

Precision Protein Nutrition in poultry: Unlocking amino acid availability through protease supplementation

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ABSTRACT

Since protein is one of the costliest and nutritionally important ingredients in chicken diets, its effective use is crucial for economic and sustainable production. However, traditional protein-based feed formulation frequently leads to increased nitrogen excretion, poor amino acid use, and increased environmental burden. Precision Protein Nutrition (PPN), a nutritional approach that uses digestible amino acid-based formulations to match dietary amino acid supply with poultry physiological requirements, has arisen in response to these issues. Exogenous protease supplementation has drawn a lot of interest in this context since it can boost amino acid availability and accelerate protein decomposition. This paper investigates the role of protease supplementation in maximizing protein use in poultry and looks at the fundamentals of PPN. The methods by which proteases improve nutrition absorption, ileal amino acid digestibility, and protein digestion are highlighted. The effects of protease on intestinal microbial ecology, gut shape, and the performance of birds fed diets low in crude protein are also included in the paper. Protease supplementation can enhance feed efficiency, support growth performance, and increase nitrogen retention while lowering the quantity of undigested protein that reaches the lower gastrointestinal tract, according to evidence from recent research. By lowering dietary protein requirements, feed costs, and nitrogen emissions from chicken production systems, protease integration into precision feeding programs also provides economic and environmental benefits. Despite variability in response linked with enzyme properties, meal composition, and manufacturing settings, protease remains a viable tool for enhancing nutrient efficiency. All things considered, the combination of PPN and protease supplements is a calculated move toward more effective, sustainable, and ecologically conscious poultry farming.

Introduction

Because it provides the amino acids required for growth, tissue development, enzyme and hormone synthesis, and the production of live-stock products like meat and eggs, protein is one of the most crucial nutritional elements in poultry production (Alabi and Adedokun, 2025). In contrast to energy, which the body can store in vast amounts, amino acid requirements must be continuously satisfied through feed in order to support a variety of metabolic activities and productivity (Corsetti *et al.*, 2024). Therefore, the effectiveness of contemporary chicken production systems depends critically on the quantity and quality of protein in the feed (Beski *et al.*, 2015).

Although it plays a vital role, poultry frequently does not use it to its full potential (Nassar, 2026). Not all dietary protein is properly digested and used for protein synthesis in the body (Islam *et al.*, 2025). Some protein is lost during digestion, whereas others are expelled after deamination due to an imbalance in amino acid supply (Awan *et al.*, 2025). In addition to raising feed prices, this inefficiency increases nitrogen excretion, which can have detrimental effects on the environment through ammonia emissions and the buildup of nitrogenous waste (Kim *et al.*, 2026).

The idea of Precision Protein Nutrition (PPN) has emerged in recent decades as a result of attention to the effectiveness of protein use (Quan *et al.*, 2025). The goal of this strategy is to supply amino acids in quantities and ratios that correspond to the physiological requirements of birds at every stage of production (Anggriawan *et al.*, 2026). PPN highlights the significance of digestible amino acid availability as a foundation for more precise ration construction, in contrast to standard formulations that typically prioritize crude protein content (Ajomiwe *et al.*, 2024). This strategy can decrease needless nutrient losses by animals while increasing protein usage (Pomar *et al.*, 2021).

The disparity between the amino acid requirements of chickens and their availability in feed ingredients presents difficulties for PPN application (Moss *et al.*, 2021). The quantity of amino acids that are truly available for absorption and metabolism might be restricted by variations in protein quality, the existence of antinutritional agents, and variations in the digestibility of feed ingredients (Gilani *et al.*, 2012). As a result, ration formulations based only on total nutrient content frequently do not accurately represent the supply of amino acids that birds really have access to (Nawab *et al.*, 2025).

The introduction of exogenous enzymes has become a popular tactic in poultry feeding to get around these restrictions (Alagawany *et al.*, 2018). Proteases have drawn the most attention among the many kinds of feed enzymes because of their capacity to improve the breakdown of proteins and the release of amino acids from feed components (Zheng *et al.*, 2023). Proteases can improve the efficiency of amino acid consumption, decrease nutrient losses, and enable ration formulations with lower protein content without sacrificing production performance (Chen *et al.*, 2023). They can also increase the digestibility of proteins (Souza *et al.*, 2025).

Based on this background, this review aimed to discuss the development of the PPN concept in poultry, focusing on the role of protease supplementation in increasing amino acid availability and utilization. This article reviews the mechanism of action of proteases in protein digestion and assesses their impact on production performance, gastrointestinal health, nutritional efficiency, and economic and environmental ramifications. It is intended that by comprehending the connection between PPN and protease supplementation, a more thorough grasp of methods for maximizing protein use in sustainable poultry production systems can be attained.

Concept of PPN in poultry

PPN represents a modern nutritional approach that focuses on optimizing protein utilization through the accurate supply of digestible amino acids according to the physiological requirements of poultry. By emphasizing nutrient efficiency rather than crude protein concentration alone, this concept aims to enhance productive performance while reducing nutrient wastage and environmental impacts.

Definition of PPN

PPN is a dietary strategy that aims to supply protein and amino acids precisely in accordance with the physiological requirements of chickens at every stage of growth and production (Moss *et al.*, 2021). In order to maintain protein synthesis, tissue maintenance, metabolic function, and optimal production performance, this notion highlights the significance of striking a balance between the amount of amino acids available in the diet and the actual needs of the livestock (Adhikari *et al.*, 2025).

PPN bases ration formulation on the bioavailability and digestibility of amino acids, in contrast to traditional methods that concentrate on crude protein concentration (Jason and David, 1997). This strategy seeks to guarantee that every important amino acid is present in the right amounts so that the fowl body can effectively use it without creating excesses or deficits of any specific nutrient (Alabi and Adedokun, 2025). Increased amino acid catabolism, reduced protein synthesis efficiency, and nitrogen loss through excretion are all consequences of amino acid imbalances (Selle *et al.*, 2023a).

Increased protein usage efficiency and less nutrient waste are made possible by PPN, which optimizes the match between amino acid supply and demand (Lee and Rochell, 2022). By lowering nitrogen excretion, this strategy not only promotes increased poultry productivity but also lowers feed costs and environmental effects (Chalova *et al.*, 2016). PPN is therefore an essential starting point for the creation of a more accurate, effective, and long-lasting poultry nutrition system (Rochell, 2023).

Basic principles of PPN

PPN is founded on the idea that protein consumption efficiency can be boosted by providing a balanced amino acid supply that matches the physiological needs of poultry (Li *et al.*, 2025). By reducing excesses or deficits of specific amino acids that can impede growth and increase nitrogen loss, this strategy aims to enhance body protein deposition (Nanto-Hara *et al.*, 2026).

The ideal protein notion, which stresses that all essential amino acids should be accessible in the proper amounts in relation to the animal's biological needs, is one of the fundamental tenets of PPN (Wu and Li, 2022). Lysine is typically employed as the reference amino acid in this notion, and other amino acid requirements are stated as a ratio to lysine (Ma *et al.*, 2025). This method makes it possible to formulate rations more precisely to support protein synthesis without creating an excess of wasted amino acids (Remus *et al.*, 2021).

Using a formulation strategy based on digestible amino acids is the next premise (Morin *et al.*, 2024). This strategy takes into account the percentage of amino acids that birds really digest and absorb, in contrast to methods that just take into account the total nutrient content (Adhikari *et al.*, 2025). This reduces uncertainty brought on by differences in feed ingredient digestibility and makes ration design more realistic of physiologically available nutrient supply (Cambra-López *et al.*, 2022).

Additionally, PPN promotes lowering the amount of crude protein in meals without sacrificing output (Rubino *et al.*, 2022). By maximizing amino acid balance and boosting protein use efficiency, this technique ensures that poultry nutritional needs are satisfied while using less traditional protein sources (Moss *et al.*, 2021). This method lowers nitrogen excretion and promotes a more sustainable poultry production system

in addition to increasing the feed compositions' economic effectiveness (Abd El-Hack *et al.*, 2025).

The relevance of PPN in the modern poultry industry

The need for nutritional techniques that maximize nutrient use more effectively has been fueled by the expansion and intensification of chicken production (Zampiga *et al.*, 2021). Because it allows for more precise ration formulation based on poultry amino acid requirements, PPN has grown in importance in this context, enhancing production while decreasing protein inefficiencies (Rochell, 2023).

By making the best use of protein sources and amino acids, PPN enhances feed cost efficiency from an economic standpoint (Moss *et al.*, 2021). By reducing unnecessary safety margins in ration production, formulations based on digestible amino acid requirements minimize the usage of high-value protein components without sacrificing livestock performance (Kim *et al.*, 2026). Given that feed costs account for the majority of total poultry production costs, this strategy is becoming more and more crucial (Broch *et al.*, 2020).

In addition to its financial advantages, PPN is essential for lowering nitrogen losses from the production system (Frountzas *et al.*, 2025). The quantity of amino acids catabolized and eliminated as nitrogenous waste is reduced when the supply of amino acids more closely matches the physiological requirements of animals (Cambra-López *et al.*, 2022). This higher nitrogen retention efficiency helps lower ammonia emissions and possible environmental contamination from fowl excrement in addition to reflecting better protein use (Kumari *et al.*, 2016).

In the context of sustainability in the livestock sector, PPN's importance is also growing (Tedeschi *et al.*, 2026). In addition to lowering the environmental effects of feed ingredient production and consumption, optimizing protein utilization allows for more productive output with less resources (Llorens *et al.*, 2024). Therefore, PPN supports the equilibrium between productivity, resource efficiency, and environmental responsibility in contemporary poultry production systems in addition to acting as a method to enhance production performance (George and George, 2023). The conceptual framework of PPN in poultry is summarized in Figure 1.

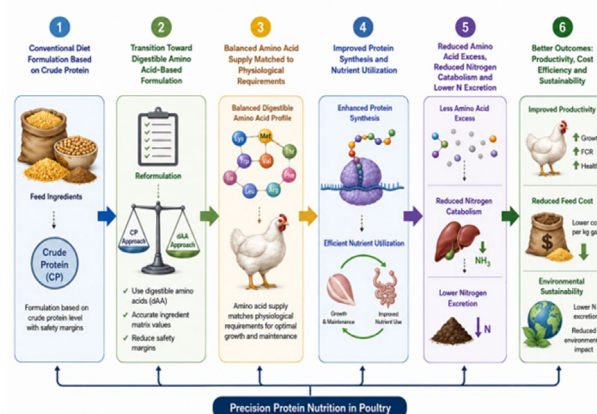


Figure 1. Conceptual framework of PPN in poultry.

Protein digestion and amino acid utilization in poultry

The efficiency of protein utilization in poultry is largely determined by the effectiveness of protein digestion and amino acid absorption within the gastrointestinal tract. Understanding physiological processes governing nutrient digestion, as well as the factors that limit amino acid availability, is essential for optimizing protein efficiency and supporting the principles of PPN.

Protein digestion process in poultry

In poultry, protein digestion is a complicated process that involves a number of enzymatic activities to transform feed protein into peptides and amino acids that the body can absorb (Macelline *et al.*, 2021). The availability of amino acids for a number of physiological processes, such as growth, tissue synthesis, immunological response, and production, is greatly influenced by the effectiveness of this mechanism (Alagawany *et al.*, 2020).

The proventriculus, which secretes pepsinogen and hydrochloric acid, is where the first stage of protein digestion starts (Yokhana *et al.*, 2016). Pepsinogen, a proteolytic enzyme that helps break down complex protein structures into simpler peptide fragments, is activated in acidic environments (Kageyama, 2002). After entering the ventricle or gizzard, the digesta is mechanically ground to expand the substrate's surface area, which boosts the activity of digestive enzymes (Bedford and Apajalahti, 2022).

Pancreatic enzymes, mainly trypsin and chymotrypsin, continue to hydrolyze proteins in the small intestine (Kurz and Seifert, 2021). Chymotrypsin acts on aromatic and hydrophobic amino acid residues, whereas trypsin breaks down peptide bonds in basic amino acid residues (Ma *et al.*, 2005). These two enzymes' activity results in oligopeptides, which are then broken down by different peptidases located on the brush boundary of enterocytes and in the intestinal lumen (Vertiprakhov *et al.*, 2023). This process gradually transforms dietary protein into dipeptides, tripeptides, and free amino acids that are suitable for absorption (Kuzmina *et al.*, 2024).

