Feed Enzyme Preparations Modulate Intestinal Microecology in Monogastric Animals and Potential Mechanisms of Action: Postprint

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Abstract

In recent years, through the application of novel molecular microbiology techniques and data analysis methods, numerous studies have demonstrated that feed enzymes not only promote the digestion and absorption of nutrients in the organism, but also affect the development of specific microorganisms in the gut. The mechanism may involve modulating the physicochemical properties of chyme and the quantity of nutrients required by gut microorganisms, while simultaneously increasing the content of substances with potential prebiotic effects, thereby influencing the development of gut microecology. This paper aims to summarize the effects of dietary supplementation with feed enzymes on the gut microecology of monogastric animals and explore the underlying mechanisms.

Full Text

Gut Microecosystem of Monogastric Animals Regulated by Feed Enzymes and Its Possible Mechanism

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Abstract

In recent years, the application of novel molecular microbiology techniques and data analysis methods has confirmed that feed enzymes not only promote nutrient digestion and absorption but also influence the development of specific intestinal microbial populations. The underlying mechanism likely involves modulation of the physicochemical properties of chyme and the availability of nutrients required by gut microbes, while simultaneously increasing the content of

substances with potential prebiotic effects, thereby shaping gut microecosystem development. This paper aims to summarize the effects of dietary feed enzyme supplementation on the gut microecosystem of monogastric animals and explore the associated mechanisms.

Key words: feed enzymes; monogastric animal; gut microecosystem

Introduction

Accumulating research has demonstrated that intestinal microorganisms play crucial roles in multiple physiological functions of livestock and poultry, including gut tissue development, immune system maturation, and nutrient digestion and absorption [1-3]. Disruption of gastrointestinal microecological balance often accompanies altered physiological function and disease onset; for instance, excessive proliferation of *Escherichia coli* represents a major cause of post-weaning diarrhea in piglets [4]. However, the diversity of gastrointestinal microbiota is influenced by numerous factors, including chyme composition, pH, transit rate, and endogenous enzymes [1,5]. Feed enzymes, primarily intended to promote dietary nutrient digestion, alter the chemical composition and physical characteristics of intestinal chyme, consequently affecting gut microbes by modifying their living environment. Nevertheless, recent feed enzyme research has predominantly focused on growth performance and nutrient digestibility, with relatively limited investigation into effects on intestinal microbiota and underlying mechanisms. Therefore, this review synthesizes domestic and international literature on feed enzymes' impact on gut microecosystem and attempts to elucidate possible mechanisms, providing theoretical support for expanding the scientific application of feed enzymes in animal production.

1.1 Modulating Physicochemical Properties of Chyme Through Substrate Degradation

Feed enzymes degrade non-starch polysaccharides (NSP) that constitute plant cell walls, disrupting the hydration shell surrounding chyme and thereby influencing its composition and physicochemical characteristics. Since microbial species differ in substrate preferences and nutritional requirements, chyme composition and structure profoundly affect intestinal microbial distribution. The relationship between feed enzymes and gut microecosystem [Figure 1: see original paper] can be understood through two aspects: first, dietary composition itself affects chyme physicochemical properties and gastrointestinal physiological function; second, feed enzymes exert regulatory effects by degrading or digesting substrates within the gastrointestinal tract, altering their content. This degradative action forms the theoretical basis for feed enzyme development and application, as their target substrates cannot be effectively degraded by endogenous digestive enzymes, thereby impairing nutrient absorption. For example, the increased chyme viscosity caused by indigestible NSP represents the most

important factor reducing feed utilization efficiency in broilers [6-8]. Elevated chyme viscosity decreases intestinal motility, slows chyme transit, and allows prolonged microbial proliferation and metabolism in the digestive tract, often accompanied by massive proliferation of harmful bacteria that compromise gut function and animal health. Supplementing NSP enzymes (e.g., xylanase and glucanase) partially or completely degrades soluble NSP, reducing chyme viscosity and modulating the microenvironment of the posterior digestive tract, consequently altering intestinal microbial composition and metabolic status [8-10]. Thus, feed enzymes influence microbial status by acting on major feed ingredients (target substrates) to modify the physicochemical state of chyme.

