

Review Article

Method of Enzyme Application and Effect on the Performance of Broilers Fed Meal-Based Diet in Ethiopia: Systematic Review

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Abstract

This seminar evaluate the effects of enzyme supplementation on the nutrient digestibility and growth performance of broiler chickens. Enzyme supplementation in broiler diets has gained significant attention in the poultry industry for its potential to improve growth performance, feed efficiency, and overall bird health. Enzymes such as xylanase, phytase, protease, β -glucanase, and mannanase have shown varying degrees of efficacy in enhancing nutrient digestibility and utilization, particularly in cereal-based diets. This review examines the role of enzyme supplementation in broiler nutrition, focusing on its impact on growth performance, feed conversion, and the reduction of anti-nutritional factors in plant-based feed ingredients. Enzyme supplementation improves feed efficiency by reducing the viscosity of non-starch polysaccharides like β -glucans and pentosans found in grains such as wheat, barley, and rye. This reduction in viscosity enhances gut health, nutrient absorption, and overall digestibility. Specific enzyme combinations, such as xylanase and phytase, have shown significant improvements in nutrient digestibility, protein accretion, bone mineralization, and mineral retention, which directly contribute to enhanced growth performance. Future research should focus on the optimization of enzyme combinations, the use of multi-enzyme products, and exploring their long-term effects on broiler health, performance, and meat quality. Enzyme supplementation represents a promising strategy for improving feed efficiency, reducing environmental pollution, and enhancing poultry production, though further studies are required to understand the full range of potential benefits.

Keywords

Broiler Diet, Enzyme, Supplementation

1. Introduction

In poultry production, the nutritional quality of the diet plays a major role in the efficient utilization of nutrients by animals, ultimately influencing broiler performance and economic returns for producers. Moreover, the increasing importance of broiler production has been attributed to environmental standards to help prevent envi-

ronmental pollution and ensure improved rearing conditions for birds. Feed costs account for about 60-75% of broiler production costs savings require knowledge of the digestive tract characteristics of poultry, nutrient requirements, digestion, and nutrient utilization at certain growth and fattening stages [1].

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They improve the energy value of feedstuff, improve the digestion, assimilation, and usage of carbohydrate, protein, fat, and phosphorus from undigested crop residues, and reduce the excretion level of P and N in non-digestible nutrients in the surrounding, resulting in decreased environmental degradation [2]. The broiler industry has experienced remarkable improvements in productivity owing to the high priority placed on genetic programming, nutrition, and management practices [3]. In order to achieve this improvement, broilers should be fed diets that can meet all their nutrient requirements. Enzyme supplementation in broiler chicken enhances feed efficiency, growth performance, and nutrient utilization, including protease, amylase, lipases, xylanase, and phytase, which have been employed in broiler diets [4]. According to Goli and AghdamShahryar [5], adding enzyme o growth performance of quail and found that supplementary multi-enzyme improved BW gain significantly and that the use of enzyme in broiler diets containing 6% crude fiber significantly increased BW compared to without enzymes. Enzyme-supplemented wheat and barley diets with multi-enzymes give rise to significant ($P<0.05$) increase in BW gain compared to those without enzymes [6].

Therefore, Supplementation with multi-enzyme tended to improve the nutritive value of the corn-soybean diet in broiler chicks (Shirmohammad and Mehri, [7]; the good incorporation between the enzyme and Japanese quail diet plays an important role in improving growth [8]. Sherif [9] declared that the diet supplemented with Avizyme, Sicozyme, Natuzyme or phytase significantly positively affected the final BW of chicks compared to a supplemented diet.

The use of these enzymes has been associated with improved nutrient digestibility and enhanced broiler performance [10, 11]. Additionally, regimes supplemented with xylanase and other enzymes had significantly higher total weight gain of chicks than an unsupplemented diet [12]. In addition, [13] showed that supplementation of the enzyme mixture (xylanase and protease) to broiler diet during the first three weeks of age improved the performance. Moreover, the use of these enzymes has been associated with improved nutrient digestibility and enhanced broiler performance [11].

Even though the proven benefits of enzyme supplementation, limited information exists on the comparative efficacy of individual enzymes versus their combined application in broiler diets. Thoughtful, additive effects of enzyme combinations are essential for maximizing broiler productivity. Therefore, this seminar aims to provide a comprehensive evaluation of the effects of enzyme supplementation on the performance of broilers fed meal-based diets.

