

Protease-mediated optimization of low-protein diets: Mechanistic insights into amino acid efficiency and gut function in poultry

Siti R. Ayuti¹, Eun J. Kim², Aswin R. Khairullah³, Amirul F.M. Azmi⁴, Zulfi N.A. Rosyada^{5*}, Rico Anggriawan⁶, Bima P. Pratama⁷

¹Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Jl. Tgk. Hasan Krueng Kalee No. 4, Kopelma Darussalam, Banda Aceh, 23111, Aceh, Indonesia.

²Department of Animal Science and Biotechnology, Kyungpook National University, Daehak-ro, Buk-gu, 41566, Daegu, Republic of Korea.

³Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km. 46 Cibinong, Bogor, 16911, West Java, Indonesia.

⁴Animal Nutrition and Production Research Group, Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Jl. Padang Tembak, Pengkalan Chepa, 16100, Kelantan, Malaysia.

⁵Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya, 60115, East Java, Indonesia.

⁶Department of Animal Husbandry, Faculty of Agriculture and Animal Husbandry, Universitas Kahuripan Kediri, Jl. Pb. Sudirman No.25, Plongko, Pare, Kediri, 64212, East Java, Indonesia.

⁷Research Center for Process Technology, National Research and Innovation Agency (BRIN), KST BJ Habibie, Serpong, South Tangerang, 15314, Banten, Indonesia.

ARTICLE INFO

Received: 06 July 2026

Accepted: 23 September 2026

*Correspondence:

Corresponding author: Zulfi Nur Amrina Rosyada
E-mail address: nur.amrina@fkf.unair.ac.id

Keywords:

Amino acid utilization, Exogenous protease, Gut health, Low-protein diet, Nutrient digestibility, Poultry nutrition.

ABSTRACT

Interest in nutritional techniques that enhance nutrient absorption while lowering environmental effects has increased due to the growing demand for sustainable poultry production. Low-protein diets (LPDs) have drawn a lot of interest as a way to reduce nitrogen excretion and feed costs without sacrificing productivity. Reducing dietary crude protein levels, however, may restrict the availability of amino acids, hinder the use of nutrients, and, if improperly balanced, have detrimental effects on gastrointestinal function. In this regard, exogenous proteases have become important feed enzymes that can improve the efficiency of amino acid consumption in chickens and improve protein digestion. With a focus on the processes underpinning enhanced amino acid efficiency and gastrointestinal function, this study highlights the state of the art addressing the role of protease supplementation in enhancing low-protein diets. By improving the digestion of dietary proteins, proteases decrease the transit of undigested protein to the lower gastrointestinal tract and increase the release and absorption of amino acids. Better nitrogen retention, increased nutritional digestibility, and more effective feed use are all facilitated by these benefits. Additionally, by promoting villus formation, preserving barrier integrity, and regulating microbial activity within the gut ecosystem, protease supplementation affects intestinal physiology. These reactions encourage a better intestinal environment for the absorption of nutrients and the general health of the digestive system. Recent research shows that adding exogenous proteases to low-protein feeding programs can maintain growth performance and production efficiency while lowering ammonia emissions and nitrogen losses. When taken as a whole, these results demonstrate the potential of proteases as a key element of precision nutrition techniques meant to enhance the sustainability of chicken production from an economic and environmental standpoint. Future studies should concentrate on improving the use of enzymes through developments in microbiome science, digestive physiology, and precision feed formulation.

Introduction

As the principal source of amino acids needed for growth, tissue development, maintenance, and productive performance, protein is one of the most important and expensive parts of poultry diets (Taylor-Bowden *et al.*, 2024). In order to provide a sufficient supply of critical amino acids, conventional chicken feeding plans have historically depended on rather high crude protein levels (Alabi and Adedokun, 2025). However, because a significant amount of ingested protein is not retained by the bird and is subsequently expelled, increasing dietary protein concentrations frequently leads to inefficient nitrogen consumption (Musigwa *et al.*, 2024). This inefficiency raises feed costs and adds to environmental issues related to manure management and nitrogen emissions (Such *et al.*, 2021).

Low-protein diets (LPDs), which aim to improve nutrient utilization while lowering reliance on protein-rich feed ingredients, have emerged as a potential nutritional strategy in response to these issues (Woyengo *et al.*, 2023). Nutritionists can now lower dietary crude protein levels while preserving a balanced amino acid profile thanks to developments in feed formulation and the availability of crystalline amino acids (Kidd *et al.*, 2021). Without sacrificing productive efficiency, well designed LPDs can reduce feed costs, reduce nitrogen excretion, and enhance the sustainability of poultry production systems (Nanto-Hara *et al.*, 2026).

Despite these benefits, there are certain drawbacks to using LPDs (Bhusal *et al.*, 2026). When the supply of digestible amino acids is insufficient, reductions in dietary crude protein may affect nitrogen metabolism, amino acid availability, and nutrient use efficiency (Chen *et al.*, 2026). Inadequate protein digestion can also increase the quantity of un-

digested substrates that enter the lower gastrointestinal system, which may have an impact on intestinal integrity, microbial activity, and general gut function (Adedokun and Olojede, 2019). These difficulties show that additional nutritional strategies are required in reduced-protein feeding programs in order to improve nutrient digestibility and sustain gastrointestinal health (Pan and Yu, 2014).

Exogenous proteases are one of the many feed additives that have drawn a lot of interest as a way to enhance the use of protein in chicken diets (da Nobrega *et al.*, 2025). These enzymes boost the availability of amino acids, improve the breakdown of dietary proteins, and lessen nutrient losses brought on by inadequate digestion (Wahid *et al.*, 2025). Beyond their effects on protein degradation, proteases may also alter intestinal architecture, microbial ecology, and nutrient absorption processes, ultimately contributing to increased digestive efficiency and gut functionality (Gibril *et al.*, 2025). Because of this, protease supplementation is now a crucial part of precision nutrition plans meant to optimize the benefits of low-protein diets (Zafar *et al.*, 2025).

A thorough grasp of the mechanisms connecting protease activity, amino acid efficiency, and gut function is still crucial for maximizing their use in poultry production, despite the fact that many studies have examined the individual effects of low-protein diets and exogenous proteases. Thus, the purpose of this study is to summarize what is currently known about the function of exogenous proteases in low-protein feeding systems, with a focus on how they affect intestinal health, growth performance, protein and amino acid use, and environmental sustainability. This study aimed to offer mechanistic insights and suggest future research areas for the creation of more effective and sustainable feeding

regimens by incorporating recent developments in poultry nutrition and digestive physiology.

Low-protein diets in poultry production

Low-protein diets have become an increasingly important nutritional strategy in modern poultry production due to their potential to improve nutrient utilization efficiency, reduce feed costs, and minimize environmental impacts. The successful implementation of this approach relies on precise amino acid supplementation and a thorough understanding of the nutritional and physiological consequences associated with dietary protein reduction.

Nutritional principles and formulation of low-protein diets

In chicken production, LPDs have become a strategic nutritional technique to increase the efficiency of protein use while lowering feed costs and nitrogen emissions into the environment (Li *et al.*, 2025). In order to create LPDs, dietary crude protein concentrations are lowered below standard levels without sacrificing the availability of digestible amino acids needed for growth, maintenance, and production (Zhang *et al.*, 2025a). This strategy is predicated on the understanding that birds require particular amino acids rather than just crude protein (Shen *et al.*, 2026).

The perfect protein concept, which provides essential amino acids in amounts that closely correspond to the bird's physiological needs, is vital to the successful application of LPDs (Anggriawan *et al.*, 2026). Nutritionists can now lower dietary protein levels while preserving a suitable amino acid balance thanks to developments in feed formulation and the improved availability of crystalline amino acids (Adhikari *et al.*, 2025). Supplementing with synthetic sources of lysine, methionine, threonine, tryptophan, valine, and other limiting amino acids reduces amino acid shortages that could hinder growth performance and helps make up for the decrease in protein-rich feed ingredients (Maynard *et al.*, 2022).

Despite these benefits, reducing dietary crude protein may change how nitrogen is metabolized and decrease the availability of bioactive nitrogen-containing molecules and non-essential amino acids (Siegert *et al.*, 2025). If amino acid requirements are not properly met, excessive protein reduction can potentially affect intestinal function, metabolic efficiency, and nutrient digestibility (Naeem, 2026). As a result, precise ingredient identification, the formulation of digestible amino acids, and a thorough comprehension of nutrient interactions throughout the gastrointestinal tract are critical to the efficacy of LPDs (Bryden and Li, 2010).

Low-protein feeding techniques are a crucial tool for maximizing nutrient consumption while promoting productive performance and environmental stewardship as poultry nutrition continues to advance toward higher precision and sustainability (Nassar, 2026).

Benefits and challenges of low-protein diets

The potential economic, environmental, and nutritional advantages of low-protein diets have drawn a lot of attention in the field of poultry nutrition (Matton *et al.*, 2025). Low-protein formulations can save feed costs and increase nutrient use efficiency by minimizing the use of high-protein feed ingredients, especially soybean meal (Son *et al.*, 2024). The profitability of chicken production systems is still impacted by changes in the price and availability of conventional protein sources, making this tactic more and more important (Osei-Akoto *et al.*, 2026).

From an environmental standpoint, cutting back on dietary crude protein reduces nitrogen consumption, which in turn reduces nitrogen excretion in manure (Nanto-Hara *et al.*, 2026). Ammonia emissions and environmental pollution are caused by the metabolism and excretion of excess dietary protein that is not used for productive purposes (Swelum *et al.*, 2021). Thus, increasing dietary protein levels while satisfying amino

acid requirements might enhance the efficiency of nitrogen consumption and promote more environmentally friendly methods of raising chickens (Alabi and Adedokun, 2025).

Despite these benefits, low-protein diets are still difficult to execute successfully (Kareem *et al.*, 2025). Even when key limiting amino acids are given in crystalline form, excessive reductions in crude protein may restrict the supply of some essential and non-essential amino acids (Kidd *et al.*, 2021). Protein synthesis, food metabolism, and tissue development can all be hampered by insufficient amino acid availability, which eventually lowers growth performance, feed efficiency, and carcass quality (Qaid and Al-Garadi, 2021). Additionally, intestinal physiology and nutrient absorption processes may be affected by low-protein diets, especially in cases of amino acid shortages or imbalances (Salahi *et al.*, 2025).

Therefore, accurate formulation techniques that take into consideration the birds' physiological needs, component variability, and digestible amino acid requirements are essential for the success of low-protein feeding regimens (Pesti and Choct, 2023). Advances in feed enzyme technology, notably the use of exogenous proteases, have opened new chances to circumvent some of these restrictions by raising protein digestibility and improving amino acid availability in reduced-protein diets (Ghavipanjeh *et al.*, 2026).