The jejunum and ileum are where the last products of protein digestion are mostly absorbed (Recoules *et al.*, 2019). Peptide transporters like PepT1 carry dipeptides and tripeptides, but certain ion gradient-dependent transporter systems absorb free amino acids (Tokutake *et al.*, 2021). Before being sent to the portal circulation, the majority of short peptides are hydrolyzed into amino acids once they have entered the enterocytes (Kidd *et al.*, 2021). In order to enable protein synthesis and other metabolic processes, the absorbed amino acids are subsequently dispersed throughout different tissues (Qaid and Al-Garadi, 2021).

Limiting factors of amino acid availability

Poultry's access to amino acids is influenced by a number of variables that affect the digestion, release, and absorption of nutrients in the digestive tract in addition to the protein content of the diet (Macelline *et al.*, 2021). These limiting variables can lower the percentage of amino acids that are actually available for the body's metabolism and protein synthesis, which lowers the effectiveness of feed protein consumption (Ravindran and Bryden, 1999).

Antinutritional factors in feed ingredients, especially those derived from plant sources, are a significant factor influencing the availability of amino acids (Khajali and Rafiei, 2024). Non-starch polysaccharides (NSPs) can make digesta more viscous and prevent digestive enzymes from interacting with protein substrates (Suyama and Gewehr, 2026). This state restricts the release of amino acids during digestion and lowers the effectiveness of protein breakdown (Morgan *et al.*, 2022). Additionally, trypsin inhibitors and other protease inhibitors can lower the activity of endogenous proteolytic enzymes, preventing the breakdown of proteins and decreasing the digestibility of amino acids (Borda-Molina *et al.*, 2019).

The creation of protein-phytate complexes is another antinutritional factor (Selle *et al.*, 2023b). The rate of amino acid breakdown and absorption may be slowed down by this interaction, which can change the structure of proteins and make them less accessible to digestive enzymes (Raza *et al.*, 2019). Phytate can interact with specific minerals involved in digestion in addition to binding protein, which further restricts poultry's ability to use nutrients (Junior *et al.*, 2024).

Another major cause of uneven amino acid availability is variation in

the quality of plant-based feed ingredients (Bryden and Li, 2010). Protein concentration, amino acid profiles, and nutritional digestibility can all be impacted by variations in plant types, growth conditions, processing techniques, and storage (Volpato *et al.*, 2022). As a result, when utilized in ration compositions, two feed items with comparable crude protein contents could not have the same nutritional value (Son *et al.*, 2024).

Protein accessibility to digestive enzymes can be impacted by interactions between dietary components as well as inherent variables in feed ingredients (Kaur *et al.*, 2022). Because of the feed matrix's intricate structure, some proteins may not come into contact with proteolytic enzymes, leaving them in a condition that is difficult to digest (Bedford and Apajalahti, 2022). This disorder increases the percentage of protein that travels to the distal portion of the digestive tract while decreasing the quantity of amino acids that can be absorbed in the small intestine (Ravindran, 2013).

Protein metabolism inefficiency

Not all of the protein that is ingested can be effectively transformed into body protein, despite the fowl digestive system's design to optimize nutrient use (Cambra-López *et al.*, 2022). Certain amino acids are not available to assist growth and synthesis due to various nutrient losses that take place during digestion and metabolism (Macelline *et al.*, 2025a). The effectiveness of protein utilization in poultry feeds is significantly hampered by this phenomena (Naeem, 2026).

Endogenous amino acid losses, or the loss of amino acids from the bird's own secretions and tissues, are a significant cause of protein loss (Ravindran, 2021). This loss includes digestive enzymes, mucoproteins, intestinal mucus, and epithelial cells that undergo natural turnover (Teng *et al.*, 2021). Some of these substances are not completely reabsorbed and are eventually eliminated, despite the fact that they play vital physiological roles in preserving mucosal integrity and digestion (Ducatelle *et al.*, 2023). Antinutritional factors, mucosal irritation, or low-quality feed can all increase the amount of endogenous losses, which lowers nitrogen retention efficiency (Myrie *et al.*, 2008).

Certain feed proteins can evade digestion in the small intestine and travel to the distal portion of the digestive tract in addition to endogenous losses (Perera and Ravindran, 2025). This undigested protein becomes a substrate for microbial fermentation, which produces different nitrogen metabolites such as ammonia, biogenic amines, and other protein breakdown products (Ravindran, 2021). This process can change the intestinal environment to become less favorable for digestive health in addition to representing a loss of possible nutrients that birds could use (Szydłowska *et al.*, 2026).

Ineffective protein metabolism may also result from an imbalance in the ration's amino acid composition (Qaid and Al-Garadi, 2021). The use of other amino acids for protein synthesis is hindered when one or more amino acids are not readily available (Iqbal *et al.*, 2025). The total efficiency of protein use is decreased when excess amino acids are deaminated and either employed as an energy source or eliminated as nitrogen compounds (Nanto-Hara *et al.*, 2026).

From a physiological perspective, inefficient protein utilization is a discrepancy between the amount of protein ingested and the percentage that is truly transformed into body tissue or animal products (Wang *et al.*, 2025). Therefore, to promote the use of PPN in contemporary chicken production systems, nutritional techniques that enhance protein digestibility, minimize endogenous amino acid losses, and maximize nutrient absorption are essential (Alabi and Adedokun, 2025).

Role of exogenous protease in poultry nutrition

Exogenous proteases have become important feed additives in poultry nutrition due to their ability to enhance protein digestion and improve amino acid availability. By complementing endogenous digestive

enzymes, proteases can increase nutrient utilization efficiency, support gut function, and contribute to more precise protein feeding strategies. Understanding their mechanisms of action and interactions with other feed enzymes is therefore essential for maximizing their nutritional benefits in poultry diets. Table 1 summarizes roles and nutritional effects of exogenous protease supplementation in poultry nutrition.

Mechanism of action

The enzymatic hydrolysis of peptide bonds in dietary proteins is the main way that exogenous proteases provide nutritional advantages (Liu *et al.*, 2023). This process helps break down complicated protein structures into smaller, easier-to-digest molecules (Zhang *et al.*, 2025). Proteases increase the amount and rate of protein destruction throughout the gastrointestinal system by enhancing the activity of endogenous digestive enzymes, especially when dietary proteins are difficult to fully digest (Oxenboll *et al.*, 2011).

Oligopeptides, dipeptides, tripeptides, and free amino acids are produced by the hydrolytic activity of proteases and are more easily absorbed through the intestinal epithelium (Romero-Garay *et al.*, 2021). By increasing the pool of absorbable nitrogenous compounds accessible for metabolic activities, this improved breakdown of protein substrates improves the availability of amino acids for tissue accretion, protein synthesis, and productive functions (Qaid and Al-Garadi, 2021). As a result, a larger percentage of dietary protein can be effectively used, lowering nutrient losses brought on by insufficient digestion (Lemme *et al.*, 2019).

By breaking up protein-associated antinutritional complexes, exogenous proteases can enhance nutrient accessibility in addition to their direct effects on protein hydrolysis (Onbaşlılar *et al.*, 2026). Proteins are either attached to substances that restrict enzymatic access and nutrient release or integrated within structural matrices in many plant-derived feed ingredients (Obianwuna *et al.*, 2024). By increasing substrate accessibility and promoting the activity of endogenous enzymes, protease supplements can partially break down these proteinaceous barriers (Zakria *et al.*, 2025).

This impact improves the release of nutrients that have been trapped and makes digestion more effective (Park *et al.*, 2020).

Effect on amino acid digestibility

The capacity of exogenous protease supplementation to improve the availability and digestibility of dietary amino acids is its main nutritional benefit (Duque-Ramírez *et al.*, 2023). Proteases improve the percentage of amino acids that are available for absorption before digesta reaches the distal intestine by encouraging a more thorough breakdown of protein substrates within the gastrointestinal tract (da Nobrega *et al.*, 2025). In poultry nutrition, where effective amino acid consumption directly affects growth performance, feed efficiency, and protein deposition, this improvement is very crucial (Nuamah *et al.*, 2026).

An increase in apparent and standardized ileal amino acid digestibility is one of the most often reported results of protease administration (Hong *et al.*, 2025). Ileal digestibility assays are a good predictor of the bird's actual supply of amino acids since they show nutrient absorption before microbial fermentation in the hindgut (Cho *et al.*, 2026). Increased proteolytic activity makes it easier for dietary proteins to be broken down completely, which increases amino acid recovery at the site of absorption and lowers the amount of undigested protein that escapes digestion (Macelline *et al.*, 2021).

A more effective use of essential amino acids, which are vital for immune system function, muscular growth, enzyme synthesis, and general productivity, frequently coincides with an increase in amino acid digestibility (Taylor-Bowden *et al.*, 2024). Improved nitrogen retention and more efficient protein synthesis can be supported by increased availability of essential amino acids such lysine, methionine, threonine, and tryptophan (Alagawany *et al.*, 2020). Consequently, instead of being catabolized and eliminated, a greater portion of the amino acids that are eaten are used in productive processes (Macelline *et al.*, 2025b).

Moreover, improved digestibility of amino acids helps to more precisely match the availability of nutrients with physiological needs (Cao

Table 1. Roles and nutritional effects of exogenous protease supplementation in poultry nutrition.

Functional	Mechanism of action	Nutritional outcome	Contribution to PPN	References
Protein hydrolysis	Cleavage of peptide bonds in dietary proteins into smaller peptides and amino acids	Improved protein digestion and nutrient availability	Enhances utilization of dietary protein resources	Liu <i>et al.</i> (2023); Zhang <i>et al.</i> (2025); Oxenboll <i>et al.</i> (2011)
Production of absorbable nitrogenous compounds	Generation of oligopeptides, dipeptides, tripeptides, and free amino acids	Increased amino acid absorption and utilization	Supports growth, tissue accretion, and productive performance	Romero-Garay <i>et al.</i> (2021); Qaid and Al-Garadi (2021); Lemme <i>et al.</i> (2019)
Reduction of protein-associated antinutritional barriers	Partial degradation of protein complexes and structural matrices	Greater accessibility of nutrients to digestive enzymes	Improves nutrient release from plant-based feed ingredients	Obianwuna <i>et al.</i> (2024); Zakria <i>et al.</i> (2025); Park <i>et al.</i> (2020)
Enhancement of amino acid digestibility	More complete digestion of dietary proteins before the distal intestine	Increased apparent and standardized ileal amino acid digestibility	Maximizes amino acid recovery and minimizes undigested protein losses	Duque-Ramírez <i>et al.</i> (2023); Hong <i>et al.</i> (2025); Cho <i>et al.</i> (2026); Macelline <i>et al.</i> (2021)
Improved essential amino acid utilization	Increased availability of lysine, methionine, threonine, and tryptophan	Better protein synthesis and nitrogen retention	Supports immune function, muscle growth, and productivity	Alagawany <i>et al.</i> (2020); Taylor-Bowden <i>et al.</i> (2024); Macelline <i>et al.</i> (2025b)
Support for reduced-crude-protein diets	Improved efficiency of amino acid utilization	Maintains performance while lowering dietary protein levels	Facilitates sustainable and economical feeding strategies	Cao <i>et al.</i> (2024); Zhang <i>et al.</i> (2026); Abdulmajid and Suliaman (2025)
Synergy with phytase	Increased accessibility of protein substrates through phytate degradation	Enhanced amino acid and phosphorus utilization	Improves overall nutrient efficiency and reduces nutrient wastage	El Enshasy <i>et al.</i> (2018); Nuamah <i>et al.</i> (2024); Walker <i>et al.</i> (2024); da Nobrega <i>et al.</i> (2025)
Synergy with carbohydrases	Breakdown of plant cell walls and reduction of digesta viscosity	Improved enzyme-substrate interaction and nutrient release	Enhances digestion of complex plant-based diets	Wan <i>et al.</i> (2023); Suyama and Gewehr (2026); Bedford and Apajalahti (2022); Singh and Kim (2021)
Multi-enzyme feeding strategy	Simultaneous targeting of protein, phytate, and fiber fractions	Increased nutrient digestibility and feed efficiency	Supports sustainable and cost-effective poultry production	Moss <i>et al.</i> (2026); He <i>et al.</i> (2026); Bist <i>et al.</i> (2024); Jha <i>et al.</i> (2026)

et al., 2024). This facilitates the adoption of diets low in crude protein while preserving sufficient amino acid availability for peak performance (Zhang et al., 2026). Therefore, in chicken production systems, protease supplementation is an efficient nutritional strategy for enhancing amino acid efficiency, optimizing protein use, and reinforcing the PPN principles (Abdulmajid and Suliaman, 2025). The mechanism by which exogenous protease improves amino acid availability is illustrated in Figure 2.