The specific objectives of this seminar are as follows:

To review the effect of enzyme supplementation on the

growth performance of broiler meal-based diet.

To review Common Enzyme Supplementation for Broiler Chickens.

2. Literature Review

2.1. Broiler Chicken Production in Ethiopia

A broiler is any chicken (*Gallus gallus domesticus*) that is breed and raised specifically for meat production. Most commercial broilers reach slaughter weight between four and seven weeks of age, although slower growing breeds reach slaughter weight at approximately 14 weeks of age. Chicken production system is an appropriate and locally available resource in livestock population. In Africa, Ethiopia is the highest in chicken population [14].

In sub-Saharan Africa, 85% of all households keep chickens under free range/ extensive system, with women owning 70% of it, providing insufficient animal protein in the form of meat and eggs as well as being a reliable source of cash income [15]. Only few research results are available on the meat production abilities of local chickens [14].

Poultry meat and egg production accounted for more than 28% of the total animal protein produced in the world in 1997 [16]. In 2020, the proportional contribution of poultry meat is believed to be increased to 40%, the major increment is being happened in the developing world [17]. In Ethiopia from the total chicken meat production local chickens with an average annual output of 72,300 metric tons [18], contribute about 99.2 % of meat production.

Day-old chickens of different populations of indigenous chickens measures live weight of 27.3g per chicken [16]. Dana [19] in adult live body weight of the different populations of indigenous local chickens also reported 1.6 kg for male and 1.3 kg for females.

According to the result of Kibret [16], who indicated that the meat production ability of indigenous chicken was limited in growth performance. Local males may reach 1.5 kg live weight at 6 months of age, and females about 30% less [16].

2.2. Enzymes in Animal Feed

Enzymes are biological catalysts composed of amino acids with vitamins and minerals. Some of the enzymes that have been used over the past several years and have the potential for use in the feed industry include cellulase β -glucanases, xylanases, and associated enzymes, phytases, proteases, lipases, and galactosidases. Most of the enzymes used in the feed industry have been applied for poultry to neutralize the effects of the viscous, non-starch polysaccharides in cereals such as barley, wheat, rye, and triticale. The benefits of using enzymes in poultry diets enhanced bird performance and feed conversion, reduced feed cost but also less environmental pollution due to re-

duced output of excreta [20].

Enzymes are produced in every living organism, from the highest developed animals and plants to the simplest unicellular forms of life, as they are essential for the metabolic process. Microorganisms are like Bacteria (*Bacillus subtilis*, *B. Lentus*, *B. Amyloliqifaciens*, *B. licheniformis* and *B. Stearothermophils*), Fungus (*Trichoderma longibrachiatum*, *Aspergillus oryzae* and *Aspergillus Niger*) and Yeast (*S. Cerevisiae*) involved in the production of enzymes. All microorganisms have a common function, which is the production of enzymes for the breakdown of plant cell wall components in the form of high polymer carbohydrates. However, fungi also produce enzymes for the degradation of various substances [20].

2.3. Methods of Enzyme Supplementation and Their Effects on Feed Efficiency

Enzymes are produced in various forms for supplementation in poultry diets. They can be used as powder, granules, and liquids. Some enzymes are thermos labile and processing at high temperatures may reduce their activity [21]. May used several methods to mix enzymes with dietary ingredients in poultry diets [22].

Powdered Enzymes in Premix; Enzyme powders are first blended into the premix and then mixed with other dietary ingredients. Direct Mixing of Powders and Granules; Enzyme powders or granules are directly combined with the primary feed ingredients. Liquid Enzymes Post-Pelleting; Liquid enzymes are sprayed onto the feed after pelleting to avoid heat-related inactivation. Liquid Enzymes in Drinking Water or Feed; Enzymes in liquid form are mixed into the drinking water or blended directly with other feed ingredients.

