Effects of Exogenous Protease on Growth Performance, Nutrient Digestibility, Health, and Environmental Emissions in Pigs and Chickens: A Postprint

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Abstract

Exogenous proteases have attracted considerable attention due to their ability to improve growth performance and health in livestock and poultry, enhance the digestibility of protein, amino acids, and other nutrients, and conserve feed resources. The effects of exogenous proteases on growth performance, nutrient digestibility, and health in pigs and chickens are influenced by factors such as animal age, diet type, and enzyme characteristics. This paper summarizes the classification of proteases, methods for enzyme activity assay, and their functions, with particular emphasis on analyzing the effects of exogenous proteases on growth performance, nutrient digestibility, and health in pigs and chickens, and discusses the impact of exogenous protease supplementation on environmental emissions in pig and chicken production, aiming to provide a theoretical basis for the efficient utilization of exogenous proteases in livestock and poultry farming.

Full Text

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Abstract

Exogenous proteases have attracted considerable attention for their ability to improve growth performance and health in livestock and poultry, enhance the digestibility of nutrients such as protein and amino acids, and conserve feed resources. The effects of exogenous protease on growth performance, nutrient digestibility, and health in pigs and chickens are influenced by factors including animal age, diet type, and enzyme characteristics. This paper provides an overview of protease classification, methods for measuring enzyme activity, and their functions, with particular emphasis on analyzing the effects of exogenous protease on growth performance, nutrient digestibility, and health in pigs and chickens. Additionally, the impact of exogenous protease supplementation on environmental emissions in pig and chicken production is discussed, aiming to provide a theoretical basis for the efficient utilization of exogenous protease in livestock production.

Keywords: exogenous protease; growth performance; nutrient digestibility; animal health; environmental emission

Feed ingredients contain various anti-nutritional factors such as phytic acid, non-starch polysaccharides, and protein inhibitors that limit nutrient utilization by animals. Feed enzymes show broad application prospects in improving growth performance, promoting animal health, and regulating gene function in monogastric animals. The global feed enzyme market is estimated to reach $1.37 billion by 2020 [1]. Currently, most proteases used in feed and livestock enterprises are microbial-derived, and their mechanisms of action have attracted widespread research attention. Numerous studies have shown that exogenous protease supplementation can improve growth performance and health in livestock and poultry, reduce the effects of anti-nutritional factors in feed, enhance nutrient digestibility (especially protein and amino acids), and decrease nitrogen content in excreta, thereby reducing environmental pollution from ammonia, nitrates, and nitrites [2-6]. However, due to variations in enzyme characteristics, feed substrate background, target animals, and application methods, experimental results have been inconsistent, with some even showing negative outcomes [7]. Therefore, this review summarizes protease classification, activity measurement methods, mechanisms of action, and functions, focusing on analyzing the effects of exogenous protease on growth performance, nutrient digestibility, health, and environmental emissions in pigs and chickens, to provide a theoretical reference for the rational and efficient utilization of exogenous protease in pig and chicken diets.

1 Protease

Proteases are a general term for enzymes that hydrolyze proteins, widely present in animal viscera, plant stems, leaves, fruits, and microorganisms. Their function is to break peptide bonds, destroy the primary structure of proteins, and hydrolyze proteins into small molecular polypeptides and amino acids. Animals can secrete a certain amount of endogenous proteases (pepsin, trypsin, chymotrypsin, etc.) to digest nutrients in feed, but large amounts of nutrients remain undigested and are excreted into the environment through feces, wasting feed resources and causing environmental pollution.

1.1 Classification of Proteases

Proteases can be classified into different types based on various criteria. First, by source: animal-derived proteases, plant-derived proteases, and microbial-derived proteases. Animal-derived proteases are mainly extracted from the pancreas of large animals such as cattle, sheep, and pigs, with high production costs, and are primarily used in medicine or as reagents. Common plant-derived proteases include papain and bromelain, extracted from unripe papaya and pineapple, respectively. Microbial-derived proteases are mainly obtained through bacterial cultivation and fungal fermentation of *Aspergillus niger*, *Aspergillus* spp., and *Bacillus* spp. Compared with the other two types, microbial-derived proteases have strong specificity, are safe and efficient, have stable properties, and are not time-controlled [8], making them the most widely used in feed and livestock enterprises. The main strains used for industrial protease production in China are *Bacillus* spp., including *Bacillus licheniformis* 2709, *Bacillus licheniformis* C1213, and *Bacillus pumilus* 289 and 209 [9].