1.2 Influencing Nutrients Required for Microbial Metabolism

Nutrients required by gut microbes originate from chyme (dietary components and endogenous secretions) that resist digestive degradation or are absorbed very slowly by the host, enabling successful competition between microbes and the host. Therefore, the degradative capacity and efficiency of enzymes determine the types and quantities of nutrients available to microbes. For instance, phytase supplementation increases available phosphorus levels in the gut [12], thereby affecting microbial populations regulated by available calcium and phosphorus. Feed enzymes modulate the supply of nutrients required for gut microecosystem by degrading target substrates. A crucial metabolic function of gut microbes is degrading and fermenting undigested food residues (non-digestible carbohydrates, etc.) and mucins produced by epithelial cells to obtain energy for growth and reproduction [13]. Dietary fiber, particularly soluble NSP, significantly affects the flow of undegraded dry matter entering the hindgut. Research indicates that colonic microbes can ferment 40-60 g of carbohydrates daily, producing volatile fatty acids (VFA) that serve as an energy source promoting microbial proliferation [14]. When the flow of undegraded dry matter into the hindgut increases substantially, it leads to massive microbial proliferation that competes with the host for available nutrients, causing microecological disturbance and nutritional diarrhea. Since gut microbiota can only compete with the host for available nutrients and different microbes have distinct substrate preferences and growth requirements, feed enzymes can regulate microbial distribution by influencing the digestive degradation status of chyme.

1.3 Increasing Substances with Prebiotic Effects

Increasing prebiotic substances represents another mechanism through which feed enzymes may influence gut microecosystem development. Plant ingredients and byproducts used in feed manufacturing contain virtually all types of polysaccharides. Supplementation with specific NSP mono-enzymes or complex enzymes can degrade these polysaccharides in the gut to generate large quantities of oligosaccharides. For example, adding xylanase to wheat-based broiler diets increased short-chain xylo-oligosaccharide content in the cecum fivefold [9]. Studies have confirmed that oligosaccharides can be metabolized by beneficial

gut bacteria and selectively promote acid-producing bacteria such as *Bifidobacterium* and *Lactobacillus* while inhibiting opportunistic pathogens. *Bifidobacterium* maintains species dominance in the gut through itself and its metabolites, excludes pathogenic bacteria, and interacts with other flora to adjust interspecies relationships, ensuring optimal microbial community composition and maintaining functional balance [15]. Campbell et al. [16] found that feeding fructooligosaccharides and xylooligosaccharides increased *Bifidobacterium* populations by 8% and 17%, respectively, while total VFA content increased by 67.7% and 74.6%, with corresponding pH reductions of 7% and 11%, creating an environment conducive to probiotic proliferation and pathogen inhibition. Among the generated VFAs, butyrate can promote growth of certain beneficial bacteria and maintain dynamic equilibrium of intestinal microbiota. The remaining oligosaccharides not utilized by beneficial bacteria can also bind and remove pathogenic bacteria from the body, preventing pathogen colonization in the gut and achieving inhibition of harmful bacteria.