Enzyme supplementation in poultry diets has been shown to positively impact certain growth parameters, like weight gain and feed intake, although it may not always affect egg production or egg quality measures such as initial egg weight, average egg weight, egg specific gravity, Haugh unit score, and mortality [23]. Enzymes like β -glucanase and xylanase play a crucial role in breaking down β -glucans and pentosans (non-starch polysaccharides) found in plant-based feed ingredients. By hydrolyzing these components, enzymes reduce the viscosity of the intestinal contents. This reduction in viscosity helps improve the digestibility of the feed, as viscous material in the gut can slow down nutrient absorption and hinder overall feed efficiency [24]. The viscosity-lowering effect of enzymes has been well-documented not only in broilers but also in laying hens [25]. When viscosity is reduced, it allows for better flow of digestive contents, which can facilitate nutrient absorption and improve feed utilization, leading to potential improvements in growth performance and feed intake.

Furthermore, enzyme supplementation enhance the digestibility of various nutrients, but the effects may vary depend-

ing on the nutrient type. Zhou *et al.* [26], reported an overall improvement of 2.9% in crude protein (CP) digestibility with enzyme addition, although the improvement was not uniform across all amino acids. Specific amino acids like lysine, methionine, and arginine showed no significant improvement in digestibility, whereas others like valine and tryptophan saw improvements of 2.3% and 3.0%, respectively Zanella *et al.* [27]. This variability suggests that enzyme efficacy may depend on the specific substrate and its interaction with enzymes.

Enzyme supplementation also showed significant improvements in the digestibility of starch, fat, and protein in hens fed on wheat and barley-based diets Oloffs *et al.*, [28]. However, the effect of enzyme addition can be influenced by the fat source used in the diet. Langhout *et al.* [29] found that the type of fat included in the diet considerably affected the performance and digestibility improvements from enzyme supplementation in wheat and rye-based diets. Moreover, enzymes also enhanced the metabolizable energy (ME) of corn-soybean-based diets, increasing it by 65 kcal/kg for broilers and turkeys [29].

As Kaczmarek *et al.* [30] reported that hominy diets had lower AME values, which can be improved with the addition of enzymes. For instance, Y. Liu and Geraert [31], enzyme combinations could increase the AME by 65 kcal/kg for broiler chickens and layers fed corn and soybean meal diets, 85 kcal/kg for wheat-based diets, 140 kcal/kg for barley-based diets and 65 kcal/kg for pig diets. These improvements in AME typically arise from better nutrient digestibility, although crude fat digestibility may not be as significantly affected by enzyme addition. Enzymes can help break down components like non-starch polysaccharides (NSPs) and phytate, making more nutrients available for absorption, thus improving overall energy utilization in animal feeds.

Enzyme supplementation in broiler chicken diets has shown various beneficial effects on both growth performance and nutrient digestibility. Sarvestani *et al.* [32] found that supplementing broiler diets with Biozyme enzyme (at a level of 0.75 kg/ton) in pellet form led to an increase in the weight of several tissues, including breast, thigh, heart, liver, and abdominal fat. This suggests that enzyme supplementation could support the growth of key body parts, particularly those involved in meat yield and fat deposition.

Additionally, breast meat yield was found to increase with the addition of Versazyme in broiler chicken diets [33]. This improvement in meat yield could be attributed to the enhanced digestibility and availability of nutrients, which may have supported muscle development and overall growth. When digestibility improvements, enzyme supplementation has shown positive effects on various feed types. Oloffs *et al.* [28], Bedford [34], Ziggers [35], and Serena *et al.* [36] all reported that enzyme addition to broiler diets based on barley, wheat, rye, and corn resulted in improved starch and nitrogen digestibility.

Enzyme supplementation, particularly combinations of xy-

lanase, phytase, protease, and organic acids, has significantly improved various aspects of broiler chicken performance, including nutrient digestibility, mineral retention, protein accretion, bone mineralization, growth performance, and feed conversion efficiency. Anwar *et al.* [37] reported that the combination of xylanase and phytase improved nutrient digestibility and retention in broilers fed on wheat-based diets. The beneficial effects of this enzyme combination were also observed in other studies (Olukosi and Adeola [38], where it resulted in improved growth performance and whole-body accretion of minerals and protein. The improvement in protein accretion specifically indicates better nutrient utilization, a direct result of phytase's ability to release more phosphorus from plant-based feed ingredients, making it more available for absorption. Bromfield *et al.* [39] found that a combination of carbohydrates and protease improved bone mineralization in broiler chicks on a wheat-based diet. This suggests that enzyme combinations can also contribute to skeletal health, which is important for overall growth and development in broilers.