Second, by optimal pH: acid proteases, neutral proteases, and alkaline proteases. Currently, neutral and acid proteases are most commonly used in the feed industry, including pepsin, rennet, and some microbial proteases. Acid proteases mainly act in the stomach, and their addition to feed can improve protein digestion and absorption rates in animals (especially young animals) and enhance their growth performance [4].

Third, by active site: aspartic proteases, thiol proteases, serine proteases, etc. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) classifies proteases into endopeptidases and exopeptidases based on their position of action on peptide chains during protein degradation; for example, aspartic proteases and serine proteases are endopeptidases [10].

1.2 Methods for Measuring Protease Activity

Protease activity is affected by many factors: (1) Temperature: proteases are active within a certain temperature range, with maximum activity at the optimal temperature. Low temperatures reduce enzymatic reaction rates, while high temperatures cause enzyme inactivation. (2) pH: proteases only show activity within a certain pH range; enzymes become inactive above or below this range, and the pH at which enzyme activity is maximal is the optimal pH. (3) Activators or inhibitors promote or inhibit enzymatic reactions. (4) Metal ions: magnesium (Mg^{2}), calcium (Ca^{2}), and other metal ions can affect protease activity to varying degrees.

Professional standards, national standards, and some corporate standards in China and abroad are not unified in their methods for measuring protease activity and definitions of enzyme activity. Professional standard ZB X 66030-87 states that the Folin method is generally used to measure protease activity, defining one unit of protease activity as the amount that hydrolyzes casein to produce 1 g of tyrosine per minute at 40°C. National standard GB/T 23527-2009 specifies using the Folin method or UV spectrophotometry to measure microbial protease activity, defining one unit of enzyme activity (U/g or U/mL) as the amount in 1 g of solid enzyme powder (or 1 mL of liquid enzyme) that hydrolyzes casein to produce 1 g of tyrosine per minute under certain temperature and pH conditions. National standard GB/T 28715-2012 specifies using spectrophotometry to measure acid and neutral protease activity, defining one unit (U) as the amount of enzyme that hydrolyzes casein to produce phenolic amino acids equivalent to 1 g of tyrosine per minute at (40±0.2)°C and corresponding pH (pH 3.0 for acid protease, pH 7.2 for neutral protease). Novozyme (Denmark) defines serine protease activity as: under conditions of pH 9.0 and 37°C, the amount of enzyme required to release 1 mol of p-nitroaniline from 1 mol/L substrate per minute is defined as one unit, i.e., 1 PROT. Shanghai Jielong Bio-Products Co., Ltd. notes that keratinase activity can be simply measured using the UV spectrophotometry method with azocasein as substrate [11].

Literature also shows inconsistencies in enzyme activity definitions and descriptions of units. Fru et al. [12] and Angel et al. [13] defined a protease activity unit as the amount of enzyme required to release p-nitroaniline from substrate per minute under conditions of pH 9.0 and 37°C. Yu et al. [14] defined one unit of protease activity as the amount that releases tyrosine from hemoglobin substrate per minute at pH 7.5 and 30°C. Bai et al. [15] defined protease activity as the amount of tyrosine produced by 1 g of protease hydrolyzing casein per minute at 37°C (U/g). Yu et al. [16] noted that the Coomassie brilliant blue staining method can be used to simply and environmentally measure ginger protease activity, defining enzyme activity as the amount of enzyme required to decompose 1 g of bovine serum albumin per minute at 37°C (U).

Currently, there is no unified standard for protease activity measurement methods, substrates, pH, temperature, and other conditions. Moreover, national standards lack standard methods for alkaline protease activity measurement, making comparisons of enzyme activity measurements difficult.