2.1 Effects of NSP Enzymes on Gut Microbiota

NSP enzymes, including xylanase, β-glucanase, cellulase, and β-mannanase, degrade high-viscosity soluble NSP into polysaccharide fragments or oligosaccharides. Research demonstrates that NSP enzymes exert prebiotic effects on beneficial bacteria while inhibiting harmful bacteria. Hu et al. [17] found that adding 200 U/kg xylanase to growing pig diets with 40% wheat replacing corn increased colonic *Lactobacillus* and *Bifidobacterium* populations by 20% and 11%, respectively, while decreasing *E. coli* and *Clostridium* by 11% and 15%. Ye et al. [18] reported that supplementing 1,000 U/kg xylanase to 50% wheat diets significantly increased cecal *Bifidobacterium* by 8.5% in weaned piglets. These studies suggest that NSP enzyme supplementation effectively modulates gut microecosystem, likely by reducing intestinal content viscosity and degrading soluble NSP into functional oligosaccharides that stimulate beneficial bacteria to produce β-glycosidases, selectively generating short-chain fatty acids like propionate and butyrate as carbon sources. This lowers gastrointestinal pH, inhibits pathogen colonization, and promotes rapid proliferation of beneficial bacteria. Similar studies by Engberg et al. [19] and Ding et al. [20] found that xylanase supplementation in all-wheat diets increased ileal *Lactobacillus* by 3.6% and 16.5%, respectively. Li [21] demonstrated that β-mannanase supplementation in corn-soybean meal diets for weaned piglets increased *Lactobacillus* and *Bifidobacterium* while reducing *E. coli*. Zhou et al. [22] showed that dietary β-glucanase for weaned piglets did not affect *Lactobacillus* counts but significantly reduced *E. coli* (by 6%). Beneficial bacteria represented by *Lactobacillus* and *Bifidobacterium*, and pathogenic bacteria represented by *E. coli*, constitute normal gut flora that play vital roles in maintaining microecological balance [23]. However, some studies found no significant effects of feed enzymes on gut microbiota. O' Connell et al. [24] reported that NSP enzyme supplementation in 70% wheat diets did not affect cecal and colonic microbial populations in growing-finishing pigs. Smith et al. [25] and Reilly et al. [26] observed similar results in all-barley

or all-oat diets supplemented with NSP enzymes. This may be due to insufficient NSP enzyme supplementation relative to high NSP content in these diets, preventing effective modulation of intestinal chyme physicochemical structure or composition and thus failing to significantly influence gut microecosystem. Hübener et al. [9] found that 400 U/kg xylanase in wheat-rye diets reduced total bacterial counts and specific bacterial populations while also decreasing total VFA content. Therefore, higher NSP enzyme doses are required for high-NSP diets to exert effects on gut microbiota.

2.2 Effects of Phytase on Gut Microbiota

Calcium and phosphorus are important regulators of gut microecosystem development, and phytase supplementation increases available phosphorus, thereby modulating gut microbiota. Ptak et al. [12] added 5,000 FTU/kg phytase to diets with normal or deficient calcium and digestible phosphorus levels. Using fluorescence in situ hybridization to detect ileal microbial populations, they found that phytase in calcium/digestible phosphorus-deficient diets significantly increased ileal total bacteria (5.0%), *Lactobacillus* (2.4%), and *Enterococcus* (1.2%). Additionally, dietary calcium/digestible phosphorus levels and phytase showed interactive effects on *Clostridium*, *Clostridium coccoides-Eubacterium rectale*, *Bifidobacterium*, and *Streptococcus-Lactococcus* populations. Both calcium-phosphorus ratio and phytase individually affected microbial community structure, with additive effects, indicating that phytase modulates total bacterial counts by increasing available phosphorus for microbial utilization. Through quantitative PCR analysis of ileal chyme, Metzler-Zebeli et al. [27] found that 1,000 FTU/kg phytase in low-phosphorus diets for growing-finishing pigs significantly increased strict anaerobes such as *Clostridium coccoides*, *Clostridium leptum*, and *Bacteroides-Prevotella-Porphyrmonas*, with a trend toward increased *Enterobacteriaceae*, while total bacterial counts remained unchanged. Wang et al. [28] used 16S rRNA analysis of ileal microbiota in weaned piglets and found that two phytase sources increased *Bifidobacterium*, *Lactobacillus*, and *Clostridium* but also increased *E. coli* and *Salmonella*. Liang et al. [29] added different phytase sources to calcium/phosphorus-deficient diets (with reduced dicalcium phosphate by 1/3) and found no significant changes in aerobic bacteria, total anaerobes, *Lactobacillus*, *Bifidobacterium*, or *E. coli* in ileum and cecum compared to normal calcium/phosphorus levels, suggesting that phytase can compensate for calcium/phosphorus deficiency by increasing available minerals for microbial reproduction, thereby maintaining stable gut microbiota. Phytase significantly affects calcium/phosphorus digestibility, mineral absorption, mucin secretion, and endogenous losses, all of which influence nutrient utilization and intestinal environment. These results demonstrate phytase' s important role in modulating gut microbiota, though its effects are clearly influenced by dietary calcium/phosphorus levels.