A study by Gružauskas *et al.* [40] showed that combining enzymes with organic acids improved body weight and feed conversion in broiler chickens. The presence of organic acids can support gut health by lowering pH, thereby enhancing the activity of enzymes and improving overall feed digestibility. Józefiak *et al.* [41] also observed positive effects of organic acids on performance in poultry. It established that the supplementation of XTRACT (a product containing enzymes) in diets where corn was included resulted in increased protein retention and decreased fat retention in chicken carcasses. This suggests that enzyme supplementation can help broilers gain more lean muscle mass while reducing fat accumulation, which is important for meat quality.

As Zakaria *et al.* [42] observed, enzyme supplementation in broiler diets enhanced meat production, including higher breast and thigh weights. This is consistent with the general trend that enzyme supplementation not only improves feed efficiency and growth performance but also enhances the overall body weight and feed conversion of broilers. Additionally, enzyme supplementation improved the absorption of starch, fat, and amino acids, making nutrients more bioavailable for the birds, which can enhance growth, feed efficiency, and overall performance. These studies collectively suggest that enzyme supplementation in broiler diets can improve nutrient utilization, potentially leading to better growth, enhanced meat yield, and overall feed efficiency.

3. Common Enzyme Supplementation for Broiler

3.1. Protease Enzyme Supplementation

Proteases are a broad and complex category of hydrolytic enzymes that are categorized according to their site of action,

the structure of the enzyme active site, and particular reaction processes. Proteases are widely distributed in broiler chickens, where they play a biochemical and physiological role in a variety of cellular and organismal functions, such as growth, adaptability, regulation, and the breakdown of proteins (Tassidis, n.d. [43]. The beneficial effects of protease enzymes can be seen on different levels. Protease supplementation in broiler diets significantly enhanced the body weight gain of broilers over days 1-18 and 19-35 as well as over the broiler chicken's overall growth phase from day 1 to 35 as the dietary protease levels rose from 0% to 0.09%. Furthermore, groups supplemented with protease had a lower and better feed conversion ratio than the control group, which was also marked by a greatly better digestibility of crude protein, dry matter, and energy.

In addition, with increasing dietary protease addition in broiler chicken diets, a number of amino acids, including histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, and cysteine, which as discussed earlier cannot be synthesized by broiler chicken, showed a considerable improvement in digested rate [44]. Secondly, protease supplementation has been demonstrated to have a positive effect on the intestinal morphology of broiler chickens. In fact, supplementation with protease is capable of increasing villus height and the villus height to crypt depth ratio in the duodenum, as well as the villus height of the jejunum, and the villus height to crypt depth ratio of the ileum [45].

Exogenous protease can play an important role in boosting the immunity of broiler chickens directly and indirectly. On a direct level, protease has been demonstrated to increase the serum IgM levels in broilers Amer *et al.* [46], while on the indirect level, the inclusion of protease in the meals of broiler chickens can significantly reduce litter moisture and reduce the risks of foot pad dermatitis due to *E.coli*. This is due to the enzyme's ability to increase protein breakdown.

An increase in the level of protease will result in an increase in the level of protein digested, which will result in less undigested protein reaching the caecum. As a consequence, microbial fermentation and ammonia production will be lower [47]. In addition to that, protease supplementation can lead to certain changes in the gut flora and has the capacity to increase the number of beneficial bacteria inhabiting the microflora such as *Bacteroidetes* [48].

3.2. Phytase Supplementation

Phytases, known as Myo-inositol hexaphosphate hydrolases, are phosphatases, which are enzymes that may catalyze the hydrolysis of phosphate ester linkages. Phytases are able to hydrolyze one or more phosphate groups from phytic acid (Lourenco *et al.*, [48]. Firstly, phytase supplementation several beneficial effects on the nutrient digestion and availability level of broilers while increasing body weight gain and decreasing feed conversion ratio.

In fact, phytase is capable of greatly increasing the ileal

digestibility of phosphorus regardless of the dose supplemented, which leads to an increased level of phosphorus in the broiler's serum Karami *et al.*, [49]. Exogenous phytase supplementation also had a great impact on calcium and phosphorus absorption even on broilers fed insufficient levels of both calcium and phosphorus. Indeed, even when the amount of calcium and phosphorus in the feed is insufficient, phytase has been proven to elevate the calcium and phosphorus availability [50].