2 Functions of Protease

Research reports on the functions of exogenous protease in feed mainly include the following aspects: (1) Directly decomposing substrates to improve the digestion and utilization of nutrients such as protein. Exogenous protease can increase the solubility of protein in soybean meal and improve amino acid metabolism rates [17-19]. (2) Enhancing the activity of endogenous enzymes in animals. Zuo et al. [20] added exogenous protease at three different levels (100, 200, 300 mg/kg) to corn-soybean meal diets, significantly increasing the activities of pepsin, pancreatic amylase, and trypsin in weaned piglets. (3) Eliminating anti-nutritional factors in feed and improving digestive capacity. Hosoyama et al. [5] showed that protease can reduce the effects of anti-nutritional factors in soybean meal and improve its nutritional value. (4) Enhancing animal immunity. Bromelain can improve anti-diarrhea ability in piglets [21]. (5) Participating in endocrine regulation in animals and improving growth performance by altering certain blood components [22]. (6) Improving the farming environment. Adding protease to livestock and poultry diets can greatly reduce nitrogen emissions in broiler feces [6].

3.1 Effects of Exogenous Protease on Growth Performance of Pigs and Chickens

Numerous studies have shown that protease can significantly improve weight gain and feed conversion ratio in broilers, thereby enhancing their growth performance. Yu et al. [14] added 125 mg/kg alkaline protease (enzyme activity 25,000 U/g) to corn-soybean meal diets, significantly improving average body weight and feed conversion ratio in broilers at 1-21 days, 22-38 days, and 1-38 days of age. Li et al. [2] added 300 mg/kg alkaline protease (enzyme activity 100,000 U/g) to corn-soybean meal diets, significantly increasing average body weight at 21 and 42 days and average daily gain (ADG) at 1-21 days and 1-42 days, while significantly reducing feed-to-gain ratio at 1-21 days. Zhuang et al. [23] added 500×10 and 600×10 low-temperature alkaline protease (from marine bacteria YS-80-122) to corn-soybean meal basal diets, resulting in ADG increases of 5.06% and 3.98%, final weight increases of 70 g and 100 g, and feed-to-gain ratio reductions of 0.05 and 0.06 in broilers, respectively. Exogenous protease also improves growth performance in animals fed diets other than corn-soybean meal types. Mahmood et al. [24] added 200 mg/kg combined protease (containing 8,000 U/g acid protease and 12,000 U/g neutral protease) to by-product-based diets, significantly increasing average daily feed intake (ADFI) and ADG at 1-21 days and improving carcass yield in broilers. However, Purshotham et al. [7] added 6,000 U/kg compressed protease to corn-soybean meal diets with 2% reduced protein level, significantly reducing ADFI and feed conversion ratio in broilers throughout the growth period.

Reports on the effects of protease supplementation in pig diets have been inconsistent. Stephenson et al. [25] added 500 mg/kg keratinase (DP100) to low-lysine corn-soybean meal-dried distillers grains with solubles (DDGS) diets for growing pigs, significantly increasing ADFI at 1-131 days but significantly decreasing carcass yield. Liu et al. [26] found that adding 300 mg/kg papain increased ADFI by 2.7% and ADG by 2.56% in piglets compared with the control group, but the differences were not significant. O' Shea et al. [27] added 200 mg/kg protease to rapeseed meal-wheat-DDGS diets for growing-finishing pigs, significantly improving ADG, ADFI, and body weight. However, McAlpine et al. [28] added 200 mg/kg protease (enzyme activity 75,000 U/g) to wheat shorts-rapeseed meal-wheat-barley diets for growing pigs, significantly reducing ADFI, ADG, and final body weight at 1-28 days.

Exogenous protease can degrade specific polypeptides in protein-rich ingredients such as meals, supplement the insufficient secretion of endogenous protease in the digestive tract of broilers and piglets, and stimulate endogenous enzyme secretion [29], thereby improving nutrient digestibility and utilization and promoting growth performance. However, factors such as animal age, diet type, and enzyme characteristics affect protease efficacy [30], and exogenous protease shows more pronounced effects on improving production performance in young animals.