Protease as a nutritional tool in poultry feeding

Exogenous proteases have gained significant attention in poultry nutrition due to their ability to enhance protein utilization and improve the nutritional value of feed ingredients. By complementing endogenous digestive enzymes, proteases facilitate more efficient protein digestion and contribute to improved amino acid availability, making them particularly valuable in low-protein feeding strategies.

Characteristics and sources of exogenous proteases

Because they can promote nutrient utilization and protein decomposition, exogenous proteases are among the most often used feed enzymes in chicken nutrition (da Nobrega *et al.*, 2025). These enzymes accelerate the breakdown of peptide bonds in dietary proteins, producing free amino acids and smaller peptides that the bird can absorb and use more easily (Youssef, 2025). Numerous parameters, including as substrate selectivity, catalytic activity, pH stability, temperature tolerance, and resilience to gastrointestinal conditions, affect how effective exogenous proteases are (Zakria *et al.*, 2025).

Proteases are often divided into serine, cysteine, aspartic, and metalloproteases according to their catalytic processes (Verma *et al.*, 2016). Because of their wide substrate range and high hydrolytic activity, serine proteases are most frequently used in feed applications (Zheng *et al.*, 2023). Proteases can also be classified as acidic, neutral, or alkaline enzymes based on their ideal pH levels, indicating which parts of the gastrointestinal tract they are best suited for (Zafar *et al.*, 2025). Therefore, the ability of a certain protease to continue to function under the physiological conditions encountered during digestion is intimately linked to its selection (Park *et al.*, 2020).

Microbial fermentation is the primary method used to create commercial feed proteases due to its effectiveness, scalability, and affordability (Katu *et al.*, 2025a). Because they can create extracellular proteases with great stability and activity, bacterial species—especially those in the genus *Bacillus*—are used extensively (Phan *et al.*, 2025). Fungal microorganisms, such as *Aspergillus* and *Trichoderma* species, are prized for their varied enzymatic profiles and are significant producers of proteolytic enzymes (Zhang *et al.*, 2025b). The production of genetically modified microbial strains that can produce proteases with higher catalytic qualities and tolerance to feed processing conditions has been made possible by advances in biotechnology (Meng *et al.*, 2025).

Next-generation proteases that target certain food proteins and en-

hance amino acid release have been developed in response to the increased interest in precision nutrition (Keohavong, 2026). These developments have extended the use of exogenous proteases beyond basic protein hydrolysis, establishing these enzymes as crucial instruments for enhancing nutrient efficiency, promoting gut health, and enabling the effective application of low-protein feeding techniques in contemporary poultry production systems (da Silva *et al.*, 2021).

Mechanisms of protein hydrolysis by exogenous proteases

The ability of exogenous proteases to catalyze the degradation of dietary proteins into smaller, more digestible components is the main factor contributing to their nutritional efficacy (Duque-Ramírez *et al.*, 2023). After ingestion, these enzymes break down complex protein structures that endogenous enzymes might not be able to fully digest by cleaving peptide bonds in protein substrates (Ajomiwe *et al.*, 2024). Protein fractions that are difficult to digest are made more accessible by this additional proteolytic activity, especially those linked to dietary ingredients produced from plants (Tejeda and Kim, 2021).

Large polypeptides are broken down into oligopeptides, dipeptides, and free amino acids as protein hydrolysis advances, all of which are effectively absorbed via the intestinal epithelium (Jeong *et al.*, 2024). Protein accretion and productive performance are supported by the increased availability of absorbable nitrogenous substances, which enhance amino acid digestibility and utilization (Perera and Ravindran, 2025). In the distal gastrointestinal tract, where excessive protein fermentation can produce unwanted compounds that harm intestinal health, enhanced protein breakdown may also lessen the flow of undigested protein (Ducatelle *et al.*, 2023).

Exogenous proteases can change the structural properties of some feed components and lessen the antinutritional effects of proteinaceous chemicals in addition to enhancing nutrient release (Gilbert *et al.*, 2018). Protease inhibitors and storage proteins found in some plant-based products impede digestion by reducing the activity of natural enzymes or limiting the availability of nutrients (Yan *et al.*, 2025). Exogenous proteases may partially break down these substances through targeted hydrolysis, increasing protein availability and promoting more effective digestion processes (Rada *et al.*, 2016).

By reducing the buildup of undigested protein substrates that are available for microbial fermentation, the hydrolytic action of proteases may further promote a more advantageous intestinal environment (Amer *et al.*, 2021). This action can assist proper nutrient absorption and preserve digestive efficiency, especially in diets designed with lower quantities of crude protein (Song *et al.*, 2023). As a result, the mechanism of protein hydrolysis goes beyond straightforward protein degradation and includes a number of physiological and biochemical reactions that together improve fowl gastrointestinal function and amino acid consumption (Kareem *et al.*, 2025). The mechanism by which exogenous proteases enhance protein hydrolysis and reduce undigested protein flow is illustrated in Figure 1.

Protease-mediated improvement of amino acid efficiency

Improving amino acid efficiency is a primary objective of modern poultry nutrition, particularly in low-protein feeding systems where nutrient utilization must be optimized. Through enhanced protein hydrolysis and increased nutrient availability, exogenous proteases play a crucial role in maximizing amino acid digestibility, improving nitrogen utilization, and supporting more efficient dietary amino acid balance. Table 1 summarizes the principal mechanisms through which exogenous proteases enhance amino acid efficiency in poultry fed low-protein diets.

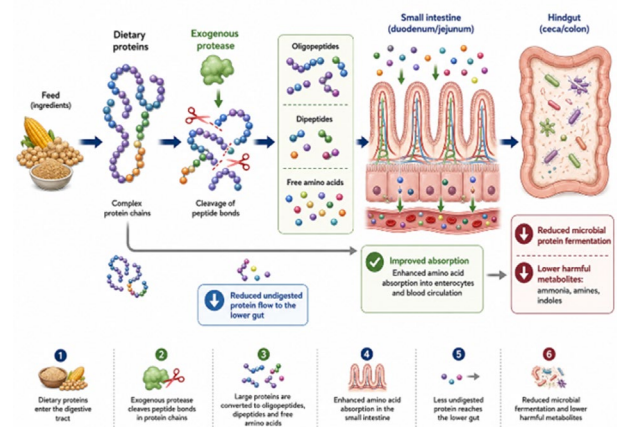


Figure 1. Mechanism of exogenous protease action on dietary protein hydrolysis in low-protein poultry diets.

Enhancement of protein and amino acid digestibility

One of the main goals of enzyme supplementation in poultry nutrition is to increase the digestibility of dietary protein (Bedford and Apajalahti, 2022). It has been demonstrated that adding exogenous proteases improves the hydrolysis of protein fractions that indigenous digestive enzymes are unable to fully break down (Alagawany *et al.*, 2018). This increases the amount of absorbable peptides and amino acids that are available in the gastrointestinal system (Taylor-Bowden *et al.*, 2024). This effect is especially significant in low-protein diets, when maintaining growth performance and production efficiency requires making the most of available nutrients (Shen *et al.*, 2026).

Ileal digestibility, which measures the percentage of nutrients absorbed prior to reaching the hindgut, is a frequently used biomarker of protein consumption (Bryan *et al.*, 2019). Reduced nutritional losses and less undigested protein entering the lower digestive system are linked to improved ileal digestibility, which is linked to a more effective conversion of dietary protein into metabolically accessible amino acids (Macelline *et al.*, 2021). Protease supplementation has been shown in numerous trials to improve the ileal digestibility of crude protein and specific amino acids, indicating increased nutrient recovery from feed constituents and higher digestive efficiency (Poudel *et al.*, 2024).

Protease supplementation improves the availability of critical amino acids needed for tissue growth, maintenance, and productive functions in addition to improving total protein digestion (Park *et al.*, 2020). In poultry diets, amino acids including lysine, methionine, threonine, and valine are often regarded as limiting nutrients, especially when crude protein levels are decreased (Adhikari *et al.*, 2025). Exogenous proteases help maintain a more balanced supply of amino acids and facilitate the effective use of resources for protein synthesis and metabolic processes by increasing the release of these amino acids from dietary proteins (Peñuela-Sierra *et al.*, 2024).

Additionally, increased digestibility of amino acids may lessen the requirement for excessive dietary protein inclusion, enabling dietitians to create more precise diets without sacrificing nutrient sufficiency (Bryden and Li, 2010). By guaranteeing a steady supply of amino acid substrates for cellular growth and physiological processes, increased nutrient availability can also promote intestine development and metabolic efficiency (Shahid *et al.*, 2026). Therefore, one of the main ways that exogenous proteases facilitate the effective application of low-protein feeding techniques in chicken production is by increasing protein digestibility and amino acid availability (Ndazigaruye *et al.*, 2019).

Nitrogen utilization and reduction of nutrient losses

Because it directly affects productive performance, feed efficiency, and environmental sustainability, efficient nitrogen utilization is a crucial

goal in chicken nutrition (Gao *et al.*, 2025). Due to inadequate protein digestion and poor amino acid absorption, a significant amount of dietary nitrogen may be unused in conventional feeding regimens (Nahm, 2007). The following excretion of this wasted nitrogen is a cause of environmental worry as well as a nutritional loss (Brink *et al.*, 2022). Therefore, increasing the total efficiency of poultry production requires nutritional techniques that improve nitrogen retention (Pesti and Choct, 2023).

By enhancing the degree of protein degradation and promoting the release of absorbable amino acids within the gastrointestinal system, exogenous proteases aid in better nitrogen use (da Nobrega *et al.*, 2025). A higher percentage of dietary nitrogen can be absorbed into bodily tissues and productive outputs instead of being excreted thanks to improved digestion (Heo *et al.*, 2023). This enhancement is especially crucial for low-protein diets, as maintaining a sufficient nutritional supply while lowering dietary crude protein levels requires optimizing the biological value of available nitrogen (Iqbal *et al.*, 2025).

Another significant effect of protease supplementation is the decrease in undigested protein entering the lower gastrointestinal system (Qiu *et al.*, 2023). Microbial fermentation of protein portions that evade digestion can produce metabolites such as amines, ammonia, and other nitrogenous substances (Yu *et al.*, 2025). Overproduction of these metabolites can reduce the effectiveness of digestion and lead to nutritional waste (Atuahene *et al.*, 2025). Exogenous proteases improve nutrient recovery and utilization by increasing protein degradation in the upper digestive tract, which lowers the amount of leftover protein accessible for microbial catabolism (Duque-Ramírez *et al.*, 2023).

