesion to plant fiber are hypothesized. Although the mechanism remains unclear, exogenous enzymes may promote cellulose degradation by increasing bacterial adhesion to fiber and breaking hydrogen bonds in rigid fiber matrices[49]. However, increased exogenous enzymes may compete with rumen microbes for feed binding sites[45], explaining negative effects at high supplementation levels. Thus, optimal enzyme efficacy requires maintaining activity without completely replacing naturally occurring rumen enzymes.

Reduced viscosity increases intake. Choct[50] found that enzymes reduced digesta viscosity in poultry; similar effects in ruminants could increase rumen content passage rate, reduce gastrointestinal fill, and improve intake. Increased intake is important for improving performance, but rapid enzyme outflow from the rumen may prevent adequate time for fiber degradation, and most enzyme activity is quickly lost in the abomasum due to low pH and pepsin. Therefore, enzyme supplementation must be controlled to reduce viscosity and increase intake while improving digestibility.

4.3. Post-Rumen Action

Post-rumen enzyme action primarily involves increasing post-rumen enzyme activity to promote feed degradation and nutrient absorption. Hristov et al.[51] first reported that approximately 30% of xylanase could escape the rumen and reach the small intestine, consistent with findings in pigs. At high supplementation levels, other enzymes may also bypass the rumen and increase polysaccharide degradation in the small intestine. Although the abomasum is thought to prevent exogenous enzymes from entering the small intestine due to low pH and pepsin, some enzymes have been reported to pass through. Morgavi et al.[47] suggested that some exogenous enzymes can survive in rumen fluid, abomasal, and small intestinal environments, with fecal polysaccharide-degrading capacity increasing linearly with enzyme activity[52]. For individual enzymes, xylanase activity is superior to cellulase in the rumen and abomasum, resulting in higher xylanase activity in small intestinal fluid. Current research on post-rumen enzyme effects is limited, and future studies should investigate rumen bypass effects and action modes of different enzymes to maximize nutrient digestibility.

Theoretically, exogenous enzymes can improve animal performance by enhancing rumen carbohydrate degradation, reducing digestive tract viscosity, and improving post-rumen nutrient absorption. However, actual post-rumen effects remain unclear, with current evidence suggesting primary action through rumen function improvement, necessitating further post-rumen research.

5. Future Prospects for Exogenous Enzyme Application in Ruminants

As intensification and scale increase, reducing production costs, improving economic efficiency, and minimizing environmental pollution will be key directions for ruminant operations. Green additives therefore have promising prospects, and exogenous enzymes that improve nutrient digestibility, particularly forage utilization, will play important roles. However, further research is needed to optimize ruminant exogenous enzymes. Based on practical production conditions, in-depth research should focus on the following aspects.

5.1. Using New Technologies to Study Rumen-Enzyme Collaboration

New technologies such as DNA and RNA sequencing analysis should be used to understand rumen microbial community structure and function. Metagenomics and proteomics should be applied to explore collaboration between exogenous and endogenous enzymes, improving the targeting and efficiency of enzyme supplementation. This will clarify the mechanisms and physiological pathways by which exogenous enzymes degrade plant cell walls and improve feed utilization in ruminants[53].

5.2. Selecting Appropriate Enzyme Types

Based on ruminant digestive physiology and dietary composition, cellulase and xylanase should be primarily selected for rumen application, as they degrade cellulose and hemicellulose into hexoses and pentoses that are further converted to volatile fatty acids for utilization. However, amylase, lipase, and protease are not recommended for rumen addition. Starch is already extensively degraded in the rumen, making exogenous amylase effects negligible. Dietary fat content is low in ruminant diets, requiring no lipase supplementation. Additionally, since increasing rumen-bypass protein is generally desired, ruminal protease is unnecessary, though post-rumen protease could be considered to improve whole-tract protein digestibility. Recent studies have found that ferulic acid esterase plays an important role in degrading ferulic acid ester bonds between hemicellulose and lignin, improving cell wall degradation[54], requiring further production trials for validation. These findings indicate that appropriate enzyme profiles must be selected based on actual ruminant production and physiological conditions to achieve optimal results.

5.3. Collaborative Action of Compound Enzyme Preparations

Understanding the characteristics of ruminant exogenous enzymes and selecting appropriate auxiliary additives can achieve optimal production effects. While exogenous enzymes can improve feed digestibility and performance to some extent, they have limitations. Combining them with other ruminant additives such as probiotics and vitamin preparations can further improve animal health and feed utilization, achieving more desirable production outcomes.

Exogenous enzyme application in ruminants is in a developing stage. Although current applications show some variability, particularly with limited effects in small ruminants, and enzymes have certain limitations such as weak acid-base resistance and temperature instability, future improvements using new technologies like metagenomics and optimized in vitro simulation methods to understand enzyme properties and mechanisms, along with advances in strain screening, enzyme profile selection, application methods, and compound preparations, will enable these eco-friendly, green enzyme preparations to play increasingly important roles in ruminant production.

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Note: Figure translations are in progress. See original paper for figures.