3.2 Effects of Exogenous Protease on Nutrient Digestibility in Pigs and Chickens

Protease can decompose macromolecular substances in diets, reduce the effects of anti-nutritional factors, and improve the digestibility of not only protein and amino acids but also other nutrients. Zhou [4] added 200 mg/kg alkaline protease (enzyme activity 75,000 PROT/g, from *Bacillus licheniformis*) to corn-soybean meal-meat and bone meal basal diets, increasing the apparent ileal digestibility of crude protein by 5.92% in 21-day-old broilers and significantly improving the apparent ileal digestibility of serine, glycine, alanine, methionine, tyrosine, and proline. Fru et al. [12] added 200 mg/kg alkaline protease (enzyme activity 75,000 PROT/g, from *Bacillus licheniformis*) to corn-soybean meal basal diets, increasing the apparent ileal digestibility of protein, fat, and energy by 5.9%, 2.5%, and 7.2%, respectively. Angel et al. [13] added 100-800 mg/kg alkaline protease (enzyme activity 75,000 PROT/g, from *Bacillus licheniformis*) to corn-soybean meal-corn gluten meal diets with reduced crude protein levels, significantly improving the digestibility of crude protein and most essential amino acids (arginine, isoleucine, leucine, etc.) and some non-essential amino acids in 7-22-day-old broilers. Stefanello et al. [31] added 15,000 U/g protease (enzyme activity 75,000 PROT/g) to soybean meal and corn-soybean meal semi-purified diets for broilers, significantly improving the apparent ileal digestibility of essential and non-essential amino acids, as well as increasing ileal and total tract digestibility of protein, dry matter, and energy. Freitas et al. [32] added 200 mg/kg protease (enzyme activity 75,000 PROT/g) to corn-soybean meal-meat and bone meal diets for broilers, significantly improving ileal digestibility of crude protein and fat. Cowieson et al. [33] added 15,000 PROT/kg serine protease to wheat-soybean meal diets (containing 100 mg/kg each of phytase and xylanase) for broilers, significantly increasing the apparent ileal corrected digestibility of nitrogen, essential amino acids, non-essential amino acids, and total amino acids. Mahmood et al. [34] added 200 mg/kg protease (containing 8,000 U/g acid protease and 12,000 U/g alkaline protease) to by-product-based diets and corn-miscellaneous meal-by-product-based diets, significantly improving apparent metabolizable energy, nitrogen-corrected apparent metabolizable energy, nitrogen retention rate, and apparent nitrogen digestibility in broilers. Zhang et al. [35] added exogenous protease at 75,000 and 150,000 PROT/g (enzyme activity 90,000 PROT/g, optimal pH 7.0-10.0, from *Bacillus licheniformis*) to corn-soybean meal diets for broilers, using the 仿生法 (simulation method) to measure nutrient digestibility. The results showed that in vitro dry matter digestibility, gross energy digestibility, and in vitro digestible energy of the total digestive tract were significantly improved, but in vitro dry matter digestibility and gross energy digestibility in the gastric digestion stage of 22-42-day-old broiler diets were significantly reduced.

Pan et al. [36] added 200 mg/kg coated compound protease (8,000 U/g) to corn-soybean meal diets for Duroc×Landrace×Yorkshire crossbred pigs, significantly improving apparent total tract digestibility of dry matter, gross energy, crude protein, and organic matter, as well as apparent ileal digestibility of crude protein, nitrogen, and most essential amino acids. Li et al. [37] mixed two acid proteases and one neutral protease at a 3:3:2 mass ratio (enzyme activities 88,000, 59,000, and 129,000 U/g, respectively) and added 1,000 mg/kg of the protease complex to corn-soybean meal, corn-miscellaneous meal, and wheat-miscellaneous meal diets for Luyan White growing pigs. The results showed no significant difference in crude protein digestibility for the corn-soybean meal diet compared with the control group, but nutrient digestibility significantly increased in the other two diet types. Wang et al. [38] added 500 and 1,000 mg/kg keratinase to corn-soybean meal diets for growing pigs, significantly improving apparent ileal digestibility of crude protein and amino acids such as lysine, methionine, tryptophan, and threonine. Ye et al. [39] added acid protease (enzyme activity 50,000 U/g, from *Aspergillus niger*) and neutral protease (from *Bacillus subtilis*) to corn-full-fat soybean-whey powder diets for piglets, showing that energy apparent digestibility increased by 6.08% and crude protein apparent digestibility increased by 7.53%. Jiang et al. [40] added a complex of three proteases at 1,000 mg/kg to corn-soybean meal, corn-miscellaneous meal, and wheat-miscellaneous meal basal diets, significantly improving apparent ileal digestibility of various amino acids including methionine and valine.