Significant environmental effects result from improved nitrogen retention (Cook *et al.*, 2011). Reduced nitrogen excretion helps to enhance air quality and lessen the environmental impact of intensive chicken production by reducing the amount of ammonia released from poultry manure (Bist *et al.*, 2023). Therefore, exogenous proteases' capacity to improve nitrogen utilization and reduce nutrient losses promotes sustainable production practices and economic efficiency, especially in feed-

ing regimens that lower dietary protein concentrations (Goswami *et al.*, 2026).

Interactions between exogenous proteases and synthetic amino acids

The extensive use of synthetic amino acids, which allow exact manipulation of amino acid profiles while lowering reliance on high-protein feed sources, has contributed to the growing adoption of low-protein diets in poultry nutrition (Barekaini *et al.*, 2023). However, the effective digestion and utilization of protein-bound amino acids included in feed ingredients is just as important to the nutritional effectiveness of this strategy as the supplementation of limiting amino acids (Adhikari *et al.*, 2025). In this situation, synthetic amino acids and exogenous proteases can work in tandem to maximize nutritional availability and raise total amino acid efficiency (Wealleans *et al.*, 2024).

By improving the release of amino acids from dietary proteins, protease supplementation expands the pool of digestible amino acids that can be absorbed (Szczurek and Świątkiewicz, 2020). Birds are able to use dietary protein more efficiently thanks to this enhanced nutrient release, which helps minimize differences between formulated and biologically accessible amino acid concentrations (Alagawany *et al.*, 2020). Therefore, a more balanced supply of amino acids throughout the digestive process can boost protein synthesis and productive performance when proteases and synthetic amino acids are used together (Beski *et al.*, 2015).

The interaction between these nutritional methods is particularly essential in diets with reduced crude protein levels, when amino acid imbalances are more likely to arise (Alabi and Adedokun, 2025). Reductions in amino acids derived from intact proteins may not be entirely compensated for by synthetic amino acids, despite the fact that they successfully address deficits of major limiting amino acids including lysine, methionine, threonine, and tryptophan (Cordero *et al.*, 2025). Proteases can assist maintain a more complete amino acid profile for metabolic processes and tissue accretion by increasing the availability of both es-

Table 1. Mechanisms by which exogenous proteases improve amino acid efficiency in low-protein poultry diets.

Mechanism	Primary action of exogenous protease	Nutritional consequences	Practical implications in low-protein diets	Representative references
Enhanced protein hydrolysis	Hydrolyzes complex dietary proteins into absorbable peptides and free amino acids beyond the capacity of endogenous enzymes	Increased crude protein digestibility and amino acid availability	Improves nutrient supply to support growth and productive performance under reduced crude protein diets	Bedford and Apajalahti (2022); Alagawany <i>et al.</i> (2018); Taylor-Bowden <i>et al.</i> (2024); Shen <i>et al.</i> (2026)
Improved ileal amino acid digestibility	Promotes amino acid digestion and absorption before nutrients reach the hindgut	Greater recovery of digestible amino acids with reduced nutrient losses	Enhances digestive efficiency and maximizes utilization of dietary protein	Bryan <i>et al.</i> (2019); Macelline <i>et al.</i> (2021); Poudel <i>et al.</i> (2024)
Greater availability of limiting amino acids	Enhances release of lysine, methionine, threonine, valine and other essential amino acids from dietary proteins	Improved amino acid balance for protein synthesis, tissue accretion, and metabolic functions	Helps maintain productive performance despite reduced dietary crude protein	Park <i>et al.</i> (2020); Adhikari <i>et al.</i> (2025); Peñuela-Sierra <i>et al.</i> (2024); Bryden and Li (2010); Ndazigaruye <i>et al.</i> (2019)
Improved nitrogen utilization	Increases incorporation of absorbed nitrogen into productive tissues through enhanced protein digestion	Higher nitrogen retention and reduced nitrogen excretion	Improves feed efficiency and biological utilization of dietary protein	Gao <i>et al.</i> (2025); da Nobrega <i>et al.</i> (2025); Heo <i>et al.</i> (2023); Iqbal <i>et al.</i> (2025)
Reduced undigested protein entering the hindgut	Increases protein degradation in the upper gastrointestinal tract, leaving less substrate for microbial fermentation	Reduced production of undesirable nitrogenous metabolites and improved nutrient recovery	Supports intestinal health while minimizing digestive nutrient losses	Qiu <i>et al.</i> (2023); Yu <i>et al.</i> (2025); Atuahene <i>et al.</i> (2025); Duque-Ramírez <i>et al.</i> (2023)
Reduced ammonia emission through lower nitrogen excretion	Improves nitrogen retention, thereby decreasing nitrogen excreted in manure	Lower ammonia generation and environmental nitrogen losses	Contributes to environmentally sustainable poultry production	Cook <i>et al.</i> (2011); Bist <i>et al.</i> (2023); Goswami <i>et al.</i> (2026)
Synergistic interaction with synthetic amino acids	Enhances digestibility of protein-bound amino acids while complementing supplemented crystalline amino acids	More balanced amino acid supply throughout digestion and improved protein synthesis	Facilitates precision formulation of reduced-protein diets with optimal amino acid balance	Barekaini <i>et al.</i> (2023); Wealleans <i>et al.</i> (2024); Szczurek and Świątkiewicz (2020); Beski <i>et al.</i> (2015)
Improved amino acid utilization efficiency	Optimizes utilization of both essential and non-essential amino acids while reducing amino acid catabolism	Enhanced metabolic efficiency, nitrogen retention, and protein accretion	Supports optimal performance with lower dietary protein inclusion and greater formulation flexibility	Alabi and Adedokun (2025); Cordero <i>et al.</i> (2025); Liu <i>et al.</i> (2024); Lambert <i>et al.</i> (2023)

sential and non-essential amino acids through boosting the digestibility of feed proteins (Park *et al.*, 2020).

Moreover, increased protein breakdown might increase the effectiveness of supplemental amino acid utilization (Liu *et al.*, 2024). A more balanced supply of digestible amino acids minimizes catabolic losses and enhances nitrogen retention by lowering the possibility of excesses or deficits of specific amino acids (Alabi and Adedokun, 2025). Nutritionists can accomplish performance goals while further lowering dietary protein concentrations thanks to this complementing impact, which can facilitate more accurate diet formulation (Lambert *et al.*, 2023). The complementary effects of exogenous proteases and synthetic amino acids on amino acid efficiency are summarized in Figure 2.

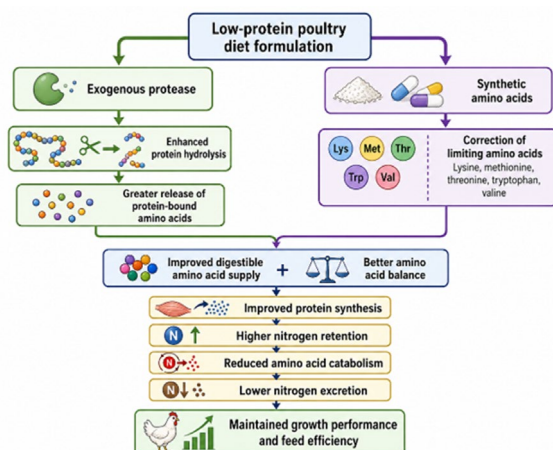


Figure 2. Complementary roles of exogenous proteases and synthetic amino acids in improving amino acid efficiency in poultry.

Effects of protease on gut function and intestinal health

In addition to enhancing protein digestibility, exogenous proteases can influence various aspects of gastrointestinal function that are closely associated with nutrient utilization and overall bird health. Their effects on intestinal morphology, microbial ecology, and nutrient absorption mechanisms highlight the broader role of proteases in maintaining gut integrity and supporting digestive efficiency in poultry. Table 2 summarizes the multifaceted effects of exogenous proteases on gut function and intestinal health in poultry.

Effects of exogenous proteases on intestinal morphology and barrier function

Intestinal integrity is a major factor in determining the health and production of chickens since the gastrointestinal tract is essential for food digestion, absorption, and host defense (Azizi *et al.*, 2026). Exogenous proteases have been shown to affect intestinal shape and barrier function in addition to their well-established effects on protein digestion (Zheng *et al.*, 2023). Particularly in birds fed low-protein diets, improvements in gut structure can improve the effectiveness of nutrient use and help maintain a stable intestinal environment (Ndazigaruye *et al.*, 2019).

An increase in villus height is one of the most commonly documented morphological reactions to protease supplementation (Nath *et al.*, 2025). Taller villi are typically linked to a larger absorptive area and improved digestive capacity. Intestinal villi serve as the main absorptive surface for

Table 2. Effects of exogenous proteases on gut function and intestinal health in poultry.

Gut health component	Mechanism of action of exogenous proteases	Physiological effects	Implications for poultry production	Representative references
Intestinal morphology	Enhanced protein digestion reduces luminal accumulation of undigested protein and supports epithelial development	Increased villus height and improved villus architecture, resulting in greater absorptive surface area	Improves nutrient utilization and supports growth performance, particularly under low-protein diets	Nath <i>et al.</i> (2025); Wang <i>et al.</i> (2025); Duangnumswang <i>et al.</i> (2021); Adabi <i>et al.</i> (2025)
Crypt architecture	Reduced intestinal stress decreases epithelial turnover and crypt cell proliferation	Lower crypt depth and improved villus-to-crypt ratio, indicating enhanced intestinal homeostasis	More dietary nutrients are allocated to growth and production rather than tissue repair	Gawel <i>et al.</i> (2025); Hossain and Amin (2026); Wickramasuriya <i>et al.</i> (2022); Onbaşilar <i>et al.</i> (2026); Kim <i>et al.</i> (2021)
Intestinal barrier function	Reduced accumulation of undigested protein limits harmful microbial fermentation and preserves mucosal integrity	Improved maintenance of tight junctions and intestinal barrier function	Strengthens defense against pathogens while maintaining efficient nutrient absorption	Wang <i>et al.</i> (2020); Caputi (2026); Zafar <i>et al.</i> (2025); Emami <i>et al.</i> (2019)
Protein fermentation in the distal intestine	Increased protein hydrolysis reduces the amount of undigested protein reaching the distal intestine	Reduced production of ammonia, biogenic amines, indoles, phenols, and other harmful fermentation products	Creates a healthier intestinal environment and improves digestive efficiency	Apajalahti and Vienola (2016); Duque-Ramirez <i>et al.</i> (2023); Aruwa <i>et al.</i> (2021)
Gut microbiota composition	Altered luminal nutrient availability reduces substrates for proteolytic bacteria while favoring beneficial microbial populations	Improved microbial balance and greater gastrointestinal homeostasis	Supports digestive efficiency and long-term gut health	Yadav and Jha (2019); Bindari and Gerber (2022); Khasanah <i>et al.</i> (2024); Aminullah <i>et al.</i> (2026); Naeem and Bourassa (2025); Grigore <i>et al.</i> (2025)
Microbial metabolism	Reduced availability of fermentable protein substrates limits production of harmful microbial metabolites	Lower epithelial stress and improved intestinal metabolic environment	Promotes intestinal stability, mucosal integrity, and productive performance	Shahid <i>et al.</i> (2026); Katu <i>et al.</i> (2025b); Azizi <i>et al.</i> (2026); Son <i>et al.</i> (2024)
Nutrient absorption	Increased availability of absorbable peptides and amino acids enhances epithelial uptake	Improved recovery of digestible nutrients and metabolic efficiency	Maximizes utilization of dietary nutrients in reduced-protein feeding systems	Musigwa <i>et al.</i> (2021); Sadr <i>et al.</i> (2025); Ababor <i>et al.</i> (2023); Abd El-Hack <i>et al.</i> (2022)
Amino acid transport mechanisms	Greater supply of digestion products enhances transporter-mediated uptake of amino acids and peptides	More efficient amino acid transport and reduced competition among transport systems	Improves utilization of dietary amino acids for growth and metabolism	Gilbert <i>et al.</i> (2007); Taylor-Bowden <i>et al.</i> (2024); Park <i>et al.</i> (2020)
Overall gut functionality	Integrated improvements in intestinal morphology, barrier integrity, microbial ecology, and nutrient absorption	Enhanced digestive capacity, intestinal homeostasis, and metabolic efficiency	Supports sustained productive performance and adaptation to low-protein feeding strategies	Wealleans <i>et al.</i> (2024); Adedokun and Olojede (2019); Jha and Mishra (2021); Oni and Oke (2025); Selle <i>et al.</i> (2023); Getahun and Yemane (2025)