2.3 Effects of Complex Enzyme Preparations on Gut Microbiota

Complex enzyme preparations can act on multiple substrates and suit various feed types, though their effects on gut microbiota show variability. Kiarie et al. [30] found that a complex enzyme (pectinase + cellulase + mannanase + xylanase + glucanase + galactosidase) significantly increased ileal *Lactobacillus* (by 14%), suggesting that hydrolysis products such as arabinose, xylose, and mannose residues generated in the gut can serve as nutrients for *Lactobacillus* metabolism and promote proliferation. Pluske et al. [5] summarized similar conclusions: carbohydrate residues produced by dietary carbohydrase supplementation can promote proliferation of beneficial gastrointestinal flora. Yi et al. [31] added a complex enzyme (amylase + protease + xylanase) to corn-soybean meal diets and found that cecal and colonic *Lactobacillus* increased significantly (by 6%) while colonic *E. coli* decreased (by 11%). Agboola et al. [32] reported that a complex enzyme (amylase + protease + xylanase) in corn-soybean meal diets significantly increased ileal *Lactobacillus*. In these studies, complex enzymes effectively increased beneficial bacteria while reducing harmful bacteria, likely by modulating chyme physicochemical properties and composition, regulating nutrient availability for microbial metabolism, and increasing potential prebiotic substances. Similarly, Högberg et al. [23] found that complex enzyme (xylanase + β-glucanase) supplementation in cereal diets increased the molar proportion of lactic acid in the ileum, indicating increased *Lactobacillus* populations. Chai et al. [33] added NSP complex enzymes (β-glucanase + β-xylanase + β-mannanase + protease) to weaned piglet diets and observed a trend toward increased fecal *Lactobacillus* (14%) and decreased *E. coli* (9%). Baurhoo et al. [34] found that a complex enzyme (protease + cellulase + xylanase + β-glucanase) had no effect on *Lactobacillus* or *E. coli* in corn-based diets. Some studies reported opposite results: Smith et al. [25] found that complex enzyme (xylanase + β-glucanase) supplementation in 67% barley or 64.5% oat diets significantly reduced ileal *Lactobacillus* (by 144% and 56%) and tended to reduce ileal *Bifidobacterium* and *Enterobacter* as well as cecal *Bifidobacterium*, *Enterobacter*, and *Lactobacillus*, though the reasons require further investigation. Compared with single enzymes, complex enzymes show more targeted degradation and adaptability to multiple substrates, more effectively regulating gut microbiota development while improving nutrient digestion and absorption. Therefore, complex enzyme preparations represent an important future direction for enzyme development.

Future Perspectives

Feed enzymes significantly influence gut microecosystem development and show promise as gut microecological modulators, though further investigation is needed. First, current research primarily focuses on promoting nutrient digestion and absorption, with limited application studies targeting gut microecosystem improvement. Second, the regulatory effects of feed enzymes on gut microecosystem are influenced by multiple factors including animal physiological status, dietary composition, and initial gut microbiota status, yet

the interactions between enzymes and these factors remain unclear. Third, the enzymatic hydrolysis rate and efficiency in the complex intestinal environment, as well as the effects of substrates and products on microbial metabolism, are poorly understood, and research methods are relatively limited. For instance, NSP enzyme degradation of NSP to produce oligosaccharides involves uncontrollable product profiles and fermentation rates, affecting microecological modulation efficacy. Recent studies have identified some enzymes that directly affect gut microbiota to achieve ideal modulation, such as alkaline phosphatase and glucose oxidase [35-37]. Therefore, strengthening research on feed enzymes for regulating gut microecosystem development and fully exploiting their dual value in promoting nutrient digestion and improving animal gut health is crucial for enhancing animal production performance and health.

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