Compared with conventional corn-soybean meal diets, exogenous protease shows more pronounced effects in miscellaneous meal-based diets. Exogenous protease primarily improves the digestibility of essential amino acids, and can also enhance the digestibility of some non-essential amino acids, crude protein, and energy. Mahagna et al. [41] showed that exogenous protease supplementation may inhibit endogenous enzyme activity. Zhang et al. [36] demonstrated that the optimal pH of exogenous protease affects enzymatic reactions. Neutral and alkaline proteases may have their activity inhibited or even be degraded in the animal stomach. Therefore, when selecting exogenous proteases, in addition to considering diet type, animal age, and enzyme properties, the interaction between endogenous and exogenous enzymes must also be considered.

3.3 Effects of Exogenous Protease on Health of Pigs and Chickens

Young animals have immature digestive systems and frequently experience indigestion. Exogenous protease can supplement the insufficient secretion of endogenous enzymes in young animals, improve protein digestion and absorption, reduce the pressure of protein digestion in the hindgut, and thereby decrease diarrhea caused by indigestion. Exogenous protease supplementation can alleviate heat, cold, and weaning stress in animals and enhance immunity. Improved protein digestibility reduces the burden on kidneys for excreting urea or urate, improving overall health status in pigs and chickens, though research results have been inconsistent, with some reporting negative effects.

3.3.1 Effects on Chicken Health Zhao et al. [42] added 200 mg/kg neutral protease (enzyme activity 10,000 U/kg) to corn-soybean meal diets with reduced crude protein levels for Roman Brown laying hens in late laying period, significantly reducing mortality. Peek et al. [43] added 25,000 U/kg protease (enzyme activity 558,700 U/mL, from *Bacillus licheniformis*) to corn-wheat-soybean meal diets, showing that protease significantly improved body weight in broilers infected with *Eimeria* coccidiosis, indicating that protease can alleviate the adverse effects of coccidiosis on broiler growth performance. Giannenas et al. [3] added 200 mg/kg protease to low crude protein level (20%) corn-corn gluten-wheat bran diets, significantly reducing *Clostridium perfringens* in cecum and ileum and *Fusobacterium necrogenes* in cecum, demonstrating that protease reduces harmful bacteria in the intestine and promotes broiler gut health. Li [44] added 32,000 U/kg protease (enzyme activity 12,220 U/g) to wheat-based diets for broilers, significantly reducing the ratio of villus height to crypt depth in the duodenum, indicating that high-dose protease has adverse effects on intestinal morphology. Wang et al. [42] added 125 mg/kg PT125™ compound protease to corn-soybean meal-cottonseed meal-wheat shorts diets for Sanhuang chickens, significantly reducing malondialdehyde (MDA) content in serum and increasing superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, demonstrating that exogenous protease supplementation improved heat stress resistance and antioxidant capacity in Sanhuang chickens.

3.3.2 Effects on Pig Health Xie et al. [43] added 1,000 mg/kg metalloprotease to nursery pig diets, reducing coughing rate, diarrhea rate, and mortality by 7.66%, 3.33%, and 3.33%, respectively, compared with the control group. Mei et al. [47] added 100 mg/kg protease (enzyme activity 50,000 U/g) to basal piglet diets, significantly improving anti-diarrhea capacity and growth performance. Kim et al. [48] showed that adding 200 mg/kg protease (enzyme activity 75,000 U/g, from *Bacillus licheniformis*) to low-protein level (23.51%) corn-soybean meal diets significantly reduced transforming growth factor- (TGF-) content in blood of piglets at 3 days post-weaning, significantly reduced white blood cell count at 7 days post-weaning, and showed a trend toward lower tumor necrosis factor- (TNF-) content in blood at 7 and 14 days post-weaning, indicating that exogenous protease supplementation can reduce inflammation in weaned piglets.