nutrition intake (Wang *et al.*, 2025). The formation of epithelial cells and the preservation of a healthy villus architecture may be supported by better protein digestion and decreased exposure to undigested substrates (Duangnumsaeng *et al.*, 2021). As a result, birds fed diets treated with protease frequently have more advantageous intestinal structures that promote effective nutrient absorption (Adabi *et al.*, 2025).

Another crucial measure of intestinal health and epithelial turnover is crypt depth (Gawel *et al.*, 2025). Proliferative cells found in the intestinal crypts are in charge of replacing damaged enterocytes and regenerating the epithelial lining (Hossain and Amin, 2026). Increased tissue turnover or intestinal stress are major causes of excessive crypt deepening, which may divert nutrients from useful processes (Wickramasuriya *et al.*, 2022). A more balanced villus-to-crypt ratio has been linked to protease supplementation, indicating decreased epithelial stress and enhanced intestinal homeostasis (Onbaşlar *et al.*, 2026). These modifications might make it possible to allocate a larger percentage of dietary nutrients to processes related to growth and production rather than tissue repair (Kim *et al.*, 2021).

Exogenous proteases may help maintain the integrity of the intestinal barrier in addition to morphological changes (Wang *et al.*, 2020). The intestinal barrier serves as a selective interface that limits the translocation of pathogens, poisons, and hazardous microbial metabolites while allowing nutritional absorption (Caputi, 2026). Proteases can prevent excessive microbial fermentation and the synthesis of substances that could jeopardize epithelial integrity by decreasing the buildup of undigested protein in the gastrointestinal tract (Zafar *et al.*, 2025). The intestinal barrier's protective role may be strengthened by a better luminal environment that supports the preservation of tight junctions and mucosal structure (Emami *et al.*, 2019).

Gut microbiota modulation

The intestinal microbiota is a dynamic and complex microbial community that is essential to gastrointestinal homeostasis, immunological control, and food metabolism (Yadav and Jha, 2019). One of the main factors affecting the organization of microbial communities is dietary composition, and variations in protein consumption can have a significant impact on microbial activity in the gastrointestinal tract (Bindari and Gerber, 2022). Therefore, the composition and metabolic processes of the gut microbiota may be indirectly influenced by nutritional techniques that enhance protein digestion, such as the use of exogenous proteases (Khasanah *et al.*, 2024).

The decrease in undigested protein entering the distal colon is linked to a significant percentage of the effects of protease supplementation on microbial ecology (Onbaşlar *et al.*, 2026). Metabolites such ammonia, biogenic amines, indoles, phenols, and branched-chain fatty acids are produced when food proteins in the upper gastrointestinal system evade enzyme digestion and become substrates for microbial fermentation (Apajalahti and Vienola, 2016). Overabundance of these substances can damage gut health and foster the growth of unwanted bacteria populations (Aruwa *et al.*, 2021). Exogenous proteases inhibit excessive proteolytic fermentation in the hindgut by increasing protein hydrolysis and amino acid absorption, which lowers the availability of fermentable protein residues (Duque-Ramírez *et al.*, 2023).

Microbial population dynamics may then be impacted by changes in the intestinal lumen's nutrition availability (Aminullah *et al.*, 2026). By reducing the selection advantage of proteolytic bacteria and promoting microorganisms linked to intestinal health and digestive efficiency, reduced protein fermentation may encourage a more balanced microbial environment (Naeem and Bourassa, 2025). Protease supplementation has often been associated with changes toward a more stable and advantageous microbial community structure, despite the fact that microbial responses can vary depending on meal content, bird age, and environmental conditions (Grigore *et al.*, 2025).

Improvements in gut health may also result from regulation of microbial metabolism in addition to compositional changes (Shahid *et al.*, 2026). Reducing potentially hazardous fermentation products can assist maintain a more suitable intestinal environment for nutrition absorption and reduce epithelial stress (Katu *et al.*, 2025b). Additionally, a healthy microbial environment may promote mucosal integrity and aid in the preservation of regular immunological and digestive processes (Azizi *et al.*, 2026). These effects are especially important in low-protein feeding regimes, because maintaining productive performance depends on intestinal stability and effective nutrition utilization (Son *et al.*, 2024).

Nutrient absorption and transport mechanisms

In order to maximize the productive value of poultry diets and guarantee that digested nutrients are efficiently used for growth, maintenance, and physiological functions, efficient nutrient absorption is crucial (Musigwa *et al.*, 2021). Exogenous proteases have an impact on processes related to nutrient absorption and transport inside the intestinal epithelium, even though their primary function is to improve protein breakdown (Sadr *et al.*, 2025). Enhanced intestinal lumen nutrient availability can affect absorptive efficiency and make it easier for the body to use amino acids from food proteins (Ababor *et al.*, 2023).

After proteolytic digestion, proteins are broken down into free amino acids and tiny peptides, which are then taken up by enterocytes' apical membrane-based transport mechanisms (Gilbert *et al.*, 2007). These transporters ensure that a variety of amino acids and peptide fractions are taken up and transferred from the intestinal lumen into epithelial cells, where they are subsequently distributed throughout the body (Taylor-Bowden *et al.*, 2024). Protease supplementation improves the availability of absorbable substrates by enhanced protein degradation, which may improve the effectiveness of transporter-mediated nutrient uptake and lessen competition between amino acids for absorption sites (Park *et al.*, 2020).

Proteases' beneficial impacts on intestinal architecture and luminal conditions further bolster the advantages of better nutrient absorption (Wealleans *et al.*, 2024). Greater interaction between nutrients and absorptive cells is made possible by a well-developed absorptive surface, which is defined by advantageous villus architecture and epithelial integrity (Adedokun and Olojede, 2019). Concurrently, the reduction of undigested protein residues in the gastrointestinal tract may reduce the build-up of metabolites formed from fermentation that may obstruct regular absorption processes (Jha and Mishra, 2021). A more conducive environment for nutrition absorption and metabolic use is produced by these combined actions (Oni and Oke, 2025).

Because the dietary supply of amino acids is more accurately matched to physiological requirements in low-protein feeding regimes, effective absorption becomes especially crucial (Selle *et al.*, 2023). Maintaining productive performance in such circumstances depends on optimizing the recovery of digestible nutrients (Getahun and Yemane, 2025). Exogenous proteases can improve the bird's ability to absorb and use food nutrients by boosting the availability of absorbable amino acids and maintaining intestinal function (Abd El-Hack *et al.*, 2022). Figure 3 illustrates the main effects of exogenous proteases on gut function and intestinal health in poultry.

Effects on growth performance and production efficiency

Among the most crucial metrics for assessing the nutritional efficacy of dietary changes in chicken production are growth performance and production efficiency (Cao *et al.*, 2024). Due to its potential to enhance nutrient utilization and boost productive performance, especially in low-protein feeding regimes, the application of exogenous proteases has garnered significant interest (Duque-Ramírez *et al.*, 2023). Proteases can

improve the efficiency with which dietary resources are transformed into bodily tissues and productive outputs by improving protein digestibility and amino acid availability (Aderibigbe *et al.*, 2020).

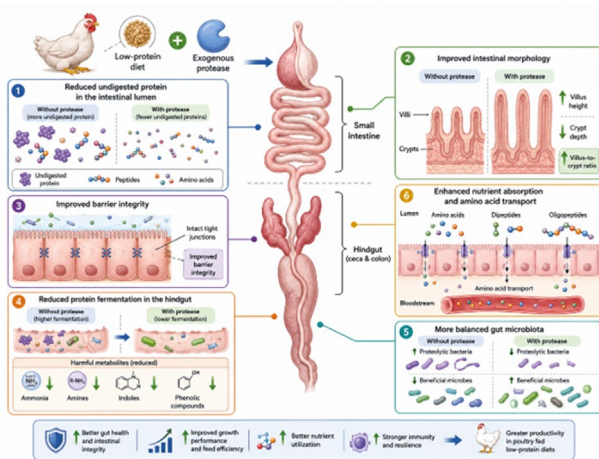


Figure 3. Effects of exogenous proteases on gut function and intestinal health in poultry fed low-protein diets.

An improvement in body weight increase is one of the main reactions linked to protease supplementation (Tajudeen *et al.*, 2022). The availability of amino acids needed for muscle growth, tissue accumulation, and metabolic processes is increased by increased hydrolysis of dietary proteins (Alabi and Adedokun, 2025). In low-protein diets, when effective use of available amino acids is necessary to maintain normal growth, improved nutrient accessibility may be particularly advantageous (Wang *et al.*, 2024). Consequently, birds eating protease-supplemented meals usually display growth performance equivalent to or exceeding that achieved with traditional protein levels (Qiu *et al.*, 2023).