Secondly, the positive effects of phytase supplementation are not only limited to digestion but to bone mineralization and litter moisture as well. The addition of phytase to the diet of broiler chickens is capable of increasing the length, ash content, and bone marrow density of the tibia while in parallel increasing the bone marrow density and concentration of the femur. Simultaneously, phytase is capable of reducing excessive litter moisture content which can help reduce the risks of foot pad dermatitis in broilers [50].

Thirdly and most importantly, exogenous phytase supplementation can have a positive impact on intestinal morphology and microbiota. On the morphological aspect, phytase supplementation can increase the villus height and width of the jejunum, as well as improve the crypt depth of the ileum and the villus height to crypt depth ratio of the ileum. The positive modifications made by phytase supplementation on the intestines also reached the microflora that can become more resilient to pathogenic bacteria due to the direct and indirect effects of phytases.

In fact, exogenous phytase can create a lower pH in the small intestines, which has a bacteriostatic effect on pathogenic bacteria. For instance, phytase has been demonstrated to directly reduce the number of Enterobacteriaceae and Helicobacteriaceae, while increasing the number of beneficial bacteria inhabiting the intestines such as the *Lactobacillus reuteri*. This specific bacterium has been linked with the production of antimicrobial substances such as reuterin, ethanol, and organic acids. In addition to that, the supplementation of phytase in a broiler's diet can improve the intestinal barrier and reduce the number of bacteria that are transferred from the lumen of the gut to the surrounding tissues [51].

In general, we can conclude that the supplementation of phytase to broiler feeds has many benefits. It improves the digestion and absorption of nutrients, especially phosphorus and calcium, which results in better growth.

Additionally, phytase lowers the moisture content of the litter and lowers the chance of footpad dermatitis while improving bone mineralization. In addition, it has a positive effect on intestinal shape and microbiota, maintaining an optimal intestinal environment and lowering the predominance of pathogenic bacteria.

3.3. Mannanase Supplementation

Mannanases also called β -mannanases are enzymes required for the breakdown of mannan, an undigestible type of

hemicellulose present in feedstuff and that represents anti-nutritive compounds that could have a deteriorating effect on different levels of the broiler's health if not digested, such as a decrease in the voluntary feed intake, nutrient absorption, metabolism, and immune system [52].

On the first hand, the positive effects of mannanase supplementation can be seen in the average daily gain and the feed conversion ratio of broilers of all age categories, by increasing the level of average metabolizable energy while increasing the level of different nutrients such as methionine, valine, and leucine [52].

Mannanase can play a very crucial role in enhancing the immunity of broiler chickens by different mechanisms. Due to their inflammatory and viscous properties, galactomannans are thought to damage the intestinal epithelial cell lining. By depolymerizing them, mannanases are able to reduce this inflammatory process [53]. In addition, mannanase supplementation has been demonstrated to increase blood serum levels of immunoglobulins A, G, and M [54].

Not only do the positive effects of mannanase supplementation have a direct impact on immunity, but indirectly as well. Indeed, adding mannanase to broiler chicken diets leads to better intestinal integrity which results in lower risks of footpad lesions [53]. In general, we can conclude that mannanase has a beneficial effect on broiler chickens by increasing nutrient availability, enhancing immunity, lowering inflammation, and improving intestinal health. It also enhances growth performance and feed efficiency.

3.4. Lysozyme Supplementation

As previously mentioned, lysozymes, also called 1,4- β -N-acetylmuramidase, are enzymes secreted in the broiler's intestines as part of the innate immunity, that cause the loss of cellular permeability and cellular death by cleaving the glycosidic connection between the N-acetylglucosamine and N-acetylmuramic acid in the bacterial peptidoglycan of the cellular wall.

Lysozyme supplementation can have many beneficial effects on broiler chicken including boosting the immune system of chickens, improving the intestinal microbiota, and promoting growth. For these reasons, lysozymes are being used as natural antibiotic substitutes [55].

Even on the genetic level, lysozyme supplementation results in a notable increase in the presence of genes related to the degradation of the external membrane and cell wall of bacteria [55]. Significant changes in gene expression can be seen in response to lysozyme supplementation and are confirmed by an increase in the expression of certain mRNAs. A wide range of genes are affected by these modifications, most notably genes producing enzymes such as copper and zinc-superoxide dismutase, and glutathione peroxidase, which are crucial parts of the antioxidant defense system.