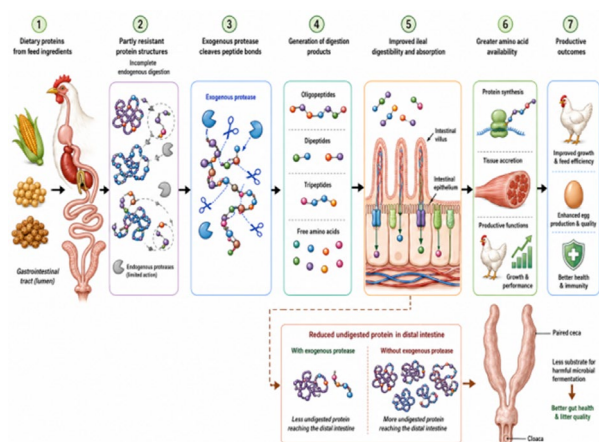


Figure 2. Mechanism of exogenous protease in improving amino acid availability in poultry.

Interaction with other feed enzymes

Combining exogenous proteases with other feed enzymes can increase their efficacy in poultry diets (Radhi et al., 2023). Improved nutrient release and a more effective digestive process result from these interactions, which are based on complimentary modes of action that target various dietary substrates (Jha et al., 2026). In contemporary poultry nutrition, this multi-enzyme approach has grown in significance, especially in diets made with a variety of plant-based foods that include intricate nutritional matrices (Soisuwan et al., 2023).

A notable example is the interaction between proteases and phytases (Borda-Molina et al., 2019). Proteins are frequently linked to phytate-containing complexes in plant-based feedstuffs, which can hinder digestion and decrease nutritional bioavailability (El Enshasy et al., 2018). Phytase lessens the anti-nutritional effects of phytate-protein interactions and releases minerals that would otherwise be inaccessible by hydrolyzing phytate molecules (Nuamah et al., 2024). By making protein substrates more accessible to proteolytic enzymes, this process creates an environment that enhances the efficiency of proteases (da Nobrega et al., 2025). Therefore, using these enzymes together can improve the availability of amino acids and phosphorus utilization, resulting in a more effective use of dietary resources (Walker et al., 2024).

Additionally, proteases work in concert with carbohydrases such as mannanase, xylanase, and β -glucanase (Wan et al., 2023). By breaking down the structural polysaccharides found in plant cell walls, these enzymes lower the physical barriers that prevent intracellular proteins from being exposed to digesting enzymes (Suyama and Gewehr, 2026). Protease activity can more easily access previously contained protein fractions when cell wall integrity is compromised (Bedford and Apajalahti, 2022). Additionally, the decrease in digesta viscosity that is frequently linked to supplementing with carbohydrase might enhance interactions between enzymes and substrates and promote the digestive tract's ability to absorb nutrients (Singh and Kim, 2021).

Proteases work in concert with phytases and carbohydrases, which emphasizes the significance of coordinated enzyme methods in poultry nutrition (Moss et al., 2026). These enzyme combinations can improve feed efficiency, boost nutrient digestibility, and aid in the development of more economical and sustainable feeding programs by concurrently addressing several obstacles to nutrient digestion (He et al., 2026). In PPN

frameworks, where enhancing nutrient availability is crucial for maximizing productive performance while reducing nutrient losses, these interactions are especially pertinent (Bist et al., 2024).

Protease supplementation in low-protein diets

Reducing dietary crude protein has become a key nutritional technique to enhance the efficiency of nitrogen consumption, lower feed costs, and lessen the environmental effect of raising chickens (Matton et al., 2025). Lowering crude protein levels, however, may jeopardize the availability of amino acids and decrease the supply of peptides and other nitrogenous molecules required for healthy development and physiological processes (Adhikari et al., 2025). Therefore, rather than only increasing dietary protein inclusion, maintaining avian performance under reduced-protein feeding regimes necessitates nutritional interventions that improve nutrient use (Oktariansyah and Kamal, 2026).

Exogenous protease supplementation has drawn a lot of interest as a strategy to help low-protein diets be implemented successfully (Law et al., 2018). Proteases enhance the nutritional value of current protein sources by increasing the release and absorption of amino acids from feed materials through improved breakdown of dietary proteins (Alabi and Adedokun, 2025). This advancement makes it possible for nutritionists to create diets that contain less crude protein while yet providing a sufficient amount of the digestible amino acids needed for optimal performance (Beski et al., 2015).

Proteases may help mitigate amino acid deficiencies that may occur when dietary protein levels are decreased, in addition to improving protein digestibility (Wang et al., 2020a). The efficiency of nutritional extraction and use is increased by improved amino acid liberation from feed proteins, which narrows the gap between the supply of amino acids and the bird's metabolic needs (Zampiga et al., 2021). Because variations in protein quality and digestibility might restrict the availability of amino acids, this effect is especially useful in formulations that primarily use plant-derived constituents (Bryan and Classen, 2020).

Productive performance often reflects the nutritional benefits of protease supplementation (Park et al., 2020). Growth rates that are equivalent to those attained with higher-protein diets can be maintained and body weight gain can be supported by improved nitrogen retention and amino acid use (Musigwa et al., 2024). Additionally, higher nutrient digestibility frequently leads to an improved feed conversion ratio (FCR), which shows that feed is converted into body mass more effectively (Zhang et al., 2023). Feed intake frequently stays constant, indicating that birds may more successfully meet their nutritional needs even when dietary crude protein is reduced (Cordero et al., 2025).

Gut health and intestinal morphology

Beyond improving protein digestibility, exogenous proteases may influence several aspects of gastrointestinal function that are closely associated with nutrient utilization and overall bird health. Their effects on intestinal morphology, microbial ecology, and gut integrity can contribute to a more efficient digestive environment, ultimately supporting nutrient absorption and productive performance.

The effect of protease on intestinal morphology and integrity

Intestinal shape is a significant factor in determining the efficiency of nutrient use in chickens since the gastrointestinal tract is crucial to the digestion and absorption of nutrients (Azizi et al., 2026). It is commonly known that the intestinal mucosa's morphological features, especially villus height and crypt depth, are markers of gut health and digesting ability (Pan and Yu, 2014). Alterations in these characteristics can alter the absorptive surface area, epithelial turnover, and overall functionality of the gut (Yegani and Korver, 2008).

Exogenous protease supplementation has been related with benefi-

cial changes in intestinal shape, notably through increases in food digestion and reductions in the accumulation of undigested protein inside the gut lumen (Zafar *et al.*, 2025). A more stable intestinal environment that promotes the growth and upkeep of intestinal villi can be produced by increased protein hydrolysis (Urban *et al.*, 2024). A larger absorptive surface, which enables more effective absorption of amino acids, peptides, and other nutrients necessary for growth and metabolism, is typically indicated by increased villus height (Ravindran and Abdollahi, 2021).

On the other hand, intestinal epithelial cell proliferation is reflected in crypt depth (Uni *et al.*, 1998). Increased tissue turnover and higher energy consumption for intestinal maintenance are frequently linked to excessive crypt growth (Hossain and Amin, 2026). By lowering luminal irritants and increasing digestive efficiency, protease supplements may help create a more balanced crypt architecture and lessen the requirement for quick epithelium regeneration (da Nobrega *et al.*, 2025). Because of this, a higher villus height-to-crypt depth ratio is often seen as an indication of better intestinal function and nutritional absorption ability (Jha and Mishra, 2021).

Proteases may help maintain the integrity of intestinal epithelial cells in addition to changing their morphology (Wang *et al.*, 2020b). Increased protein digestibility limits the generation of potentially hazardous metabolites produced by microbial protein fermentation by reducing the flow of undigested substrates to the lower gastrointestinal tract (Dittoe *et al.*, 2022). By maintaining selective permeability and shielding intestinal tissues from excessive microbial and inflammatory assaults, a more favorable luminal environment can enhance epithelial barrier function (Wickramasuriya *et al.*, 2022).

The effect of proteases on gut microbial ecology

The intestinal microbiota is a dynamic and intricate ecology that is essential for immunological control, nutrition metabolism, and gastrointestinal homeostasis (Pan and Yu, 2014). The amount and quality of nutrients that enter the intestinal lumen have a significant impact on the makeup and activity of microbial populations (Marmion *et al.*, 2021). The microbial environment in the gastrointestinal system can therefore be indirectly shaped by dietary changes that alter food digestion (Clavijo and Flórez, 2018).

By enhancing protein digestion in the upper gastrointestinal tract and lowering the quantity of undigested protein that reaches the distal intestine, exogenous protease supplementation may have an impact on intestinal flora (Zheng *et al.*, 2023). Proteolytic microbial fermentation can use excess dietary protein that escapes enzymatic digestion as a substrate, producing metabolites such as amines, ammonia, and other nitrogenous substances that may have a negative impact on gut health (Singh and Kim, 2021). Proteases alter the ecological conditions that control microbial growth and activity by increasing protein use, which reduces the availability of these substrates (Amer *et al.*, 2021).

A decrease in the growth of bacteria that preferentially use proteinaceous substrates is frequently linked to a decrease in the transit of undigested protein to the hindgut (Zafar *et al.*, 2025). This change can lessen the buildup of fermentation products that could endanger intestinal health and promote a more balanced microbial community (Yoon *et al.*, 2026). Additionally, better food digestion can foster the microbial populations linked to a stable and healthy gut environment (Ogwuegbu *et al.*, 2025).

Through indirect routes, protease supplementation may potentially improve intestine function by modifying microbial communities (Lourenco *et al.*, 2020). Improved barrier integrity, improved food digestion, and a positive relationship between the host and its gut microbes can all be facilitated by a more balanced microbiota (Aruwa *et al.*, 2021). These actions may enhance the effectiveness of food consumption and lessen physiological stress in the gastrointestinal tract (Oke *et al.*, 2025).

Implications of proteases on digestive health and nutrient utilization efficiency

The principal contact between food nutrients and the physiological processes supporting poultry development, maintenance, and production is the gastrointestinal system (Adedokun and Olojede, 2019). As a result, the effectiveness of nutrient digestion, absorption, and utilization is directly related to the general health of the digestive system (Ducatelle *et al.*, 2023). Any interference with gastrointestinal function can raise metabolic expenses, reduce nutrient availability, and eventually lower productivity (Suyama and Gewehr, 2026).

Through coordinated interactions between digestive enzymes, intestinal shape, epithelial integrity, and resident microbial communities, a properly functioning gastrointestinal tract promotes efficient digestion (Pan and Yu, 2014). Together, these elements govern the intestine's ability to draw nutrients from feed and move them into the bloodstream (Kuzmina *et al.*, 2024). Instead of being wasted due to inadequate digestion or excessive endogenous secretions, nutrients can be used more effectively for tissue accumulation, metabolic processes, and productive functions when gastrointestinal health is preserved (Aruwa *et al.*, 2021).

By improving protein digestion and lowering the buildup of undigested substrates in the intestinal lumen, protease supplements may help to improve gastrointestinal health (Dessimoni *et al.*, 2019). The possibility of excessive microbial protein fermentation and the ensuing generation of unwanted metabolites that can negatively impact intestinal function is reduced when residual protein concentrations are lower (Predescu *et al.*, 2024). This improves the luminal environment, which facilitates digestion and aids in preserving the gastrointestinal tract's physiological stability (Zhang *et al.*, 2020).