Another important metric that shows how well nutrients are used is the feed conversion ratio (FCR) (Ramankevich *et al.*, 2025). A higher percentage of swallowed feed can be used for productive processes because to improved digestibility, which also lowers nutrient losses (Suyama and Gewehr, 2026). Exogenous proteases can improve feed efficiency and lower the amount of feed needed to reach a particular level of weight gain by promoting the release and absorption of amino acids from dietary proteins (Bedford and Apajalahti, 2022). In commercial poultry farms, where feed costs account for the majority of production costs, these advancements are very beneficial (Nassar, 2026).

Protease supplementation may affect carcass traits in addition to growth performance (Wealleans *et al.*, 2024). Improvements in protein utilization can facilitate more effective muscle accretion, and adequate amino acid availability is essential for lean tissue deposition and carcass growth (Kenchaiwong *et al.*, 2026). Therefore, enhanced production outcomes and positive carcass output may result from increased nutritional efficiency, especially when dietary crude protein levels are decreased without sacrificing amino acid balance (Navidshad *et al.*, 2024).

Protease supplementation's productive advantages frequently result in financial gains (Peñuela-Sierra *et al.*, 2024). Feed formulation costs can be decreased while maintaining desired performance levels through increased nutrient utilization, decreased dietary protein requirements, and improved feed efficiency (Belkhanchi *et al.*, 2023). Additionally, there are more chances for cost optimization because costly protein-rich foods can be partially substituted without negatively impacting growth or carcass characteristics (Vlaicu *et al.*, 2024). In contemporary poultry systems, where profitability depends on attaining high levels of production efficiency while minimizing resource inputs, these financial advantages are becoming more and more significant (Marmelstein *et al.*, 2024).

Environmental and sustainability implications

Concerns about the environmental effects of intensive production

systems, especially those related to nutrient waste and emissions, have grown as the demand for poultry products has increased (Górny *et al.*, 2023). Among these issues, nitrogen pollution continues to be a significant problem since it contaminates the air, land, and water (Boakye-Yiadom *et al.*, 2024). As a result, nutritional techniques that improve the efficiency of nutrient consumption are now crucial to the sustainable production of poultry (Nassar, 2026). In this regard, using low-protein diets in conjunction with exogenous proteases presents a viable strategy for lowering the environmental impact of chicken operations without sacrificing productivity (Ndazigaruye *et al.*, 2019).

A sizable amount of the nitrogen that poultry eat is not stored for development or production and is instead expelled as manure (Ball *et al.*, 2024). In addition to representing a loss of important nutrients, excessive nitrogen excretion raises the possibility of environmental contamination through nitrogen leaching, volatilization, and buildup in nearby ecosystems (Zamani *et al.*, 2026). Exogenous proteases increase nitrogen retention in the animal by increasing amino acid utilization and protein digestibility, which lowers the quantity of nitrogen discharged into the environment (Leinonen and Williams, 2015). In low-protein feeding regimes, where improving the effectiveness of dietary nitrogen usage is a main goal, this enhancement becomes especially pertinent (de Rauglaudre *et al.*, 2023).

Reduced nitrogen excretion is closely connected with lower ammonia emissions from poultry litter (Brink *et al.*, 2022). Ammonia is known to be a significant environmental contaminant and is produced by the microbial breakdown of nitrogenous substances in manure (Swelum *et al.*, 2021). In poultry plants, high ammonia concentrations can have a detrimental impact on worker health and bird welfare as well as air quality and ecosystem acidification (do Amaral Vercellino *et al.*, 2025). By reducing the amount of nitrogen available for microbial conversion into ammonia, improved nutrient utilization brought about by protease supplementation lowers emission levels and promotes a healthier production environment (Nahm, 2007).

The use of proteases in low-protein diets promotes better sustainable feed utilization in addition to its impact on nitrogen management (Oketch *et al.*, 2025). Reduced reliance on protein-rich feed ingredients, which are frequently linked to significant resource requirements during production, processing, and shipping, is made possible by improved nutrient digestibility (Klasing *et al.*, 2025). By increasing productivity while reducing resource inputs and waste production, increasing the efficiency with which nutrients are taken from available feed resources is consistent with the principles of sustainable livestock production (Hamouda *et al.*, 2025).

Future production systems are anticipated to heavily rely on nutritional interventions that increase nutrient efficiency as environmental regulations and sustainability goals gain importance in the chicken industry (Osei-Akoto *et al.*, 2026). An effective way to strike a balance between environmental responsibility and productive performance is to incorporate exogenous proteases into low-protein dietary plans (Ndazigaruye *et al.*, 2019). Protease supplementation helps create more ecologically robust and sustainable chicken production systems by decreasing nitrogen losses, ammonia emissions, and resource-use efficiency (Amer *et al.*, 2021).

Current limitations and future perspectives

Exogenous proteases have been shown to be beneficial in low-protein poultry diets, however there are still a number of issues with their continuous use and optimization in industrial production settings (Cao *et al.*, 2024). One of the main obstacles is still the variability in the biological reactions to protease supplementation, since the degree of improvement in intestinal health, growth performance, and nutrient digestibility can vary greatly between trials (Park *et al.*, 2020). These discrepancies are frequently explained by variations in the birds' physiological states, man-

agement techniques, feed composition, and enzyme properties (Ebrahimi et al., 2026).

The type of feed ingredients utilized in diet formulation has a significant impact on the effectiveness of exogenous proteases (Hong et al., 2025). The amino acid content, structural complexity, and enzymatic hydrolysis susceptibility of protein sources differ significantly (Naeem, 2026). Compared to highly digestible protein sources, ingredients with large concentrations of heat-damaged proteins, fiber-associated protein fractions, or antinutritional factors may react differently to protease supplementation (Bryan and Classen, 2020). As a result, it is not always possible to extrapolate the nutritional value produced by a particular protease across feeding regimes, underscoring the necessity of ingredient-specific assessment and enzyme characterisation (Oxenboll et al., 2011).

Variations in protease reactions are also influenced by bird-related factors (Zafar et al., 2025). Nutrient requirements and digestive capacity can be influenced by physiological stage, age, health, growth pace, and genetic background (Zhang et al., 2023). The ability of contemporary poultry strains to use nutrients generated through enzymatic hydrolysis may be impacted by their unique metabolic and nutritional traits (Cowieson et al., 2006). Therefore, to maximize enzyme application across various production systems, a more thorough understanding of host-enzyme interactions is required (Gao et al., 2025).

New possibilities for enhancing the use of exogenous proteases have been made possible by recent developments in precision nutrition (Zavelinski et al., 2024). Nutritionists can more precisely alter dietary protein and amino acid amounts by taking into consideration the nutrient contributions produced by enzyme activity through the incorporation of enzyme matrix values into feed formulation (Alabi and Adedokun, 2025). However, because diet composition, ingredient quality, and environmental factors can all affect enzyme performance, establishing trustworthy matrix values is still difficult (Zaefarian et al., 2021). To improve formulation precision, future studies should concentrate on improving prediction models that incorporate physiological and dietary factors with enzyme reactions (Cao et al., 2024).

New developments in systems science and molecular biology present new paths for the advancement of protease research (Lindberg et al., 2021). Omics-based methods, such as transcriptomics, proteomics, metabolomics, and genomes, offer potent instruments for examining the molecular processes behind enzyme-mediated enhancements in intestinal function and nutrient utilization (Zhou et al., 2025). The identification of metabolic pathways, regulatory networks, and host responses related to protease supplementation can be made easier by these technologies (Zheng et al., 2021). Our knowledge of how exogenous enzymes affect microbial ecology and microbial-derived metabolites in the gastrointestinal tract is also being enhanced by developments in microbiome research (Kogut, 2019). Integrating microbiome investigations with nutritional and physiological assessments may uncover unique mechanisms through which proteases contribute to digestive efficiency and gut health (Oni and Oke, 2025).

Therefore, multidisciplinary approaches combining nutrition, microbiology, physiology, and molecular sciences should be used in future study instead of standard performance evaluations (Ghaleghafi et al., 2025). These initiatives will support the ongoing development of sustainable low-protein feeding systems and precision poultry nutrition by helping to create more focused and efficient enzyme strategies (Vlaicu et al., 2024).

Conclusion

In chicken production, the use of exogenous proteases has become a successful nutritional method for improving the effectiveness of low-protein diets. Proteases enhance nutrition utilization and assist the maintenance of productive performance under low crude protein condi-

tions by encouraging the breakdown of dietary proteins and increasing the availability of digestible amino acids. These outcomes lead to better overall feed utilization, less nutrient losses, and more effective nitrogen retention.

Beyond their involvement in protein digestion, exogenous proteases exert positive effects on gastrointestinal function through numerous interrelated processes. Increased protein breakdown limits excessive protein fermentation and creates a more favorable intestinal environment by lowering the buildup of undigested substrates in the intestinal tract. Enhancements in nutritional absorption, intestinal shape, barrier integrity, and microbial balance further promote digestive efficiency and help to preserve gut health.

The concepts of sustainable poultry nutrition are also consistent with the incorporation of proteases into low-protein feeding programs. The environmental effects of intensive poultry production, especially ammonia emissions and nutrient waste, can be lessened via better amino acid usage and less nitrogen excretion. Simultaneously, improved feed efficiency and a possible decrease in dietary protein inclusion provide financial benefits that sustain commercial poultry systems over the long run.

Acknowledgement

This study was partially supported by the Research, Technology, and Community Service Program of the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia in the 2023 fiscal year under the Domestic Collaboration Research Scheme (grant number: 0536/E5/PG.02.00/2023).