Additionally, the increased levels of interferon-gamma, interleukin-10, and interleukin-18 after lysozyme supplementa-

tion prove that lysozyme positively affects immune response-related gene expression. The higher expression of these immune-related genes suggests an improvement in immune system performance, possibly making the chicken more resistant to pathogens and inflammatory stimuli [56].

Lysozymes are also known for directly decreasing the severity of necrotic enteritis lesions. Indeed, many experiments have proven that lysozyme is capable of significantly reducing the ileal *Clostridium* counts and several butyrate-producing clostridia. Lysozymes are also proven to have an antibacterial effect by reducing several types of clostridia such as *Clostridium perfringens*, *Clostridium botulinum*, and even *Listeria monocytogenes* [57].

In conclusion, supplementing lysozyme has a wide range of advantages for broiler chicks. Notably, it strengthens the immune system and favorably affects the production of genes linked to bacterial destruction, antioxidative defense, and immunity. This improves the chicken's overall health by making it more capable of fighting pathogens and reacting to inflammatory triggers. Additionally, lysozymes exhibit a direct antibacterial impact, significantly lowering the incidence of dangerous bacteria including *Listeria Monocytogenes* and *Clostridium* species. Lysozyme supplementation greatly impacts broiler chicken performance overall [57].

3.5. Multi-enzyme Supplementation on the Immunity

While we demonstrated that the supplementation of specific enzymes could play a key role in the development of the skeletal, metabolic, and immune systems of broiler chickens, the supplementation of a multi-enzyme mixture can also have positive impacts on the same levels in broiler chickens, and especially on the immune system.

Enzyme supplementation can be beneficial in reducing viral infections such as the Newcastle disease virus. The Newcastle disease virus is a highly pathogenic viral disease of poultry that belongs to the family of Paramyxoviridae and results in huge morbidity and mortality levels, which causes significant economic losses every year among farms in third-world countries [58].

Broiler chicken's hemagglutination inhibition antibody titer against the Newcastle disease virus can be considerably improved by supplementing enzymes. In parallel, supplementing enzymes can also slightly improve the hemagglutination inhibition antibody titer of broiler chicken receiving avian influenza vaccines [59]. Not only multi-enzyme supplementation is effective against bacteria and viruses, but also against parasites. In fact, enzymes can play an important role in reducing necrotic lesions induced by coccidiosis, a devastating disease caused by the *Eimeria* parasites that are responsible for over 3 billion dollars of losses in morbidity and mortality every year [60].

It has been demonstrated that supplementing an enzyme mixture containing amylase, protease, and xylanase to broiler

chicks reduced lesion scores of *Eimeria acervulina* and *Eimeria maxima* in the intestines [61].

Alfalfa, despite its high fiber content, is a natural source of xanthophylls, which can enhance the orange-yellow color of broiler meat. (Ponte *et al.* [62]) demonstrated that cellulase and xylanase supplementation improved the nutritive value of alfalfa-based diets and enhanced skin pigmentation in broilers. This could appeal to consumers who prefer organic chickens with naturally enhanced skin color. Lastly, Zamani *et al.*, [63], reported that locally developed enzyme supplementation significantly boosted BW and BW gain in broilers. Conducting sensory tests to compare the use of synthetic versus natural coloring agents in broiler meat could further clarify the role of enzymes in improving consumer acceptance.

4. Negative Effects of Enzyme Supplementation in Broiler Chickens

While the supplementation of enzymes numerous positive impacts and advantages on broiler chickens, several studies have been conducted to try to prove the opposite. In fact, many researchers still believe that the supplementation of enzymes to broiler chickens does not have any beneficial effects; while some others think that enzyme supplementation to broiler chickens may have a negative impact on broilers. Although the negative impacts of enzyme supplementation are negligible compared to the positive impacts, they are still significant in order to understand how to correctly supplement enzymes to broilers. Firstly, different studies suggested that the supplementation of proteases has no impact on the nutrient digestibility, feed conversion ratio, body weight gain, and average feed intake in the first 14 days of life of broilers [64].