The efficiency of nutrition utilization can also be positively impacted by improved gastrointestinal conditions (Zhang *et al.*, 2020). A healthy intestinal environment facilitates efficient nutrient absorption, lowers maintenance needs related to immune activation and tissue repair, and improves birds' overall ability to transform dietary nutrients into useful products (Azizi *et al.*, 2026). Therefore, instead of supporting compensatory physiological reactions, a larger percentage of nutrients consumed can be allocated to growth and production (Son *et al.*, 2024).

Environmental and economic implications

Protease supplementation can increase the efficiency of protein use, which has wide-ranging effects on poultry production performance, the economy, and the environment (Poudel *et al.*, 2023). Due to growing feed ingredient costs and the need for more sustainable livestock practices, improving nutrient use in contemporary production systems is becoming more and more crucial (Nassar, 2026).

Reduced nitrogen excretion into the environment is one of the main advantages of improving protein digestibility (Nanto-Hara *et al.*, 2026). The amount of nitrogen expelled through feces is decreased when feed protein is more effectively absorbed and digested (Heo *et al.*, 2023). This decrease in nitrogen excretion lowers the possibility of soil and water pollution, which is frequently linked to intensive animal husbandry, and lowers ammonia emissions from litter (Song *et al.*, 2025).

Proteases increase the availability of digestible amino acids, which allows for more optimal protein usage from a nutritional standpoint (Park *et al.*, 2020). Increased nitrogen retention and the effectiveness of body protein deposition are supported by this, allowing poultry to grow or produce more from the same amount of feed protein (Njeri *et al.*, 2026). Therefore, it is possible to raise the biological efficiency of protein source use without increasing the ration's crude protein amount (Gao *et al.*, 2025).

Additionally, these advantages offer financial benefits for feed formulation (Pesti and Choct, 2023). Rations with a lower crude protein concentration or a smaller safety margin in amino acid formulations can be

used when protein digestibility is increased (Kim *et al.*, 2026). This tactic may lower feed costs without compromising production performance by lowering reliance on premium protein source supplies, which are often somewhat pricey (Zhao *et al.*, 2025).

The use of proteases in the context of sustainability is consistent with initiatives to enhance resource efficiency and lessen the poultry industry's environmental impact (Wu *et al.*, 2026). By lowering nitrogen waste and improving feed conversion efficiency, optimizing protein consumption promotes a more ecologically friendly livestock system in addition to minimizing nutrient losses during the production process (Osei-Akoto *et al.*, 2026). As a result, protease supplementation can be seen as an essential part of a sustainable nutrition plan that incorporates environmental responsibility, economic efficiency, and productivity (Ghavipanjeh *et al.*, 2026). The broader effects of protease supplementation on gut health, nutrient efficiency, and sustainability are shown in Figure 3.

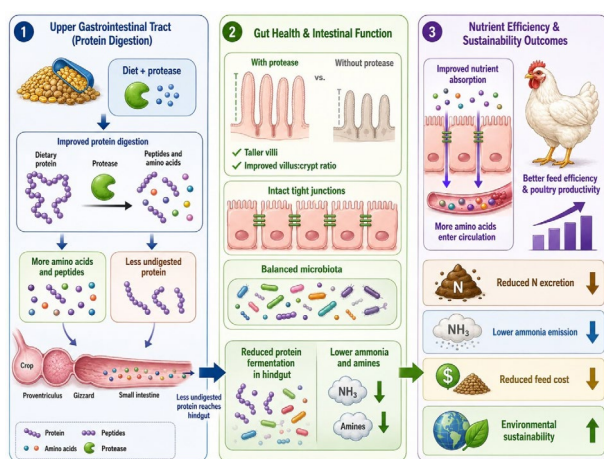


Figure 3. Effects of protease supplementation on gut health, nutrient efficiency, and sustainability in poultry.

Limitations and variability of protease response

While poultry protein utilization and amino acid availability have been demonstrated to be significantly increased by protease addition, the outcome is not necessarily uniform across production settings (Peñuela-Sierra *et al.*, 2024). Variability in protease efficiency is determined by complicated relationships between enzyme properties, ration composition, livestock circumstances, and the experimental parameters used in distinct investigations (Lin *et al.*, 2023). Table 2 summarizes major

factors contributing to the variability of exogenous protease responses in poultry nutrition.

The properties of the protease itself are a significant source of variance (Park *et al.*, 2020). Proteases' capacity to break down feed proteins can be impacted by variations in enzyme origin, substrate selectivity, catalytic activity, pH stability, and resistance to temperature and pelleting procedures (Chen *et al.*, 2023). As a result, it is not always possible to extrapolate a protease product's efficacy to other settings or feed compositions (Zaefarian *et al.*, 2021).

The response to protease supplementation is also strongly influenced by the content of feed ingredients (Hong *et al.*, 2025). The degree to which enzymes can accelerate protein decomposition depends on the complexity of the feed matrix, the presence of antinutritional agents, and protein sources with different digestibility levels (Bryan and Classen, 2020). Generally speaking, diets with poor-quality or enzymatically inaccessible protein have a higher potential response than diets with readily digested components (Law *et al.*, 2018).

Additionally, the amount of advantages from protease supplements may vary depending on the protein level of the ration (Poudel *et al.*, 2023). Because each increase in amino acid availability has a higher biological value, higher protein digestibility frequently has a greater impact in rations with lower protein concentration (Naeem, 2026). On the other hand, because dietary requirements have been satisfied, the reaction to protease may be more constrained in rations with adequate or surplus protein (Qiu *et al.*, 2023).

Physiological factors in animals also contribute to variances in enzyme response (Cowieson and Klueenter, 2019). The effectiveness of protease activity and the use of digested nutrients can be impacted by age, health, digestive capacity, production level, and environmental factors (Ghavipanjeh *et al.*, 2026). For instance, because their digestive tracts are still developing, young birds frequently react differently than adults (Kuzmina *et al.*, 2024).

Further evidence that the response to protease is contextual comes from the variations in reported outcomes between research (Naeem, 2026). Different results can be obtained depending on the bird species, ration formulation, enzyme dosage, study design, and observed data (Cao *et al.*, 2024). Protease benefits must therefore be interpreted carefully, taking into consideration the unique conditions that underlie each study (Park *et al.*, 2020). Optimizing the use of proteases in PPN systems in poultry requires an understanding of the variables influencing this response variability (Iqbal *et al.*, 2025).

Table 2. Major factors contributing to the variability of exogenous protease responses in poultry nutrition.

Factor category	Specific factors	Influence on protease response	Practical implications	References
Protease characteristics	Enzyme origin, substrate specificity, catalytic activity, pH stability, thermal resistance, pelleting stability	Determine the ability of protease to hydrolyze dietary proteins under different gastrointestinal and feed-processing conditions	Performance of one protease product may not be directly transferable to other diets or production systems	Park <i>et al.</i> (2020); Chen <i>et al.</i> (2023); Zaefarian <i>et al.</i> (2021)
Feed ingredient composition	Protein source, feed matrix complexity, antinutritional factors, protein digestibility	Influences enzyme accessibility to substrates and extent of protein degradation	Greater responses are often observed in diets containing poorly digestible proteins or high levels of antinutritional compounds	Bryan and Classen (2020); Law <i>et al.</i> (2018); Hong <i>et al.</i> (2025)
Dietary protein level	Low-, adequate-, or high-protein diets	Alters the biological value of improvements in amino acid availability	Low-protein diets typically show greater benefits from protease supplementation than protein-sufficient diets	Poudel <i>et al.</i> (2023); Naeem (2026); Qiu <i>et al.</i> (2023)
Animal physiological factors	Age, health status, digestive capacity, production stage, environmental conditions	Affect endogenous enzyme secretion, nutrient absorption, and utilization efficiency	Young birds may exhibit different responses due to immature digestive systems	Cowieson and Klueenter (2019); Kuzmina <i>et al.</i> (2024); Ghavipanjeh <i>et al.</i> (2026)
Experimental conditions	Bird species, enzyme dosage, diet formulation, study design, measured parameters	Influence the magnitude and interpretation of observed responses	Results from different studies should be compared cautiously due to methodological differences	Cao <i>et al.</i> (2024); Park <i>et al.</i> (2020); Naeem (2026)
Production environment	Management practices and production system characteristics	Modifies interactions among enzymes, diets, and birds	Protease efficacy may vary among commercial and experimental settings	Peñuela-Sierra <i>et al.</i> (2024); Lin <i>et al.</i> (2023); Iqbal <i>et al.</i> (2025)

Future perspectives

Protein and amino acid management strategies are becoming more accurate because to advancements in contemporary poultry nutrition (Adhikari *et al.*, 2025). In this regard, it is anticipated that the usage of proteases will develop from simple feed additives to an essential part of data-driven nutritional formulation systems, improving production sustainability and biological efficiency (da Nobrega *et al.*, 2025).

The idea of precision enzyme nutrition, a method for employing enzymes customized to the properties of raw materials, the physiological requirements of livestock, and particular production goals, is one prospective development avenue (Yao *et al.*, 2026). This method improves the precision of forecasting nutrient release and the effectiveness of their utilization by chickens by enabling more targeted protease usage depending on the substrate profile present in the diet Wu *et al.*, 2026).

The application of artificial intelligence-based feed formulation and nutritional modeling is made possible by advances in digital technology (Abdel-Wareth and Ahmed, 2026). The accuracy of ration design can be increased by incorporating information on livestock performance, nutrient digestibility, raw material composition, and enzyme response into predictive models (de Oliveira *et al.*, 2025). By dynamically adjusting the dosage and nutrient matrix, these systems can maximize the utilization of proteases, leading to increased biological and economic efficiency (Nath *et al.*, 2025).

Additionally, a generation of proteases with increased stability and specificity is anticipated as a result of advancements in enzyme technology (Ravindran, 2013). Enzymes with greater activity across the pH and temperature ranges of the fowl digestive tract as well as a more efficient capacity to break down challenging-to-digest proteins have been made possible by developments in protein engineering and contemporary biotechnology (Azizi *et al.*, 2026). These developments should increase the use of proteases on a variety of feed ingredients with different properties (Zheng *et al.*, 2023).

Integrating proteases with formulation systems based on digestible amino acids is another significant opportunity (Macelline *et al.*, 2021). The contribution of proteases to amino acid release and utilization can be more precisely represented in formulation models as digestibility values increasingly serve as the foundation for ration creation (Naeem, 2026). This method promotes effective protein use while lowering nitrogen loss to the environment by better aligning the delivery of nutrients with the physiological requirements of chickens (da Nobrega *et al.*, 2025).

All things considered, developments in formulation technology, enzyme biotechnology, and data-driven nutrition management systems will have a major impact on the future of protease use in poultry nutrition (Cao *et al.*, 2024). The incorporation of these developments may enhance proteases' function as a crucial tool in achieving a PPN system that is more effective, flexible, and long-lasting (Liu *et al.*, 2025).

Conclusion

Precision Protein Nutrition has become a significant nutritional strategy for increasing poultry's efficiency of protein use. This idea allows for more precise ration design than methods based only on crude protein by highlighting the supply of digestible amino acids that satisfy the physiological requirements of animals. The use of PPN improves the efficiency of increasingly scarce nutritional resources in addition to supporting the optimization of production performance.

In this context, exogenous protease supplementation is a useful tactic to improve feed protein breakdown and poultry amino acid availability. Proteases can enhance nutrient utilization, cut nitrogen losses, and facilitate the adoption of reduced protein feeds without sacrificing production by improving protein digestibility. By enhancing the intestinal environment and boosting nutrient absorption efficiency, proteases not only provide nutritional advantages but also favorably impact digestive

health.