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Ababor, S., Tamiru, M., Alkhtib, A., Wamatu, J., Kuyu, C.G., Teka, T.A., Terefe, L.A., Burton, E., 2023. The Use of Biologically Converted Agricultural Byproducts in Chicken Nutrition. *Sustainability* 15, 14562. doi: 10.3390/su151914562.
- Abd El-Hack, M.E., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Soliman, M.M., Youssef, G.B.A., Taha, A.E., Soliman, S.M., Ahmed, A.E., El-Kott, A.F., Al Syaad, K.M., Swelum, A.A., 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poult. Sci.* 101, 101696. doi: 10.1016/j.psj.2022.101696.
- Adabi, S.G., Raei, H., Torshizi, M.A.K., 2025. Can dietary protease ameliorate performance and gut integrity in broilers fed on a diet low in digestible lysine? *Poult. Sci.* 104, 105759. doi: 10.1016/j.psj.2025.105759.
- Aderibigbe, A., Cowieson, A.J., Sorbara, J.O., Pappenberger, G., Adeola, O., 2020. Growth performance and amino acid digestibility responses of broiler chickens fed diets containing purified soybean trypsin inhibitor and supplemented with a monocomponent protease. *Poult. Sci.* 99, 5007–5017. doi: 10.1016/j.psj.2020.06.051.
- 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.
- Aminullah, N., Langar, H., Mahaq, O., Azizi, M.N., Zahir, A., 2026. Microbial ecology, functional implications, and associated factors influencing poultry intestinal health. *Vet. Immunol. Immunopathol.* 293, 111065. doi: 10.1016/j.vetimm.2026.111065.
- Anggriawan, 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. *Lett. Anim. Biol.* 6, 67–75. doi: 10.62310/liab.v6i1.329.
- Apajalahti, J., Vienola, K., 2016. Interaction between chicken intestinal microbiota and protein digestion. *Anim. Feed Sci. Technol.* 221, 323–330. doi: 10.1016/j.anifeeds.2016.05.004.
- 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.
- Atuahene, D., Sam, B.A., Idan, F., Sana, S.S., Knop, R., Suthar, T., Kumar, H., Shaikh, A.M., 2025. Prebiotics, Probiotics, and Synbiotics in Pigs and Poultry: A Review of Gut Health, Performance,

- and Environmental Outcomes. *Vet. Sci.* 12, 1054. doi: 10.3390/vetsci12111054.
- 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.
- Ball, M.E.E., Wright, L.P., Wilson, K., Richmond, H., Cummings, R., Smyth, S., Davison, M., Forbes, K., Thompson, J., Bryson, P., 2024. The Nutrient Content of Litter and Manure from Different Poultry Systems—Updating and Establishing the Nutrient Profile. *Sustainability* 16, 6633. doi: 10.3390/su16156633.
- Barekatin, R., Chrystal, P.V., Nowland, T., Moss, A.F., Howarth, G.S., Van, T.T.H., Moore, R.J., 2023. Negative consequences of reduced protein diets supplemented with synthetic amino acids for performance, intestinal barrier function, and caecal microbiota composition of broiler chickens. *Anim. Nutr.* 13, 216–228. doi: 10.1016/j.aninu.2023.01.011.
- 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.
- Belkhanchi, H., Ziat, Y., Hammi, M., Ifguis, O., 2023. Formulation, optimization of a poultry feed and analysis of spectrometry, biochemical composition and energy facts. *S. Afr. J. Chem. Eng.* 44, 31–41. doi: 10.1016/j.sajce.2023.01.005.
- 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.
- Bhusal, P., Sajid, G.A., Paudel, S., 2026. Addressing therapeutic challenges in poultry production, with particular emphasis on free-range and organic systems: The potential of controlled-release delivery systems (CRDS). *Poult. Sci.* 105, 106503. doi: 10.1016/j.psj.2026.106503.
- Bindari, Y.R., Gerber, P.F., 2022. Centennial Review: Factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance. *Poult. Sci.* 101, 101612. doi: 10.1016/j.psj.2021.101612.
- Bist, R.B., Subedi, S., Chai, L., Yang, X., 2023. Ammonia emissions, impacts, and mitigation strategies for poultry production: A critical review. *J. Environ. Manag.* 328, 116919. doi: 10.1016/j.jenvman.2022.116919.
- Boakye-Yiadom, K.A., Ilari, A., Bisinella, V., Duca, D., 2024. Environmental Impact of Poultry Manure Gasification Technology for Energy and Ash Valorization. *Sustainability* 16, 9941. doi: 10.3390/su16229941.
- Brink, M., Janssens, G.P.J., Demeyer, P., Bağcı, Ö., Delezie, E., 2022. Reduction of dietary crude protein and feed form: Impact on broiler litter quality, ammonia concentrations, excreta composition, performance, welfare, and meat quality. *Anim. Nutr.* 9, 291–303. doi: 10.1016/j.aninu.2021.12.009.
- Bryan, D.D.S.L., Abbott, D.A., Van Kessel, A.G., Classen, H.L., 2019. In vivo digestion characteristics of protein sources fed to broilers. *Poult. Sci.* 98, 3313–3325. doi: 10.3382/ps/pez067.
- Bryan, D.D.S.L., Classen, H.L., 2020. In Vitro Methods of Assessing Protein Quality for Poultry. *Animals* 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.
- 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.
- Caputi, V., 2026. Functional role of the enteric nervous system in poultry intestinal health and production. *Poult. Sci.* 105, 106563. doi: 10.1016/j.psj.2026.106563.
- Chen, S., Liang, R., Fan, M., Lu, L., Zhou, J., 2026. Effects of dietary standardized ileal digestible crude protein levels on growth performance, meat quality, and Cecal microbiota of Arbor Acres broilers. *Poult. Sci.* 105, 107084. doi: 10.1016/j.psj.2026.107084.
- Cook, K.L., Rothrock, M.J., Eiteman, M.A., Lovanh, N., Sistani, K., 2011. Evaluation of nitrogen retention and microbial populations in poultry litter treated with chemical, biological or adsorbent amendments. *J. Environ. Manag.* 92, 1760–1766. doi: 10.1016/j.jenvman.2011.02.005.
- Cordero, P., Ramirez-Tolosa, G., Duflocq, 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* 15, 1027. doi: 10.3390/ani15071027.
- Cowieson, A.J., Hrubý, M., Pierson, E.E., 2006. Evolving enzyme technology: impact on commercial poultry nutrition. *Nutr. Res. Rev.* 19, 90–103. doi: 10.1079/NRR2006121.
- 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 (Basel)* 15, 1059. doi: 10.3390/ani15071059.
- da Silva, J.M.S., de Oliveira, N.R., Gouveia, A.B.V.S., Vieira, R.A., dos Santos, R.O.F., Minafra, C.S., dos Santos, F.R., 2021. Effect of protease supplementation on the digestibility of amino acids in animal-origin meals for broiler diets. *Czech J. Anim. Sci.* 66, 29–37. doi: 10.17221/134/2020-CJAS.
- de Rauglaudre, T., Méda, B., Fontaine, S., Lambert, W., Fournel, S., Létourneau-Montminy, M.P., 2023. Meta-analysis of the effect of low-protein diets on the growth performance, nitrogen excretion, and fat deposition in broilers. *Front. Anim. Sci.* 4, 1214076. doi: 10.3389/fanim.2023.1214076.
- do Amaral Vercellino, R., de Alencar Nääs, I., de Moura, D.J., de Souza Granja Barros, J., da Silva Lima, N.D., 2025. A multifactorial way to assess litter stability and ammonia risk in broiler production: effects across age (7–39 days) and thermal environments. *Ital. J. Anim. Sci.* 24, 2520–2530. doi: 10.1080/1828051X.2025.2586295.
- Duangnumswang, Y., Zentek, J., Boroojeri, F.G., 2021. Development and Functional Properties of Intestinal Mucus Layer in Poultry. *Front. Immunol.* 12, 745849. doi: 10.3389/fimmu.2021.745849.
- 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., Peñaflera-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.
- Ebrahimi, M., Daneshyar, M., Payvastegan, S., Ahmadi, H., 2026. Association and variation in performance, nutrient digestibility, carcass traits, and intestinal development of broiler chickens by various male-to-female ratios. *Vet. Anim. Sci.* 32, 100628. doi: 10.1016/j.vas.2026.100628.
- Emami, N.K., Calik, A., White, M.B., Young, M., Dalloul, R.A., 2019. Necrotic Enteritis in Broiler Chickens: The Role of Tight Junctions and Mucosal Immune Responses in Alleviating the Effect of the Disease. *Microorganisms* 7, 231. doi: 10.3390/microorganisms7080231.
- 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.
- Gawel, A., Bobrek, K., Madej, J.P., 2025. Early posthatch feeding influences small intestine development in broiler chickens. *Poult. Sci.* 104, 105861. doi: 10.1016/j.psj.2025.105861.