In addition, there is evidence that supplementing external protease to broiler chicken's diet may cause the pancreas to secrete its natural proteolytic enzymes less frequently, which can cause the digestibility of proteins to decrease as a result of this action, while in parallel, there was no evidence of a protease effect or an interaction between feed and protease on the amount of ammonia in the excreta [48]. Secondly, some enzymes available in commercial blends have been demonstrated to have significantly negative impacts on all levels of broiler chicken's health. For instance, alpha-galactosidase enzyme supplementation has significantly decreased body weight, weight gain, and relative growth of broiler chickens [46].

Second, most importantly, the limitation of the use of enzyme supplementations is due to the different physiological nature and environment of the broiler chicken's intestinal tract. The first physiological limitation to the beneficial effects of enzymes is the pH. The majority of exogenous enzymes require a pH of 4 to 6 in order to have full efficacy Zakaria *et al.* [42], while the pH of the proventriculus and

gizzard fluctuate between 2.5 and 3.5, the pH of the jejunum between 6.5 to 7 and the pH of the ileum between 7.0 and 7.5 [65]. This pH variety can cause the loss of functionality of enzymes partially or totally, depending on the specific location within the intestinal tract. The second natural physiologic limitation for the efficacy of enzymes is time. The average retention time of feed within the intestinal tract from the esophagus to the end of the ileum is on average 3 to 4 hours [42]. Meanwhile, the digesta does not take more than 60 to 90 minutes from the crop to the gizzard, which will provide only a brief timeframe for enzyme activity [65]. This process emphasizes the importance of adapting better feeding strategies for the broilers in order to increase the passage time in a way to get a full mode of action of enzymes without having an extremely long passage time that can encourage bacterial growth.

5. Conclusion and Future Perspectives

Enzyme supplementation in broiler diets has gained significant attention in the poultry industry for its potential to improve growth performance, feed efficiency, and overall bird health. Enzymes such as xylanase, phytase, protease, β -glucanase, and mannanase have shown varying degrees of efficacy in enhancing nutrient digestibility and utilization, particularly in cereal-based diets. This review examines the role of enzyme supplementation in broiler nutrition, focusing on its impact on growth performance, feed conversion, and the reduction of anti-nutritional factors in plant-based feed ingredients. Enzyme supplementation improves feed efficiency by reducing the viscosity of non-starch polysaccharides like β -glucans and pentosans found in grains such as wheat, barley, and rye. This reduction in viscosity enhances gut health, nutrient absorption, and overall digestibility. Specific enzyme combinations, such as xylanase and phytase, have shown significant improvements in nutrient digestibility, protein accretion, bone mineralization, and mineral retention, which directly contribute to enhanced growth performance. Furthermore, the addition of organic acids alongside enzymes has been found to further improve gut health, support enzyme activity, and optimize nutrient absorption.

Protease and phytase supplementation, in particular, have shown to elevate phosphorus and calcium absorption, improve bone mineralization, and reduce litter moisture, decreasing the risk of footpad dermatitis. Additionally, protease supplementation can improve intestinal morphology, enhance immunity, and reduce microbial fermentation, contributing to better overall health. The benefits of enzyme supplementation extend to the improvement of meat yield, particularly in the breast and thigh regions, suggesting enhanced muscle development. Despite the significant benefits, the effects of enzyme supplementation are influenced by various factors including the source of fat, enzyme formulation, and diet composition. Future research should focus on the optimization of enzyme combinations, the use of multi-enzyme prod-

ucts, and exploring their long-term effects on broiler health, performance, and meat quality. Enzyme supplementation represents a promising strategy for improving feed efficiency, reducing environmental pollution, and enhancing poultry production, though further studies are required to understand the full range of potential benefits.

Abbreviations

AME	Apparent Metabolizable Energy
BW	Body Weight
IgM	Immunoglobulin M
ME	Metabolizable Energy
NDV	Newcastle Disease Virus
NSPs	Non-Starch Polysaccharides
pH	Potential of Hydrogen