A thorough strategy for maximizing protein use in contemporary chicken production systems is provided by the integration of the PPN concept with protease usage. Increased biological efficiency, lower feed formulation costs, and less environmental effect from nitrogen excretion are all made possible by their combination. Thus, the use of proteases in the context of PPN can be viewed as a viable tactic to promote future poultry productivity that is more effective, competitive, and sustainable.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

- Abd El-Hack, M.E., Allam, A.A., Aldhalmi, A.K., Kamal, M., Arif, M., Alawam, A.S., Rudayni, H.A., Taha, A.E., Swelum, A.A., Elolimi, A.A., Madkour, M., Ashour, E.A., 2025. Integrating metabolomics for precision nutrition in poultry: optimizing growth, feed efficiency, and health. *Front. Vet. Sci.* 12, 1594749. doi: 10.3389/fvets.2025.1594749.
- Abdel-Wareth, A.A.A., Ahmed, A.A., 2026. Advancing Poultry Nutrition: AI Innovations for Sustainable Nutrient Requirements of Poultry: A Review. *Agriculture* 16, 450. doi: 10.3390/agriculture16040450.
- Abdulmajid, S., Suliaman, S.H., 2025. Impact of protease supplementation on growth performance and economic efficiency in broiler chicks. *Univ. Thi-Qar J. Agric. Res.* 14, 293–305. doi: 10.54174/15khfa70.
- Adedokun, S.A., Olojede, O.C., 2019. Optimizing Gastrointestinal Integrity in Poultry: The Role of Nutrients and Feed Additives. *Front. Vet. Sci.* 5, 348. doi: 10.3389/fvets.2018.00348.
- Adhikari, R., Rochell, S.J., Kriseldi, R., Silva, M., Greiner, L., Williams, C., Matton, B., Anderson, A., Erf, G.F., Park, E., Haydon, K., Lee, J., 2025. Recent advances in protein and amino acid nutritional dynamics in relation to performance, health, welfare, and cost of production. *Poult. Sci.* 104, 104852. doi: 10.1016/j.psj.2025.104852.
- Ajomiwe, N., Boland, M., Phongthai, S., Bagiyal, M., Singh, J., Kaur, L., 2024. Protein Nutrition: Understanding Structure, Digestibility, and Bioavailability for Optimal Health. *Foods* 13, 1771. doi: 10.3390/foods13111771.
- Alabi, T., Adedokun, S., 2025. Amino Acid Nutrition in Poultry: A Review. *Animals (Basel)* 15, 3323. doi: 10.3390/ani15223323.
- Alagawany, M., Elnesr, S.S., Farag, M.R., 2018. The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. *Iran. J. Vet. Res.* 19, 157–164.
- Alagawany, M., Elnesr, S.S., Farag, M.R., Tiwari, R., Yatoo, M.I., Karthik, K., Michalak, I., Dhama, K., 2020. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health - a comprehensive review. *Vet. Q.* 41, 1–29. doi: 10.1080/01652176.2020.1857887.
- Amer, S.A., Beheiry, R.R., Fattah, D.M.A., Roushdy, E.M., Hassan, F.A.M., Ismail, T.A., Zaitoun, N.M.A., Abo-Elmaaty, A.M.A., Metwally, A.E., 2021. Effects of different feeding regimens with protease supplementation on growth, amino acid digestibility, economic efficiency, blood biochemical parameters, and intestinal histology in broiler chickens. *BMC Vet. Res.* 17, 283. doi: 10.1186/s12917-021-02946-2.
- Anggrawan, R., Candra, D.A., Lestari, N.A., Amam, A., Prastiya, R.A., Akmal, Y., 2026. Ideal Protein Concept in broiler chicken nutrition: Advances in essential amino acid profiling, modeling approaches, and sustainable feed formulation: Ideal Protein Concept in broiler chicken nutrition. *Lett. Anim. Biol.* 6, 67–75. doi: 10.62310/liab.v6i1.329.
- Aruwa, C.E., Pillay, C., Nyaga, M.M., Sabiu, S., 2021. Poultry gut health - microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. *J. Anim. Sci. Biotechnol.* 12, 119. doi: 10.1186/s40104-021-00640-9.
- Awan, A., Bartlett, A., Blakeley-Ruiz, J.A., Richie, T., Theriot, C.M., Kleiner, M., 2025. Dietary protein from different sources escapes host digestion and is differentially modified by gut microbiota. *Food Funct.* 16, 7154–7168. doi: 10.1039/d5fo01132a.
- Azizi, M.N., Aminullah, N., Alam, S., Danish, F., 2026. Poultry gut health: industry drivers, management determinants, and emerging research directions. *Vet. Anim. Sci.* 31, 100566. doi: 10.1016/j.vas.2026.100566.
- Bedford, M.R., Apajalahti, J.H., 2022. The role of feed enzymes in maintaining poultry intestinal health. *J. Sci. Food Agric.* 102, 1759–1770. doi: 10.1002/jsfa.11670.
- Beski, S.S.M., Swick, R.A., Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. *Anim. Nutr.* 1, 47–53. doi: 10.1016/j.aninu.2015.05.005.
- Bist, R.B., Bist, K., Poudel, S., Subedi, D., Yang, X., Paneru, B., Mani, S., Wang, D., Chai, L., 2024. Sustainable poultry farming practices: a critical review of current strategies and future prospects. *Poult. Sci.* 103, 104295. doi: 10.1016/j.psj.2024.104295.
- Borda-Molina, D., Zuber, T., Siegert, W., Camarinha-Silva, A., Feuerstein, D., Rodehutschord, M., 2019. Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens. *Poult. Sci.* 98, 2906–2918. doi: 10.3382/ps/pez038.
- Broch, J., Nunes, R.V., Alhotan, R.A., Pesti, G.M., 2020. Comparison of formulation costs for poultry feeds based on crude protein versus the digestible lysine to protein ratio. *Cienc. Anim. Bras.* 21, e-58858. doi: 10.1590/1809-6891v21e-58858.
- Bryan, D.D.S.L., Classen, H.L., 2020. In Vitro Methods of Assessing Protein Quality for Poultry. *Animals (Basel)* 10, 551. doi: 10.3390/ani10040551.
- Bryden, W.L., Li, X., 2010. Amino acid digestibility and poultry feed formulation: expression, limitations and application. *R. Bras. Zootec.* 39, 279–287. doi: 10.1590/S1516-35982010001300031.
- Cambra-López, M., Marín-García, P.J., Lledó, C., Cerisuelo, A., Pascual, J.J., 2022. Biomarkers and De Novo Protein Design Can Improve Precise Amino Acid Nutrition in Broilers. *Animals (Basel)* 12, 935. doi: 10.3390/ani12070935.
- Cao, K.X., Deng, Z.C., Li, S.J., Yi, D., He, X., Yang, X.J., Guo, Y.M., Sun, L.H., 2024. Poultry Nutrition: Achievement, Challenge, and Strategy. *J. Nutr.* 154, 3554–3565. doi: 10.1016/j.tjnut.2024.10.030.