- Getahun, A., Yemane, N., 2025. Review on impact of dietary fiber on poultry performance. *S. Asian J. Life Sci.* 13, 41–49. doi: 10.17582/journal.sajls/2025/13.41.49.
- Ghaleghafi, A., Khorshidi, K.J., Jafari, M.A., 2025. Nutritional and managerial interventions on growth performance, nutrient transport-related genes and behavioral responses in heat stressed broiler chickens. *Vet. Anim. Sci.* 31, 100561. doi: 10.1016/j.vas.2025.100561.
- Ghaviapanjeh, M., Payvastegan, S., Mirghelani, 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.
- Gibrial, B.A.A., Tu, X., Chai, X., Xu, J., 2025. Gut microbiota composition and dietary interventions modulate abdominal fat deposition in poultry: Mechanisms and applications. *Poult. Sci.* 104, 105754. doi: 10.1016/j.psj.2025.105754.
- Gilbert, M.S., Jssennaggar, N., Kies, A.K., van Mil, S.W.C., 2018. Protein fermentation in the gut: implications for intestinal dysfunction in humans, pigs, and poultry. *Am. J. Physiol. Gastrointest. Liver Physiol.* 315, G159–G170. doi: 10.1152/ajpgi.00319.2017.
- Gilbert, E.R., Li, H., Emmerson, D.A., Webb, K.E., Wong, E.A., 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. *Poult. Sci.* 86, 1739–1753. doi: 10.1093/ps/86.8.1739.
- Górny, R.L., Ławniczek-Wałczyk, A., Klimkowicz-Pawlas, A., Górny, R.L., Ławniczek-Wałczyk, A., Piechowicz, L., Olkowska, E., Potrykus, M., Tankiewicz, M., Krupka, M., Siebielec, G., Wolska, L., 2023. Intensive poultry farming: A review of the impact on the environment and human health. *Sci. Total Environ.* 858, 160014. doi: 10.1016/j.scitotenv.2022.160014.
- Goswami, P.K., Salahuddin, M., Abdel-Wareth, A.A.A., Lohakare, J., 2026. Greenhouse gas sequestration in poultry farming: Strategies for sustainable production and environmental impact mitigation. *Poult. Sci.* 105, 106646. doi: 10.1016/j.psj.2026.106646.
- Grigore, D.M., Mircea, M.L., Pogurschi, E.N., 2025. Toward Sustainable Broiler Production: Evaluating Microbial Protein as Supplementation for Conventional Feed Proteins. *Agriculture* 15, 1486. doi: 10.3390/agriculture15141486.
- Hamouda, R.E., Youssef, I.M., Gharib, H.B., El-Menawey, M.A., Youssif, M.A.M., Osman, M.A., Abdel-Aziz, Y.A., Rudayni, H.A., Allam, A.A., Alawam, A.S., Khafaga, A.F., Abd El-Hack, M.E., Abdel-Halim, A.A., 2025. Effects of an enhanced feeding model on productivity and sustainability of broilers and hybrid chickens under Egyptian small-scale family systems. *Poult. Sci.* 104, 105845. doi: 10.1016/j.psj.2025.105845.
- 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.O., 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.
- Jeong, J.W., Lee, S.Y., Lee, D.Y., Kim, J.H., Yun, S.H., Lee, J., Mariano, E., Moon, S.S., Hur, S.J., 2024. Analytical Methods and Effects of Bioactive Peptides Derived from Animal Products: A Mini-Review. *Food Sci. Anim. Resour.* 44, 533–550. doi: 10.5851/kosfa.2024.e31.
- 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.
- Kareem, D.U., Adegoke, A.V., Amos, A.T., Adeyeye, E.A., Idowu, O.P.A., Akande, L.M., Abdulfar, Q.O., Orbuh, A.T., Aboderin, A.T., Oso, A.O., Idowu, O.M.O., 2025. Implementing reduced-protein diets for broiler chickens in emerging economies: supplementation with only the first three limiting amino acids. *Trop. Anim. Health Prod.* 57, 144. doi: 10.1007/s11250-025-04396-6.
- Katu, J.K., Tóth, T., Varga, L., 2025a. Enhancing the Nutritional Quality of Low-Grade Poultry Feed Ingredients Through Fermentation: A Review. *Agriculture* 15, 476. doi: 10.3390/agriculture15050476.
- Katu, J.K., Tóth, T., Ásványi, B., Hatvan, Z., Varga, L., 2025b. Effect of Fermented Feed on Growth Performance and Gut Health of Broilers: A Review. *Animals (Basel)* 15, 1957. doi: 10.3390/ani15131957.
- Kenchiawong, W., Kananit, S., Chankitisakul, V., Boonkum, W., 2026. Effects of Genetic Selection on Growth, Nutritional Value, and Amino Acid Profiles of Breast Muscle and Blood in Black-Boned Chickens. *Animals* 16, 581. doi: 10.3390/ani16040581.
- Keohavong, B., 2026. Digital innovation integration into biotechnology for development of sustainable protein frontiers for poultry nutrition in a circular bioeconomy. *Poult. Sci.* 105, 106276. doi: 10.1016/j.psj.2025.106276.
- Khasanah, H., Kusianto, D.E., Purnamasari, L., Cruz, J.F.D., Widianingrum, D.C., Hwang, S.G., 2024. Modulation of chicken gut microbiota for enhanced productivity and health: A review. *Vet. World* 17, 1073–1083. doi: 10.14202/vetworld.2024.1073-1083.
- 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, D.Y., Kim, J.H., Choi, W.J., Han, G.P., Kil, D.Y., 2021. Comparative effects of dietary functional nutrients on growth performance, meat quality, immune responses, and stress biomarkers in broiler chickens raised under heat stress conditions. *Anim. Biosci.* 34, 1839–1848. doi: 10.5713/ab.21.0230.
- Klasing, K.C., Dozier III, W.A., Mateos, G.G., Persia, M.E., Walzem, R.L., Sakomura, N., Reis, M., Viana, G., Riveros, R., Elkin, R.G., Angel, C.R., Korver, D.R., 2025. Informal nutrition symposium: Overview of the nutrient requirements of poultry, 10th revised edition. *J. Appl. Poult. Res.* 34, 100616. doi: 10.1016/j.japr.2025.100616.
- Kogut, M.H., 2019. The effect of microbiome modulation on the intestinal health of poultry. *Anim. Feed Sci. Technol.* 250, 32–40. doi: 10.1016/j.anifeeds.2018.10.008.
- Lambert, W., Berrocoso, J.D., Swart, B., van Tol, M., Bruininx, E., Willems, E., 2023. Reducing dietary crude protein in broiler diets positively affects litter quality without compromising growth performance whereas a reduction in dietary electrolyte balance further improves litter quality but worsens feed efficiency. *Anim. Feed Sci. Technol.* 297, 115571. doi: 10.1016/j.anifeeds.2023.115571.
- Leinonen, I., Williams, A.G., 2015. Effects of dietary protease on nitrogen emissions from broiler production: a holistic comparison using Life Cycle Assessment. *J. Sci. Food Agric.* 95, 3041–3046. doi: 10.1002/jsfa.7202.
- Li, D., Zhang, X., Zhao, Z., Wang, S., Wang, J., Wang, H., 2025. Integrated Assessment of Productive, Environmental, and Social Performances of Adopting Low-Protein Diets Technology for Laying Hens. *Animals* 15, 146. doi: 10.3390/ani15020146.
- Lindberg, D., Kristoffersen, K.A., Wubshet, S.G., Hunnes, L.M.G., Dalsnes, M., Dankel, K.R., Høst, V., Afseth, N.K., 2021. Exploring Effects of Protease Choice and Protease Combinations in Enzymatic Protein Hydrolysis of Poultry By-Products. *Molecules* 26, 5280. doi: 10.3390/molecules26175280.
- Liu, H., Xu, K., Wang, H., Lin, H., Yang, X., Wang, X., Zhao, J., Ma, B., Shu, Q., Lu, Y., Jiao, H., 2024. Effects of different forms of amino acid supplementation on the performance and intestinal barrier function of laying hens fed a low-protein diet. *Poult. Sci.* 103, 104375. doi: 10.1016/j.psj.2024.104375.
- 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.
- Marmelstein, S., Costa, I.P.d.A., Terra, A.V., Silva, R.F.d., Capela, G.P.d.O., Moreira, M.A.L., Junior, C.d.S.R., Gomes, C.F.S., Santos, M.d., 2024. Advancing Efficiency Sustainability in Poultry Farms through Data Development Analysis in a Brazilian Production System. *Animals* 14, 726. doi: 10.3390/ani14050726.
- Matton, B., Verhelle, A., Vlerick, L., Keirsschieter, K., Saremi, 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 (Basel)* 15, 1753. doi: 10.3390/ani15121753.
- Maynard, C.W., Kidd, M.T., Chrystal, P.V., McQuade, L.R., McInerney, B.V., Selle, P.H., Liu, S.Y., 2022. Assessment of limiting dietary amino acids in broiler chickens offered reduced crude protein diets. *Anim. Nutr.* 10, 1–11. doi: 10.1016/j.aninu.2021.11.010.
- Meng, S., Miao, A., Wu, S., Du, X., Gao, F., 2025. Genetically modified chickens as bioreactors for protein-based drugs. *Front. Genome Ed.* 6, 1522837. doi: 10.3389/fgeed.2024.1522837.
- Musigwa, S., Cozannet, P., Asiamah, C.A., Wu, S.B., 2024. Effects of Dietary Protein Levels, Net Energy Levels, and Essential Amino Acid-to-Two Protein Ratios on Broiler Performance. *Animals (Basel)* 14, 3065. doi: 10.3390/ani14213065.