3.4 Effects of Protease on Environmental Emissions

Undigested protein in animal diets is excreted through feces and urine, decomposing into ammonia, nitrates, and nitrites in the environment. Ammonia volatilization can cause acid rain, while nitrates and nitrites pollute water sources and soil, directly or indirectly threatening human health. Ghazi et al. [49] added *Aspergillus*-derived protease to soybean meal, incubated it at 50°C and pH 4.5 for 2 hours, then added it to diets at 290 mg/kg, finding that apparent ileal digestibility and apparent retention rate of nitrogen in broilers significantly increased. Wang et al. [50] added 1,000 mg/kg keratinase (400,000 U/g) to soybean meal-based and miscellaneous meal-based diets for broilers, significantly increasing nitrogen retention rates in both diet types. Pan et al. [36] showed that exogenous protease supplementation significantly reduced fecal nitrogen excretion (3.7 vs. 4.7 g/d), showed a trend toward reduced urinary nitrogen excretion (2.9 vs. 4.7 g/d), and significantly increased nitrogen retention rate (0.73% vs. 0.61%). Protease can increase digestion and absorption of protein ingredients, improve protein and amino acid digestibility, and thereby reduce environmental nitrogen emissions. Leinonen et al. [6] used life cycle assessment (LCA) to holistically study the environmental impacts of protease in crop cultivation, feed production, broiler rearing (water and energy use, gas emissions), and manure/waste management, showing that protease use can reduce protein feed usage and soybean cultivation, decreasing greenhouse gas emissions such as carbon dioxide (maximum reduction 12%, average reduction 5%). Oxenboll et al. [51] used LCA to specifically investigate whether protease application in poultry feed affects the environment across all steps of livestock production, including feed production, broiler marketing, and manure utilization. Adding 200 mg/kg exogenous protease (enzyme activity 15,000 PROT/kg) to corn-soybean meal-meat and bone meal diets for broilers reduced emissions of ammonia, nitrous oxide, and nitrogen monoxide, with more pronounced effects in low-protein diets. Additionally, acidification, eutrophication, and greenhouse gas emissions were significantly reduced. Overall, dietary protease supplementation can improve protein and nitrogen utilization, reduce ammonium salt emissions, and lessen manure pollution of the atmosphere, water, and soil.

4 Problems in Exogenous Protease Research

Current research on exogenous protease has many limitations: (1) Limited enzyme-producing strains (mainly *Bacillus subtilis* and *Bacillus licheniformis*), with unstable protease properties and variable enzyme activities from fermentation. (2) Most microbial protease research focuses on alkaline and metalloproteases with narrow pH ranges, and studies on action sites in the digestive tract of livestock and poultry and their relationship with endogenous enzymes are insufficient. (3) Inconsistent enzyme activity measurement methods and animal experimental conditions reduce the credibility of research results on protease supplementation levels.

Saarelainen et al. [52] and Saleh et al. [53] found that the hydrolytic activity of carbohydrate enzymes can be inhibited or the enzymes themselves digested when protease is present, making it crucial to screen for optimal enzyme profiles [54-55]. Therefore, effective evaluation of feed proteases requires in vitro simulation of protease effects at different concentrations, pH levels, and sources, as well as effects of protease supplementation in different diet types and ratios, to identify optimal combinations that conserve protease resources and improve catalytic efficiency, followed by animal experiments for verification. Simulating the digestive process of protease in animals through biomimetic methods [36] can determine action sites (stomach, small intestine, or large intestine) and explore technologies such as protease encapsulation to deliver protease directly to action sites, preventing decomposition by endogenous enzymes or activity inhibition. Additionally, identifying protease receptors at the molecular level and improving enzyme-receptor binding capacity can enhance enzymatic reaction efficiency. In summary, many aspects require further investigation across all stages of feed protease production, processing, transportation, storage, and use. In production, new enzyme-producing strains can be developed to produce thermostable and acid-resistant proteases, and new methods to increase yield and quality of proteases from various strains can be explored. During processing, appropriate materials (sodium sulfate, dextrin, etc.) can be used to encapsulate protease to improve production efficiency and resist damage from gastric acid. Improvements are also needed in enzyme activity measurement and enzyme addition and usage. Furthermore, enzymes currently widely used in the market are produced by genetically engineered microorganisms, but there is no unified understanding in China regarding the necessity of safety evaluation for genetically modified feed enzymes. Although the EU and other countries have established regulations for genetically modified food and feed production and use, they do not cover safety evaluation systems and standards for enzymes produced by genetically engineered microorganisms, necessitating the establishment of unified safety evaluation systems for genetically modified proteases.

5 Conclusion

With deepening research on feed protease preparations, their advantages in improving nutrient digestibility (especially protein), enhancing animal growth performance, and conserving protein ingredients have gradually gained attention. Exogenous protease has attracted increasing interest from livestock and feed enterprises, with microbial-derived protease being the most promising due to its low production cost, clear functions, and environmental friendliness.

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