- Chalova, V.I., Kim, J., Patterson, P.H., Ricke, S.C., Kim, W.K., 2016. Reduction of nitrogen excretion and emission in poultry: A review for organic poultry. *J. Environ. Sci. Health B* 51, 230–235. doi: 10.1080/03601234.2015.1120616.
- Chen, S., Maulu, S., Wang, J., Xie, X., Liang, X., Wang, H., Wang, J., Xue, M., 2023. The application of protease in aquaculture: Prospects for enhancing the aquafeed industry. *Anim. Nutr.* 16, 105–121. doi: 10.1016/j.aninu.2023.11.001.
- Cho, I., Yoon, J.H., Jung, H.J., Kong, C., 2026. Ileal Amino Acid Digestibility in Various Protein Sources Fed to Broiler Chickens. *Animals* 16, 779. doi: 10.3390/ani16050779.
- Clavijo, V., Flórez, M.J.V., 2018. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poult. Sci.* 97, 1006–1021. doi: 10.3382/ps/pep359.
- Cordero, P., Ramirez-Tolosa, G., Dufflocq, P., Herrera-Alcaino, S., Guzmán-Pino, S.A., 2025. Reduced Dietary Protein and Essential Amino Acids Impair Growth Performance and Increase Lysine Sensitivity in Broiler Chickens. *Animals (Basel)* 15, 1027. doi: 10.3390/ani15071027.
- Corsetti, G., Pasini, E., Scarabelli, T.M., Romano, C., Singh, A., Scarabelli, C.C., Dioguardi, F.S., 2024. Importance of Energy, Dietary Protein Sources, and Amino Acid Composition in the Regulation of Metabolism: An Indissoluble Dynamic Combination for Life. *Nutrients* 16, 2417. doi: 10.3390/nu16152417.
- Cowieson, A.J., Kluever, A.M., 2019. Contribution of exogenous enzymes to potentiate the removal of antibiotic growth promoters in poultry production. *Anim. Feed Sci. Technol.* 250, 81–92. doi: 10.1016/j.anifeeds.2018.04.026.
- da Nobrega, I.P.T., Teixeira, L.D.V., Fascina, V.B., Bittencourt, L.C., 2025. Effect of Protease Supplementation in Diets with or Without Copper Sulfate and Formaldehyde on the Standardized Digestibility of Amino Acids in Broiler Chickens. *Animals* 15, 1059. doi: 10.3390/ani15071059.
- de Oliveira, C.R.C., Rabello, C.B., Rodrigues, P.B., da Costa Lopes, C., Silva, B.A., Lopes, E.C., Ludke, M.D.C.M.M., de Souza, L.F.A., Nascimento, J.C.D.S., da Silva, W.A., Ribeiro, A.G., Carvalho, L.R.R.A., Dos Santos, M.J.B., 2025. Nutritional composition and prediction of energy values of poultry offal meal and meat and bone meal for broilers. *Trop. Anim. Health Prod.* 57, 335. doi: 10.1007/s11250-025-04580-8.
- Dessimoni, G.V., Dalólio, F.S., Moreira, J., Teixeira, L.V., Bertechini, A.G., Hermes, R.G., 2019. Protease Supplementation under Amino Acid Reduction in Diets Formulated with Different Nutritional Requirements for Broilers. *Rev. Bras. Cienc. Avic.* 21, 1–8. doi: 10.1590/1806-9061-2017-0707.
- Dittoe, D.K., Olson, E.G., Ricke, S.C., 2022. Impact of the gastrointestinal microbiome and fermentation metabolites on broiler performance. *Poult. Sci.* 101, 101786. doi: 10.1016/j.psj.2022.101786.
- Ducatelle, R., Goossens, E., Eeckhaut, V., Van Immerseel, F., 2023. Poultry gut health and beyond. *Anim. Nutr.* 13, 240–248. doi: 10.1016/j.aninu.2023.03.005.
- Duque-Ramirez, C.F., Javierre, J.A., Peña-Sierra, L.M., Diaz-Vargas, M., 2023. Effect of exogenous protease on performance, nutrient digestibility, intestinal histomorphometric, meat quality characteristics, carcass yield in broilers fed low protein diets. *Trop. Anim. Health Prod.* 55, 190. doi: 10.1007/s11250-023-03562-y.
- El Enshasy, H., Dailin, D.J., Abd Manas, N.H., Wan Azlee, N.I., Eyahmalay, J., Yahaya, S.A., Abd Malek, R., Siwapiragam, V., Sukmawati, D., 2018. Current and Future Applications of Phytases in Poultry Industry: A Critical Review. *J. Ad. VetBio Sci. Tech.* 3, 65–74. doi: 10.31797/vetbio.455687.
- Frountzas, M., Stefanakoudakis, D., Mela, E., Theodorou, P., Theodoropoulos, G.E., Theodorou, D., Toutouzias, K.G., 2025. Peripheral Parenteral Nutrition and Personalized Nutritional Approach After Colorectal Resection Surgery: A Comprehensive Review of Current Evidence. *Medicina* 61, 1459. doi: 10.3390/medicina61081459.
- Gao, Z., Zheng, J., Xu, G., 2025. Molecular Mechanisms and Regulatory Factors Governing Feed Utilization Efficiency in Laying Hens: Insights for Sustainable Poultry Production and Breeding Optimization. *Int. J. Mol. Sci.* 26, 6389. doi: 10.3390/ijms26136389.
- George, A.S., George, A.S.H., 2023. Optimizing Poultry Production Through Advanced Monitoring and Control Systems. *Partn. Univers. Int. Innov.* 1, 77–97. doi: 10.5281/zenodo.10050352.
- Ghavanpanjeh, M., Payvastegan, S., Mirghelenj, S.A., Froushani, S.M.A., Delfani, N., 2026. Effects of protease and lipase supplementation in starter diet on growth performance, small intestine morphology, and immunity in young broilers. *Vet. Anim. Sci.* 33, 100720. doi: 10.1016/j.vas.2026.100720.
- Gilani, G.S., Xiao, C.W., Cockell, K.A., 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *Br. J. Nutr.* 108, S315–S332. doi: 10.1017/S0007114512002371.
- He, X., Wang, X., Wu, J., Zhang, X., Ghane, A.E., Wei, S., Zhang, H., Fan, Y., Ye, H., Zhu, Y., 2026. Effect of supplementation of phytase and its combination with xylanase, amylase, and protease on growth performance, carcass quality and nutrient utilization of yellow-feathered broilers fed diets with lower nutritional density. *Poult. Sci.* 105, 106392. doi: 10.1016/j.psj.2026.106392.
- Heo, Y.J., Park, J., Kim, Y.B., Kwon, B.Y., Kim, D.H., Song, J.Y., Lee, K.W., 2023. Effects of dietary protein levels on performance, nitrogen excretion, and odor emission of growing pullets and laying hens. *Poult. Sci.* 102, 102798. doi: 10.1016/j.psj.2023.102798.
- Hong, J., Perez-Palencia, J., Patterson, R., Levesque, C., 2025. Effects of protease supplementation on the ileal digestibility of amino acids for protein ingredients in broiler chickens. *Poult. Sci.* 104, 105605. doi: 10.1016/j.psj.2025.105605.
- Hossain, M.E., Amin, N.B., 2026. Proliferation dynamics of enterocytes: Implications for intestinal barrier function, performance, health, and welfare in broiler chickens. *Poult. Sci.* 105, 107128. doi: 10.1016/j.psj.2026.107128.
- Iqbal, W., Ayalew, H., Li, S., Wang, X., Xu, C., Yaqoob, M.U., Ali, A., Zhang, H., 2025. Optimizing low crude protein diet: Evaluating Glycine as a conditionally essential Amino Acid. *Poult. Sci.* 104, 105163. doi: 10.1016/j.psj.2025.105163.
- Islam, R., Bhakta, S., Khatun, A., Feroz, T., Siddique, M.P., 2025. Microalgal diversity, nutritional profiles, and applications in poultry nutrition: A comprehensive review. *Anim. Nutr.* 22, 426–441. doi: 10.1016/j.aninu.2025.02.010.
- Jason, L.E., David, H.B., 1997. Use of the Ideal Protein Concept for Precision Formulation of Amino Acid Levels in Broiler Diets. *J. Appl. Poult. Res.* 6, 462–470. doi: 10.1093/japr/6.4.462.
- Jha, R., Korver, D., Kim, W.K., Marchal, L., Gibbs, K., Halley, J., 2026. The inflammation saga: Breakthrough nutritional insights for poultry. *Poult. Sci.* 105, 106200. doi: 10.1016/j.psj.2025.106200.
- Jha, R., Mishra, P., 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. *J. Anim. Sci. Biotechnol.* 12, 51. doi: 10.1186/s40104-021-00576-0.
- Junior, D.T.V., Genova, J.L., Kim, S.W., Saraiva, A., Rocha, G.C., 2024. Carbohydrases and Phytase in Poultry and Pig Nutrition: A Review beyond the Nutrients and Energy Matrix. *Animals (Basel)* 14, 226. doi: 10.3390/ani14020226.
- Kageyama, T., 2002. Pepsinogens, progastricins, and prochymosins: structure, function, evolution, and development. *Cell. Mol. Life Sci.* 59, 288–306. doi: 10.1007/s00018-002-8423-9.
- Kaur, L., Mao, B., Beniwal, A.S., Abhilasha, Kaur, R., Chian, F.M., Singh, J., 2022. Alternative proteins vs animal proteins: The influence of structure and processing on their gastro-small intestinal digestion. *Trends Food Sci. Technol.* 122, 275–286. doi: 10.1016/j.tifs.2022.02.021.
- Khajali, F., Rafiei, F., 2024. A review of plant anti-nutritional factors in animal health and production: The classification, biological properties, and the passivation strategy. *J. Agric. Food Res.* 18, 101290. doi: 10.1016/j.jafr.2024.101290.
- Kidd, M.T., Maynard, C.W., Mullenix, G.J., 2021. Progress of amino acid nutrition for diet protein reduction in poultry. *J. Anim. Sci. Biotechnol.* 12, 45. doi: 10.1186/s40104-021-00568-0.
- Kim, J.C., Sarem, B., Siegart, W., Sulabo, R., Tatekura, S., 2026. Steering nitrogen emission from poultry production through precision nutrition. *Poult. Sci.* 105, 106864. doi: 10.1016/j.psj.2026.106864.
- Kumari, K.N.R., Chandra, K.R., Kumar, D.S., 2016. Effect of protein levels in diet on production and emissions in WLH layer. *Adv. Anim. Vet. Sci.* 4, 357–362.
- Kurz, A., Seifert, J., 2021. Factors Influencing Proteolysis and Protein Utilization in the Intestine of Pigs: A Review. *Animals* 11, 3551. doi: 10.3390/ani11233551.