- Musigwa, S., Morgan, N., Swick, R., Cozannet, P., Wu, S.B., 2021. Optimisation of dietary energy utilisation for poultry – a literature review. *World's Poultry Sci. J.* 77, 5–27. doi: 10.1080/00439339.2020.1865117.
- 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.
- Naeem, M., Bourassa, D., 2025. Probiotics in Poultry: Unlocking Productivity Through Microbiome Modulation and Gut Health. *Microorganisms* 13, 257. doi: 10.3390/microorganisms13020257.
- Nahm, K.H., 2007. Feed formulations to reduce N excretion and ammonia emission from poultry manure. *Bioresour. Technol.* 98, 2282–2300. doi: 10.1016/j.biortech.2006.07.039.
- Nanto-Hara, F., Ema, T., Ohtsu, H., 2026. Amino-Acid-Balanced Low-Protein Diets Reduce Nitrogen Excretion Without Affecting Growth Performance in Broilers. *Animals (Basel)* 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.
- Navidshad, B., Sadeghi, N., Mokarrami, T., Hesari, A.K., Aghjehgheshlagh, F.M., 2024. Effects of Reduced Dietary Crude Protein and Amino Acids on the Performance, Carcass Characteristics and Intestinal Morphology of Broiler Chickens. *Poult. Sci. J.* 12, 259–269. doi: 10.22069/psj.2024.22294.2060.
- Ndazigaruye, G., Kim, D.H., Kang, C.W., Kang, K.R., Joo, Y.J., Lee, S.R., Lee, K.W., 2019. Effects of Low-Protein Diets and Exogenous Protease on Growth Performance, Carcass Traits, Intestinal Morphology, Cecal Volatile Fatty Acids and Serum Parameters in Broilers. *Animals (Basel)* 9, 226. doi: 10.3390/ani9050226.
- Oketch, E.O., Yu, M., Nawarathne, S.R., Chaturanga, N.C., Maniragua, V., Sta Cruz, B.G., Seo, E., Lee, J., Park, H., Lee, H., Heo, J.M., 2025. Multiprotease supplementation in laying hen diets: Impact on performance, egg quality, digestibility, gut histomorphology, and sustainability. *Poult. Sci.* 104, 104977. doi: 10.1016/j.psj.2025.104977.
- Onbaşlar, E.E., Yalçın, S., Batur, B., Yalçın, S., Kılıç, 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 (Basel)* 16, 465. doi: 10.3390/ani16030465.
- Oni, A.I., Oke, O.E., 2025. Gut health modulation through phytochemicals in poultry: mechanisms, benefits, and applications. *Front. Vet. Sci.* 12, 1616734. doi: 10.3389/fvets.2025.1616734.
- Osei-Akoto, A., Abdel-Wareth, A.A., Salahuddin, M., 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-Nji, F., 2011. Use of a Protease in Poultry Feed Offers Promising Environmental Benefits. *Int. J. Poult. 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-Abanza, J.N., Ali, M., Cabañas-Ojeda, J., Yang, Y., Alfaro-Wisquillo, M.C., Quintana-Ospina, G.A., Vasanthakumari, B.L., Wealleans, 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.
- Phan, H.V., Pham, H.H.S., Ngo, L.H., Tran, N.T., Ho, D.T., Nguyen, K.D.T., Tran, L.N., Nguyen, H.X., 2025. Isolation and selection of indigenous chicken-derived *Bacillus subtilis* strains as potential probiotic alternatives to antibiotics against Gram-negative enteropathogens. *J. Adv. Vet. Anim. Res.* 12, 53–63. doi: 10.5455/javar.2025.1871.
- Poudel, I., Hodge, V.R., Wamsley, K.G.S., Roberson, K., Adhikari, P.A., 2024. Effects of protease enzyme supplementation and varying levels of amino acid inclusion on productive performance, egg quality, amino acid digestibility, and economics of egg production in Hy-Line W-36 laying hen from 50 to 70 wk of age. *J. Appl. Poult. Res.* 33, 100452. doi: 10.1016/j.japr.2024.100452.
- Qaid, M.M., Al-Garadi, M.A., 2021. Protein and Amino Acid Metabolism in Poultry during and after Heat Stress: A Review. *Animals* 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.
- Rada, V., Lichoniková, M., Foltyn, M., Safarik, I., 2016. The Effect of Exogenous Protease in Broiler Diets on the Apparent Ileal Digestibility of Amino Acids and on Protease Activity in Jejunum. *Acta Univ. Agric. Silv. Mendel. Brno* 64, 1645–1652. doi: 10.11118/actaun201664051645.
- Ramankevich, A., Danko, S., Banaszekiewicz, R., Kasperek, K., Zięba, G., 2025. Residual Feed Intake as a Behavioral, Nutritional and Economic Criterion in Poultry Production. *Animals (Basel)* 15, 1115. doi: 10.3390/ani15081115.
- Sadr, V.S., Quinteros, J.A., Liu, S.Y., Barekatain, R., 2025. Nutrient transporters in broiler chickens: intestinal gene expression profiles, functional roles, and influencing factors. *J. Animal. Sci. Biotechnol.* 16, 165. doi: 10.1186/s40104-025-01302-w.
- Salahi, A., Shahir, M.H., Attia, Y.A., Fahmy, K.N.E., Bovera, F., Tufarelli, V., 2025. Impact of low-protein diets on broiler nutrition, production sustainability, gene expression, meat quality and greenhouse gas emissions. *J. Appl. Anim. Res.* 53, 2473419. doi: 10.1080/09712119.2025.2473419.
- Selle, P.H., Macelline, S.P., Chrystal, P.V., Liu, S.Y., 2023. A reappraisal of amino acids in broiler chicken nutrition. *World's Poultry Sci. J.* 79, 429–447. doi: 10.1080/00439339.2023.2234342.
- Shahid, M.A.H., Jha, R., Mishra, B., 2026. Changes in the gut microbiome, metabolic pathways, and intestinal gene expression during the peak, mid, and decline egg production phases in laying hens. *Poult. Sci.* 105, 106372. doi: 10.1016/j.psj.2026.106372.
- Shen, Z., Bao, H., Zhang, H., Yuan, D., Huang, W., Hou, S., Xie, M., Wang, M., 2026. Effects of Low-Protein Diets on Growth Performance, Intestinal Morphology, Apparent Ileal Amino Acid Digestibility and Jejunal Amino Acid Transporter Gene Expression in Heat-Stressed Growing Male Pekin Ducks. *Animals* 16, 1247. doi: 10.3390/ani16081247.
- Siebert, W., Omotoso, A., Hofmann, P., Rodehutschord, M., 2025. Relevance of nonessential amino acids in low crude protein diets for broiler chickens – an updated review. *Nutr. Res. Rev.* 38, 961–972. doi: 10.1017/S0954422425100176.
- 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, X., Anas, M.A., Kurniawati, A., Hanim, C., Muhlisin, Aprianto, M.A., Madani, A.M.A., Wang, Q., Chen, H., 2023. Effects of reduced-protein diets with protease supplementation on growth, carcass yield, intestinal morphology, organ development, nutrient digestibility, and blood biochemical of broiler chickens. *Transl. Anim. Sci.* 7, txad098. doi: 10.1093/tas/txad098.
- Such, N., Pál, L., Striffler, P., Horváth, B., Koltay, I.A., Rawash, M.A., Farkas, V., Mezőlák, Á., Wágner, L., Dublec, K., 2021. Effect of Feeding Low Protein Diets on the Production Traits and the Nitrogen Composition of Excreta of Broiler Chickens. *Agriculture* 11, 781. doi: 10.3390/agriculture11080781.
- 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.
- Swelum, A.A., El-Saadony, M.T., Abd El-Hack, M.E., Ghanima, M.M.A., Shukry, M., Alhotan, R.A., Hussein, E.O.S., Suliman, G.M., Ba-Awad, H., Ammari, A.A., Taha, A.E., El-Taraby, K.A., 2021. Ammonia emissions in poultry houses and microbial nitrification as a promising reduction strategy. *Sci. Total Environ.* 781, 146978. doi: 10.1016/j.scitotenv.2021.146978.
- Szczurek, W., Świątkiewicz, S., 2020. Standardised Ileal Amino Acid Digestibility in Field Pea Seeds of Two Cultivars Differing in Flower Colour for Broiler Chickens: Effects of Bird Age and Microbial Protease. *Animals* 10, 2099. doi: 10.3390/ani10112099.
- Tajudeen, H., Hosseindoust, A., Ha, S.H., Moturi, J., Mun, J.Y., Lee, C.B., Park, J.W., Lokhande, A., Ingale, S.L., Kim, J.S., 2022. Effects of dietary level of crude protein and supplementation of protease on performance and gut morphology of broiler chickens. *Eur. Poult. Sci.* 86, 1–12. doi: 10.1399/eps.2022.355.
- Taylor-Bowden, T., Bhogju, S., Khatwenge, 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.
- Tejeda, O.J., Kim, W.K., 2021. Role of Dietary Fiber in Poultry Nutrition. *Animals (Basel)* 11, 461. doi: 10.3390/ani11020461.
- Verma, S., Dixit, R., Pandey, K.C., 2016. Cysteine Proteases: Modes of Activation and Future Prospects as Pharmacological Targets. *Front. Pharmacol.* 7, 107. doi: 10.3389/fphar.2016.00107.
- Vlaicu, P.A., Untea, A.E., Oancea, A.G., 2024. Sustainable Poultry Feeding Strategies for Achieving Zero Hunger and Enhancing Food Quality. *Agriculture* 14, 1811. doi: 10.3390/agriculture14101811.
- Wahid, Z.R.A., Jameel, Y.J., Kadhim, L.I., 2025. Effects of supplemental exogenous protease enzyme on growth performance of broiler chickens in reduced protein-energy diet. *Kerbal J. Vet. Med. Sci.* 1, 104–108. doi: 10.65639/kjvm.25.045.
- Wang, B., Zhang, X., Liu, Y., Gao, M., Wang, M., Wang, Y., Yang, X., Guo, Y., 2024. Assessment of the dietary amino acid profiles and the relative biomarkers for amino acid balance in the low-protein diets for broiler chickens. *J. Anim. Sci. Biotechnol.* 15, 157. doi: 10.1186/s40104-024-01108-2.
- Wang, J., Wu, Y., Zhou, T., Feng, Y., Li, L.A., 2025. Common factors and nutrients affecting intestinal villus height-A review. *Anim. Biosci.* 38, 1557–1569. doi: 10.5713/ab.25.0002.
- 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., 2020. 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.
- Wealleans, A.L., Ashour, R.A., Ishma, M.A.A., Al-Amaireh, S., Gonzalez-Sanchez, D., 2024. Comparative effects of proteases on performance, carcass traits and gut structure of broilers fed diets reduced in protein and amino acids. *J. Anim. Sci. Technol.* 66, 457–470. doi: 10.5187/jast.2023.e20.
- 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.
- Woyengo, T.A., Knudsen, K.E.B., Børsting, C.F., 2023. Low-protein diets for broilers: Current knowledge and potential strategies to improve performance and health, and to reduce environmental impact. *Anim. Feed Sci. Technol.* 297, 115574. doi: 10.1016/j.anifeedsci.2023.115574.
- Yadav, S., Jha, R., 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *J. Anim. Sci. Biotechnol.* 10, 2. doi: 10.1186/s40104-018-0310-9.
- Yan, Z., Liu, Z., Zhou, C., Tan, Z., 2025. Anti-Nutritional Factors of Plant Protein Feeds for Ruminants and Methods for Their Elimination. *Animals (Basel)* 15, 1107. doi: 10.3390/ani15081107.
- Youssef, I., 2025. Use of Enzymes in Poultry Diets to Improve the Productive Performance and Reduce the Diet Cost. *J. Vet. Med. Res.* 32, 29–33. doi: 10.21608/jvmr.2025.381481.1129.
- Yu, T., Humbert, F., Li, D., Savarin, A., Zhang, M., Cui, Y., Wang, H., Dong, T., Wu, Y., 2025. Effects of Chicken Protein Hydrolysate as a Protein Source to Partially Replace Chicken Meal on Gut Health, Gut Microbial Structure, and Metabolite Composition in Cats. *Vet. Sci.* 12, 388. doi: 10.3390/vetsci12040388.
- 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-Meat-Based Diets. *Metabolites* 15, 445. doi: 10.3390/metabo15070445.
- Zakria, M., Leghari, I.H., Rajput, N., Soomro, S.A., Pirzadeh, 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/2025/13.572.582.
- Zamani, S.M., Diarra, M.S., Chen, W., Dutta, A., 2026. Valorization of poultry litter through biochar production: Linking feedstock chemistry, pyrolysis conditions, and application performance for the Canadian poultry industry. *J. Environ. Manag.* 405, 129668. doi: 10.1016/j.jenvman.2026.129668.
- Zavelinski, V.A.B., Vieira, V.I., Bassi, L.S., de Almeida, L.M., Schramm, V.G., Maiorka, A., de Oliveira, S.G., 2024. The effect of protease supplementation in broiler chicken diets containing maize from different batches on growth performance and nutrient digestibility. *Animal* 18, 101363. doi: 10.1016/j.animal.2024.101363.
- 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, H., Xuan, Y., Guo, D., Zeng, Q., Bai, S., Liu, Y., Ding, X., Zhang, K., Wang, J., 2025a. Effects of dietary low protein levels and amino acid patterns on production performance, egg quality and intestinal function in laying hens. *Poult. Sci.* 104, 105578. doi: 10.1016/j.psj.2025.105578.
- Zhang, L., Liu, Y., Xie, C., Huang, X., Deng, P., Li, C., Wan, W., Dai, Q., Hu, Y., Ma, Y., Zhang, X., Jiang, G., 2025b. Effects of enzymes originating from *Trichoderma reesei* on the performance, organ index, serum biochemical indices, and intestinal health homeostasis of broiler chicken. *Front. Vet. Sci.* 12, 1550463. doi: 10.3389/fvets.2025.1550463.
- Zheng, A., Zhang, A., Chen, Z., Pirzadeh, S.A., Chang, W., Cai, H., Bryden, W.L., Liu, G., 2021. Molecular mechanisms of growth depression in broiler chickens (*Gallus gallus domesticus*) mediated by immune stress: a hepatic proteome study. *J. Anim. Sci. Biotechnol.* 12, 90. doi: 10.1186/s40104-021-00591-1.
- 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.
- Zhou, H., Quach, A., Nair, M., Abasht, B., Kong, B., Bowker, B., 2025. Omics based technology application in poultry meat research. *Poult. Sci.* 104, 104643. doi: 10.1016/j.psj.2024.104643.