- Kuzmina, I.V., Tolpygo, S.M., Kotov, A.V., Shoiibonov, B.B., Zamolodchikova, T.S., Ovchinnikova, N.V., 2024. Intestinal digestion in poultry compared to other animal species with a diverse diet. *Front. Physiol.* 15, 1358524. doi: 10.3389/fphys.2024.1358524.
- Law, F.L., Zulkifli, I., Soleimani, A.F., Liang, J.B., Awad, E.A., 2018. The effects of low-protein diets and protease supplementation on broiler chickens in a hot and humid tropical environment. *Asian-Australas. J. Anim. Sci.* 31, 1291–1300. doi: 10.5713/ajas.17.0581.
- Lee, D.T., Rochell, S.J., 2022. Precision intestinal nutrition: knowledge and gaps regarding the role of amino acids during an enteric challenge. *Poult. Sci.* 101, 101674. doi: 10.1016/j.psj.2021.101674.
- Lemme, A., Hiller, P., Klahsen, M., Taube, V., Stegemann, J., Simon, I., 2019. Reduction of dietary protein in broiler diets not only reduces n-emissions but is also accompanied by several further benefits. *J. Appl. Poult. Res.* 28, 867–880. doi: 10.3382/japr/pfz045.
- Li, J., Hou, M., Yuan, W., Zhang, X., Wu, X., Li, Y., Jiang, R., Li, D., Guo, Y., Kang, X., Gong, Y., Wang, Y., Tian, Y., 2025. Establishment of an Amino Acid Nutrition Prediction Model for Laying Hens During the Brooding and Early-Growing Period. *Animals (Basel)* 15, 3178. doi: 10.3390/ani15213178.
- Lin, Y., Lourenco, J.M., Olukosi, O.A., 2023. The effects of protease, xylanase, and xylo-oligosaccharides on growth performance, nutrient utilization, short-chain fatty acids, and microbiota in Eimeria-challenged broiler chickens fed low-protein diet. *Poult. Sci.* 102, 102789. doi: 10.1016/j.psj.2023.102789.
- Liu, G., Castro, F.L.S., Kim, W.K., 2023. Applied Research Note: Exogenous protease supplementation to reduced-energy, reduced-protein, and reduced-amino acid diets for broiler chickens from days 1 to 42. *J. Appl. Poult. Res.* 32, 100362. doi: 10.1016/j.japr.2023.100362.
- Liu, H., Faruk, M.U., Smith, A., Aureli, R., Chatelle, C., 2025. Research note: A novel protease improves egg production and fecal nutrient utilization in laying hens fed corn- or wheat-based diet. *Poult. Sci.* 104, 105013. doi: 10.1016/j.psj.2025.105013.
- Llorens, B., Pomar, C., Goyette, B., Rajagopal, R., Andretta, I., Latorre, M.A., Remus, A., 2024. Precision feeding as a tool to reduce the environmental footprint of pig production systems: a life-cycle assessment. *J. Anim. Sci.* 102, skae225. doi: 10.1093/jas/skae225.
- Lourenco, J.M., Nunn, S.C., Lee, E.J., Dove, C.R., Callaway, T.R., Azain, M.J., 2020. Effect of Supplemental Protein on Growth Performance and Excreta Microbiome of Broiler Chicks. *Microorganisms* 8, 475. doi: 10.3390/microorganisms8040475.
- Ma, W., Tang, C., Lai, L., 2005. Specificity of trypsin and chymotrypsin: loop-motion-controlled dynamic correlation as a determinant. *Biophys. J.* 89, 1183–1193. doi: 10.1529/biophysj.104.057158.
- Ma, Y.B., Zou, W.G., Ai, C.X., Liu, S.T., Luo, X., You, W.W., Ke, C.H., 2025. Ideal Protein-Based Estimation of Essential Amino Acid Requirements and The Role of Lysine Supplementation in Low-Protein Feed Formulations of Abalone. *Curr. Dev. Nutr.* 9, 107528. doi: 10.1016/j.cdnut.2025.107528.
- Macelline, S.P., Chrystal, P.V., Liu, S.Y., Selle, P.H., 2021. The Dynamic Conversion of Dietary Protein and Amino Acids into Chicken-Meat Protein. *Animals (Basel)* 11, 2288. doi: 10.3390/ani11082288.
- Macelline, S.P., Liu, S.Y., Selle, P.H., 2025a. Attrition of amino acids in the life span of broiler chickens. *Anim. Nutr.* 22, 201–213. doi: 10.1016/j.aninu.2025.05.004.
- Macelline, S.P., Selle, P.H., Toghyani, M., Liu, S.Y., 2025b. The cost of amino acid catabolism for energy utilization in broiler chickens. *Poult. Sci.* 104, 105199. doi: 10.1016/j.psj.2025.105199.
- Marmion, M., Ferone, M.T., Whyte, P., Scannell, A.G.M., 2021. The changing microbiome of poultry meat: from farm to fridge. *Food Microbiol.* 99, 103823. doi: 10.1016/j.fm.2021.103823.
- Matton, B., Verhelle, A., Vlerick, L., Keirsschiet, K., Sarem, B., 2025. Feeding Broilers with Reduced Dietary Crude Protein or Reduced Soybean Meal Diets Has No Negative Impact on the Performance and Carcass Traits While Reducing the Feed Global Warming Potential. *Animals* 15, 1753. doi: 10.3390/ani15121753.
- Morgan, N., Bhuiyan, M.M., Hopcroft, R., 2022. Non-starch polysaccharide degradation in the gastrointestinal tract of broiler chickens fed commercial-type diets supplemented with either a single dose of xylanase, a double dose of xylanase, or a cocktail of non-starch polysaccharide-degrading enzymes. *Poult. Sci.* 101, 101846. doi: 10.1016/j.psj.2022.101846.
- Morin, G., Pinel, K., Heraud, C., Le-Garrec, S., Wayman, C., Dias, K., Terrier, F., Lanuque, A., Fontagné-Dicharry, S., Seiliez, I., Beaumatin, F., 2024. Precision formulation, a new concept to improve dietary amino acid absorption based on the study of cationic amino acid transporters. *iScience* 27, 108894. doi: 10.1016/j.isci.2024.108894.
- Moss, A.F., Chrystal, P.V., Cadogan, D.J., Wilkinson, S.J., Crowley, T.M., Choct, M., 2021. Precision feeding and precision nutrition: a paradigm shift in broiler feed formulation? *Anim. Biosci.* 34, 354–362. doi: 10.5713/ab.21.0034.
- Moss, A.F., Dao, H.T., Kim, E., Morgan, N., Dersjant-Li, Y., Ghane, A., 2026. Effects of Phytase, Xylanase, Amylase and Protease Inclusion at Full Matrix Specifications in Diets for Broiler Chickens. *Appl. Sci.* 16, 2862. doi: 10.3390/app16062862.
- Musigwa, S., Cozannet, P., Asiamah, C.A., Wu, S.B., 2024. Effects of Dietary Protein Levels, Net Energy Levels, and Essential Amino Acid-to-True Protein Ratios on Broiler Performance. *Animals (Basel)* 14, 3065. doi: 10.3390/ani14213065.
- Myrie, S.B., Bertolo, R.F., Sauer, W.C., Ball, R.O., 2008. Effect of common antinutritive factors and fibrous feedstuffs in pig diets on amino acid digestibilities with special emphasis on threonine. *J. Anim. Sci.* 86, 609–619. doi: 10.2527/jas.2006.793.
- Naeem, M., 2026. Processed animal proteins in poultry nutrition: nutritional value, digestibility, safety, and applications. *Trop. Anim. Health Prod.* 58, 389. doi: 10.1007/s11250-026-05193-5.
- Nanto-Hara, F., Ema, T., Ohtsu, H., 2026. Amino-Acid-Balanced Low-Protein Diets Reduce Nitrogen Excretion Without Affecting Growth Performance in Broilers. *Animals* 16, 494. doi: 10.3390/ani16030494.
- Nassar, F.S., 2026. Strategic role of poultry production sciences in shaping the future of global food security and strengthen sustainability. *Poult. Sci.* 105, 106617. doi: 10.1016/j.psj.2026.106617.
- Nath, S.K., Islam, M.S., Hossain, M.T., Islam, M.M., Dipa, R.A., Saha, T., Hossain, M.S., Sarker, S., Talukder, S., 2025. Matrix-Dosed Protease Supplementation Enhances Growth Performance, Nutrient Utilization, and Economic Return in Broiler Chickens. *Poultry* 4, 61. doi: 10.3390/poultry4040061.
- Nawab, A., Dao, T.H., Chrystal, P.V., Cadogan, D., Wilkinson, S., Kim, E., Crowley, T., Barekatin, R., Moss, A.F., 2025. Evaluation of Precision Feeding to Enhance Broiler Growth Performance. *Animals* 15, 2433. doi: 10.3390/ani15162433.
- Njeri, F.M., Kiarie, E.G., Ellis, J.L., 2026. Significance of specialty protein ingredients in feeding programs for broiler chickens on nitrogen utilization and excretion-A meta-analysis. *Poult. Sci.* 105, 106262. doi: 10.1016/j.psj.2025.106262.
- Nuamah, E., Okon, U.M., Jeong, E., Mun, Y., Cheon, I., Chae, B., Odoi, F.N.A., Kim, D.W., Choi, N.J., 2024. Unlocking Phytate with Phytase: A Meta-Analytic View of Meat-Type Chicken Muscle Growth and Bone Mineralization Potential. *Animals* 14, 2090. doi: 10.3390/ani14142090.
- Nuamah, E., Okon, U.M., Kim, J., Song, G., Kang, D., Lee, H., Shim, K., 2026. Functional Amino Acid Supplementation Drives Early Growth and Gut Maturation in Broilers: A Meta-Analysis. *Animals* 16, 1207. doi: 10.3390/ani16081207.
- Obianwuna, U.E., Chang, X., Oleforuh-Okoleh, V.U., Onu, P.N., Zhang, H., Qiu, K., Wu, S., 2024. Phytobiotics in poultry: revolutionizing broiler chicken nutrition with plant-derived gut health enhancers. *J. Anim. Sci. Biotechnol.* 15, 169. doi: 10.1186/s40104-024-01101-9.
- Ogwuegbu, M.C., Ali, L.C., Chukwudi, P., Innocent, T.Z., Mthiyane, D.M.N., Okpanachi, U., Machebe, N.S., 2025. Modulating the gut microbiome: strategies for improved poultry health and productivity. *Agric. Food Secur.* 14, 60. doi: 10.1186/s40066-025-00582-9.
- Oke, O.E., Fasasi, L.O., Opopoye, I.O., Akosile, O.A., 2025. The role of gut microbiota-derived metabolites in modulating poultry immunometabolism. *Front. Physiol.* 16, 1700406. doi: 10.3389/fphys.2025.1700406.
- Oktariansyah, Y., Kamal, M., 2026. Alternative Feed Use in Poultry Chickens: Impacts on Feed Efficiency and Physiological Parameters—A Systematic Literature Review. *J. Nat. Sci.* 7, 41–53.

- doi: 10.34007/jonas.v7i1.1110.
- Onbaşlılar, E.E., Yalçın, S., Batur, B., Yalçın, S., Kılıçlı, İ.B., Bakıcı, C., Bakır, B., Kartal, Y.K., Sel, T., 2026. Effects of Xylanase and Protease Supplementation on Growth Performance, Meat Quality, Gut Health, Cecal Fermentation, and Bone Traits in Broiler Chickens. *Animals* 16, 465. doi: 10.3390/ani16030465.
- Osei-Akoto, A., Abdel-Wareth, A.A.A., Salahuddin, P., Goswami, P.K., Lohakare, J., 2026. Sustainable Poultry Production Through Novel Nutrition and Circular Resource Management. *Sustainability* 18, 3673. doi: 10.3390/su18083673.
- Oxenboll, K., Pontoppidan, K., Fru-Njii, F., 2011. Use of a Protease in Poultry Feed Offers Promising Environmental Benefits. *Int. J. Poultry Sci.* 10, 842–848. doi: 10.3923/ijps.2011.842.848.
- Pan, D., Yu, Z., 2014. Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes* 5, 108–119. doi: 10.4161/gmic.26945.
- Park, J.H., Lee, S.I., Kim, I.H., 2020. The effect of protease on growth performance, nutrient digestibility, and expression of growth-related genes and amino acid transporters in broilers. *J. Anim. Sci. Technol.* 62, 614–627. doi: 10.5187/jast.2020.62.5.614.
- Peñuela-Sierra, L.M., Aragão-Neto, V.L., Lozano-Cruz, P., Mejía-Abaunza, J.N., Ali, M., Cabañas-Ojeda, J., Yang, Y., Alfaro-Wisquillo, M.C., Quintana-Ospina, G.A., Vasanthakumari, B.L., Weal-leans, A., Lao, Y., Oviedo-Rondón, E.O., 2024. Multiprotease improves amino acid release in vitro, energy, and nutrient utilization in broilers fed diets varying in crude protein levels. *Poult. Sci.* 103, 103546. doi: 10.1016/j.psj.2024.103546.
- Perera, W.N.U., Ravindran, V., 2025. Role of feed additives in poultry nutrition: Historical, current and future perspectives. *Anim. Feed Sci. Technol.* 326, 116371. doi: 10.1016/j.anifeedsci.2025.116371.
- Pesti, G.M., Chocot, M., 2023. The future of feed formulation for poultry: Toward more sustainable production of meat and eggs. *Anim. Nutr.* 15, 71–87. doi: 10.1016/j.aninu.2023.02.013.
- Pomar, C., Andretta, I., Remus, A., 2021. Feeding Strategies to Reduce Nutrient Losses and Improve the Sustainability of Growing Pigs. *Front. Vet. Sci.* 8, 742220. doi: 10.3389/fvets.2021.742220.
- Poudel, I., Hodge, V.R., Wamsley, K.G.S., Roberson, K.D., Adhikari, P.A., 2023. Effects of protease enzyme supplementation and varying levels of amino acid inclusion on productive performance, egg quality, and amino acid digestibility in laying hens from 30 to 50 weeks of age. *Poult. Sci.* 102, 102465. doi: 10.1016/j.psj.2022.102465.
- Predecus, N.C., Stefan, G., Rosu, M.P., Papuc, C., 2024. Fermented Feed in Broiler Diets Reduces the Antinutritional Factors, Improves Productive Performances and Modulates Gut Microbiome—A Review. *Agriculture* 14, 1752. doi: 10.3390/agriculture14101752.
- Qaid, M.M., Al-Garadi, M.A., 2021. Protein and Amino Acid Metabolism in Poultry during and after Heat Stress: A Review. *Animals (Basel)* 11, 1167. doi: 10.3390/ani11041167.
- Qiu, K., Chen, J., Zhang, G., Chang, W., Zheng, A., Cai, H., Liu, G., Chen, Z., 2023. Effects of Dietary Crude Protein and Protease Levels on Performance, Immunity Capacity, and AA Digestibility of Broilers. *Agriculture* 13, 703. doi: 10.3390/agriculture13030703.
- Quan, W., Zhou, J., Wang, J., Huang, J., Du, L., 2025. Machine Learning-Driven Precision Nutrition: A Paradigm Evolution in Dietary Assessment and Intervention. *Nutrients* 18, 45. doi: 10.3390/nu18010045.
- Radhi, K.S., Arif, M., Rehman, A.U., Faizan, M., Almohmadi, N.H., Youssef, I.M., Swelum, A.A., Suliman, G.M., Tharwat, M., Ebrahim, A., Abd El-Hack, M.E., Mahrose, K.M., 2023. Growth performance of broiler chickens fed diets supplemented with amylase and protease enzymes individually or combined. *Open Vet. J.* 13, 1425–1435. doi: 10.5455/OVJ.2023.v13.i11.5.
- Ravindran, V., 2013. Feed enzymes: The science, practice, and metabolic realities. *J. Appl. Poult. Res.* 22, 628–636. doi: 10.3382/japr.2013-00739.
- Ravindran, V., 2021. Progress in ileal endogenous amino acid flow research in poultry. *J. Anim. Sci. Biotechnol.* 12, 5. doi: 10.1186/s40104-020-00526-2.
- Ravindran, V., Abdollahi, M.R., 2021. Nutrition and Digestive Physiology of the Broiler Chick: State of the Art and Outlook. *Animals (Basel)* 11, 2795. doi: 10.3390/ani1102795.
- Ravindran, V., Bryden, W.L., 1999. Amino acid availability in poultry—in vitro and in vivo measurements. *Aust. J. Agric. Res.* 50, 889–908. doi: 10.1071/AR98174.
- Raza, A., Bashir, S., Tabassum, R., 2019. An update on carbohydrases: growth performance and intestinal health of poultry. *Heliyon* 5, e01437. doi: 10.1016/j.heliyon.2019.e01437.
- Recoules, E., Lessire, M., Labas, V., Duclos, M.J., Combes-Soia, L., Lardic, L., Peyronnet, C., Quinsac, A., Narcy, A., Réhault-Godbert, S., 2019. Digestion dynamics in broilers fed rapeseed meal. *Sci. Rep.* 9, 3052. doi: 10.1038/s41598-019-38725-1.
- Remus, A., Hauschild, L., Létourneau-Montminy, M.P., Pomar, C., 2021. Estimating Amino Acid Requirements in Real-Time for Precision-Fed Pigs: The Challenge of Variability among Individuals. *Animals (Basel)* 11, 3354. doi: 10.3390/ani1123354.
- Rochell, S.J., 2023. 80 Precision Nutrition to Enhance Poultry Performance and Health: the Role of Functional Amino Acids. *J. Anim. Sci.* 101, 175. doi: 10.1093/jas/skad281.212.
- Romero-Garay, M.G., Montalvo-González, E., Hernández-González, C., Soto-Dominguez, A., Becerra-Verdin, E.M., De Lourdes García-Magaña, M., 2021. Bioactivity of peptides obtained from poultry by-products: A review. *Food Chem.* 13, 100181. doi: 10.1016/j.fochx.2021.100181.
- Rubino, M., Jin, J., Gramlich, L., 2022. Safety and impact of peripheral parenteral nutrition on nutrient delivery in patients with nutrition risk: A prospective observational study. *Nutr. Clin. Pract.* 37, 1162–1171. doi: 10.1002/ncp.10764.
- Selle, P.H., Macelline, S.P., Chrystal, P.V., Liu, S.Y., 2023a. A reappraisal of amino acids in broiler chicken nutrition. *World's Poultry Sci. J.* 79, 429–447. doi: 10.1080/00439339.2023.2234342.
- Selle, P.H., Macelline, S.P., Chrystal, P.V., Liu, S.Y., 2023b. The Contribution of Phytate-Degrading Enzymes to Chicken-Meat Production. *Animals (Basel)* 13, 603. doi: 10.3390/ani13040603.
- Singh, A.K., Kim, W.K., 2021. Effects of Dietary Fiber on Nutrients Utilization and Gut Health of Poultry: A Review of Challenges and Opportunities. *Animals* 11, 181. doi: 10.3390/ani11010181.
- Soisuwan, K., Plaimast, H., Thongnum, A., Chotnirat, S., Nopparatmairee, M., 2023. Effect of natural multi-enzyme supplementation on growth performance, gut microflora, carcass characteristics, and meat quality of broilers reared in tropical climates. *J. Anim. Health Prod.* 11, 129–138. doi: 10.17582/journal.jahp/2023/11.2.129.138.
- Son, J., Lee, W.D., Kim, C.H., Kim, H., Hong, E.C., Kim, H.J., 2024. Effect of Dietary Crude Protein Reduction Levels on Performance, Nutrient Digestibility, Nitrogen Utilization, Blood Parameters, Meat Quality, and Welfare Index of Broilers in Welfare-Friendly Environments. *Animals (Basel)* 14, 3131. doi: 10.3390/ani14213131.
- Song, J.Y., Heo, Y.J., Park, J., Lee, H.K., Kim, Y.B., Kwon, B.Y., Kim, D.H., Lee, K.W., 2025. Effects of Dietary Additives on Nitrogen Balance, Odor Emissions, and Yolk Corticosterone in Laying Hens Fed Low-Protein Diets. *Animals (Basel)* 15, 2021. doi: 10.3390/ani15142021.
- Souza, L.J., Barbosa, J.A.L., Júnior, H.M., Pereira, F.A., Tse, M.L.P., Ruiz, U.S., 2025. The Addition of an Acid Protease Improved the Digestibility of Crude Protein and Amino Acids of Soybean Meal, but Not of Corn, in Piglets. *Animals* 15, 3037. doi: 10.3390/ani15203037.
- Suyama, M.S., Gewehr, C.E., 2026. Dietary fiber in poultry nutrition: impacts on physiology, digestive metabolism and on productive performance. *Trop. Anim. Health Prod.* 58, 160. doi: 10.1007/s11250-026-04970-6.
- Szydlowska, A., Sionek, B., Kołożyn-Krajewska, D., 2026. Interactions Between Plant Proteins and Gut Microbiota as Determinants of Intestinal Health. *Microorganisms* 14, 540. doi: 10.3390/microorganisms14030540.
- Taylor-Bowden, T., Bhogoj, S., Khwatenge, C.N., Nahashon, S.N., 2024. The Impact of Essential Amino Acids on the Gut Microbiota of Broiler Chickens. *Microorganisms* 12, 693. doi: 10.3390/microorganisms12040693.
- Tedeschi, L.O., Mendes, E.D.M., Fernandes, M.H.M.R., 2026. Nutrition as the Intelligent Nexus: Integrating Precision Farming into Sustainable Ruminant Systems. *Agriculture* 16, 1379. doi: 10.3390/agriculture16131379.
- Teng, P.Y., Yadav, S., Shi, H., Kim, W.K., 2021. Evaluating endogenous loss and standard ileal digestibility of amino acids in response to the graded severity levels of E. maxima infection. *Poult. Sci.* 100, 101426. doi: 10.1016/j.psj.2021.101426.
- Tokutake, Y., Taciak, M., Sato, K., Toyomizu, M., Kikusato, M., 2021. Effect of dipeptide on intestinal peptide transporter 1 gene expression: An evaluation using primary cultured chicken intestinal epithelial cells. *Anim. Sci. J.* 92, e13604. doi: 10.1111/asj.13604.
- Uni, Z., Platin, R., Sklan, D., 1998. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. *J. Comp. Physiol. B* 168, 241–247. doi: 10.1007/s003600050142.
- Urban, J., Kareem, K.Y., Atanasov, A.G., Matuszewski, A., Bień, D., Ciborowska, P., Rygalo-Galewska, A., Michalczuk, M., 2024. Postbiotics, a natural feed additive for growth performance, gut microbiota and quality of poultry products. *Curr. Res. Biotechnol.* 8, 100247. doi: 10.1016/j.crbiot.2024.100247.
- Vertiprakhov, V.G., Trukhachev, V.I., Ovchinnikova, N.V., 2023. Trypsin cycling in poultry is associated with metabolic regulation. *Front. Physiol.* 14, 1226546. doi: 10.3389/fphys.2023.1226546.
- Volpato, J.A., Ribeiro, L.B., Torezan, G.B., da Silva, I.C., Martins, I.O., Genova, J.L., de Oliveira, N.T.E., Carvalho, S.T., Carvalho, P.L.O., Vasconcellos, R.S., 2022. Characterization of the variations in the industrial processing and nutritional variables of poultry by-product meal. *Poult. Sci.* 101, 101926. doi: 10.1016/j.psj.2022.101926.
- Walker, H., Vartiainen, S., Apajalahti, J., Taylor-Pickard, J., Nikodinoska, I., Moran, C.A., 2024. The Effect of including a Mixed-Enzyme Product in Broiler Diets on Performance, Metabolizable Energy, Phosphorus and Calcium Retention. *Animals* 14, 328. doi: 10.3390/ani14020328.
- Wan, J., Shahid, M.S., Yuan, J., 2023. The Comparative Effects of Supplementing Protease Combined with Carbohydrase Enzymes on the Performance and Egg n-3 Deposition of Laying Hens Fed with Corn-Flaxseed or Wheat-Flaxseed Diets. *Animals (Basel)* 13, 3510. doi: 10.3390/ani13223510.
- Wang, M., Macelline, S.P., Liu, S.Y., Selle, P.H., 2025. Fat deposition and growth performance in broiler chickens are diversely influenced by maize or wheat following dietary crude protein reductions plausibly involving insulin. *J. Anim. Sci. Biotechnol.* 16, 179. doi: 10.1186/s40104-025-01314-6.
- Wang, Q.D., Zhang, K.Y., Zhang, Y., Bai, S.P., Ding, X.M., Wang, J.P., Peng, H.W., Tian, G., Xuan, Y., Su, Z.W., Zeng, Q.F., 2020a. Effects of dietary protein levels and protease supplementation on growth performance, carcass traits, meat quality, and standardized ileal digestibility of amino acid in Pekin ducks fed a complex diet. *Poult. Sci.* 99, 3557–3566. doi: 10.1016/j.psj.2020.03.047.
- Wang, Q.D., Li, S., Zhang, K.Y., Zhang, Y., Bai, S.P., Ding, X.M., Wang, J.P., Peng, H.W., Tian, G., Xuan, Y., Su, Z.W., Zeng, Q.F., 2020b. Protease supplementation attenuates the intestinal health damage caused by low-protein diets in Pekin ducks. *Poult. Sci.* 99, 6630–6642. doi: 10.1016/j.psj.2020.10.012.
- Wickramasuriya, S.S., Park, I., Lee, K., Lee, Y., Kim, W.H., Nam, H., Lillehoj, H.S., 2022. Role of Physiology, Immunity, Microbiota, and Infectious Diseases in the Gut Health of Poultry. *Vaccines* 10, 172. doi: 10.3390/vaccines10020172.
- Wu, G., Li, P., 2022. The "ideal protein" concept is not ideal in animal nutrition. *Exp. Biol. Med. (Maywood)* 247, 1191–1201. doi: 10.1177/15353702221082658.
- Wu, Y.H.S., Lin, Y.L., Chen, Y.C., 2026. Protein hydrolysates derived from poultry by-products as nutraceuticals: Bioactive potential, limitations, safety considerations, and commercialization perspectives. *Poult. Sci.* 105, 106956. doi: 10.1016/j.psj.2026.106956.
- Yao, Y.Y., Ye, Y., Xiong, K., Mao, S.C., Jiang, J.W., Chen, Y.Q., Li, X., Liu, H.B., Liu, L.C., Cai, B., Song, S., 2026. Current Progress and Future Directions of Enzyme Technology in Food Nutrition: A Comprehensive Review of Processing, Nutrition, and Functional Innovation. *Foods* 15, 402. doi: 10.3390/foods15020402.
- Yegani, M., Korver, D.R., 2008. Factors affecting intestinal health in poultry. *Poult. Sci.* 87, 2052–2063. doi: 10.3382/ps.2008-00091.
- Yokhana, J.S., Parkinson, G., Frankel, T.L., 2016. Effect of insoluble fiber supplementation applied at different ages on digestive organ weight and digestive enzymes of layer-strain poultry. *Poult. Sci.* 95, 550–559. doi: 10.3382/ps/pev336.
- Yoon, J.H., Lourenco, J., Olukosi, O.A., 2026. Interactive effects of dietary crude protein reduction and resistant starch inclusion on growth performance and cecal microbial fermentation in broiler chickens. *Poult. Sci.* 105, 106677. doi: 10.1016/j.psj.2026.106677.
- Zaefarian, F., Cowieson, A.J., Pontoppidan, K., Abdollahi, M.R., Ravindran, V., 2021. Trends in feed evaluation for poultry with emphasis on in vitro techniques. *Anim. Nutr.* 7, 268–281. doi: 10.1016/j.aninu.2020.08.006.
- Zafar, M.S., Qaisrani, S.N., Saima, Hayat, Z., Nauman, K., 2025. Impacts of Protease Sources on Growth and Carcass Response, Gut Health, Nutrient Digestibility, and Cecal Microbiota Profiles in Broilers Fed Poultry-by-Product-Meal-Based Diets. *Metabolites* 15, 445. doi: 10.3390/metabo15070445.
- Zakria, M., Leghari, I.H., Rajput, N., Soomro, S.A., Pirzado, S.A., 2025. Assessing the effect of dietary protease supplementation with low amino acid diet on growth, gut enzyme activity, immunity, meat quality and bone morphometry in broiler chicken. *J. Anim. Health Prod.* 13, 572–582. doi: 10.17582/journal.jahp/2025/13.3.572.582.
- Zampiga, M., Calini, F., Sirri, F., 2021. Importance of feed efficiency for sustainable intensification of chicken meat production: implications and role for amino acids, feed enzymes and organic trace minerals. *World's Poultry Sci. J.* 77, 639–659. doi: 10.1080/00439339.2021.1959277.
- Zhang, C., Hao, E., Chen, X., Huang, C., Liu, G., Chen, H., Wang, D., Shi, L., Xuan, F., Chang, D., Chen, Y., 2023. Dietary Fiber Level Improve Growth Performance, Nutrient Digestibility, Immune and Intestinal Morphology of Broilers from Day 22 to 42. *Animals (Basel)* 13, 1227. doi: 10.3390/ani13071227.
- Zhang, C., Wu, K., Wang, F., Yang, S., Li, J., Xie, M., Yin, Y., Yao, K., 2026. Effects of Amino Acid Supplementation in Low-Protein Diets on Productive Performance, Digestive Function, and Intestinal Health of Laying Hens. *Animals* 16, 1232. doi: 10.3390/ani16081232.
- Zhang, H., Wielen, N.V., Hee, B.V., Wang, J., Hendriks, W., Gilbert, M., 2020. Impact of Fermentable Protein, by Feeding High Protein Diets, on Microbial Composition, Microbial Catabolic Activity, Gut Health and beyond in Pigs. *Microorganisms* 8, 1735. doi: 10.3390/microorganisms8111735.
- Zhang, Y., Yu, S., Liu, C., Jiang, S., Wang, H., Cheng, Y., Guo, Y., Qian, H., 2025. Optimizing Enzymatic Processes for Enhanced Nutritional and Organoleptic Properties of Chicken Bones. *Foods* 14, 1217. doi: 10.3390/foods14071217.
- Zhao, W.H., Jang, S.Y., Kim, I.H., 2025. Optimizing Broiler Performance and Feed Cost Efficiency: Impact of 1,3-Diacylglycerol Supplementation at Different Energy Levels. *Vet. Sci.* 12, 633. doi: 10.3390/vetsci12070633.
- Zheng, M., Bai, Y., Sun, Y., An, J., Chen, Q., Zhang, T., 2023. Effects of Different Proteases on Protein Digestion In Vitro and In Vivo and Growth Performance of Broilers Fed Corn-Soybean Meal Diets. *Animals (Basel)* 13, 1746. doi: 10.3390/ani13111746.