Author Contributions

Teshome Werku is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

References

- [1] N. P. KULEILE, "EFFECTS OF DIET DILUTION, FEED TEXTURE AND FARM FORMULATED DIETS ON BROILER PERFORMANCE, METABOLIC DISORDERS, GASTRO-INTESTINAL TRACT DEVELOPMENT AND COST BENEFITS," 2024.
- [2] Y. Dersjant-Li, R. Davin, T. Christensen, and C. Kwakernaak, "Effect of two phytases at two doses on performance and phytate degradation in broilers during 1-21 days of age," *PLoS One*, vol. 16, no. 3, p. e0247420, 2021.
- [3] M. R. Barekatain and R. A. Swick, "Composition of more specialised pre-starter and starter diets for young broiler chickens: a review," *Anim. Prod. Sci.*, vol. 56, no. 8, pp. 1239-1247, 2016.
- [4] M. A. Arshad *et al.*, "Supplementation of bile acids and lipase in broiler diets for better nutrient utilization and performance: Potential effects and future implications-A review," *Ann. Anim. Sci.*, vol. 21, no. 3, pp. 757-787, 2021.
- [5] S. Goli and H. AghdamShahryar, "Effect of enzymes supplementation (rovabio and kemin) me on some blood biochemical parameters, performance and carcass characterizes in broiler chickens," 2015.
- [6] Q. Zeng *et al.*, "Effects of a multi-enzyme complex on growth performance, nutrient utilization and bone mineralization of meat duck," *J. Anim. Sci. Biotechnol.*, vol. 6, pp. 1-8, 2015.

- [7] F. Shirmohammad and M. Mehri, "Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks," *African J. Biotechnol.*, vol. 10, no. 38, pp. 7541-7547, 2011.
- [8] M. J. Chimote, B. S. Barmase, A. S. Raut, A. P. Dhok, and S. V Kuralkar, "Effect of supplementation of probiotic and enzymes on performance of Japanese quails," *Vet. World*, vol. 2, no. 6, p. 219, 2009.
- [9] K. El Sherif, "Performance of broiler chicks fed plant protein diets supplemented with commercial enzymes," *J. Anim. Poult. Prod.*, vol. 34, no. 4, pp. 2819-2834, 2009.
- [10] A. Aderibigbe, A. J. Cowieson, J. O. Sorbara, and O. Adeola, "Growth phase and dietary α -amylase supplementation effects on nutrient digestibility and feedback enzyme secretion in broiler chickens," *Poult. Sci.*, vol. 99, no. 12, pp. 6867-6876, 2020.
- [11] Y. A. Attia *et al.*, "The impact of multi-enzyme fortification on growth performance, intestinal morphology, nutrient digestibility, and meat quality of broiler chickens fed a standard or low-density diet," *Front. Vet. Sci.*, vol. 9, p. 1012462, 2022.
- [12] D. S. Gade, M. V Dhumal, M. G. Nikam, and D. Bhosale, "Influence of different levels of xylanase enzyme on performance, litter quality and economics of broiler chicken," *Int. J. Agri. Sci. Res.*, vol. 7, pp. 73-82, 2017.
- [13] A. Kocher, J. M. Hower, and C. A. Moran, "A dual-enzyme product containing protease in broiler diet: Efficacy and tolerance," *J. Appl. Anim. Nutr.*, vol. 3, p. e6, 2015.
- [14] D. Tadelle, C. Kijora, and K. J. Peters, "Indigenous chicken ecotypes in Ethiopia: growth and feed utilization potentials," *Int. J. Poult. Sci.*, vol. 2, no. 2, pp. 144-152, 2003.
- [15] E. B. Sonaiya, "Family poultry and food security: research requirements in science, technology and socioeconomics," 2020.
- [16] B. Kibret, "In situ characterization of local chicken eco-type for functional traits and production system in Fogera woreda, Amhara Regional State," 2008, *Haramaya University*.
- [17] C. L. Delgado, M. W. Rosegrant, H. Steinfeld, S. K. Ehui, and C. Courbois, *Livestock to 2020: The next food revolution*, vol. 61. Intl Food Policy Res Inst, 1999.
- [18] H. Teklewold, L. Dadi, A. Yami, and N. Dana, "Adopting poultry breeds in the highlands of Ethiopia," *Ethiop. Inst. Agric. Res.*, vol. 26, 2006.
- [19] N. Dana, *Breeding programs for indigenous chicken in Ethiopia: Analysis of diversity in production systems and chicken populations*. Wageningen University and Research, 2011.
- [20] F. M. Khattak, T. N. Pasha, Z. Hayat, and A. Mahmud, "Enzymes in poultry nutrition," *J. Anim. Pl. Sci.*, vol. 16, no. 1-2, pp. 1-7, 2006.
- [21] M. R. Bedford, F. G. Silversides, and W. D. Cowan, "Process stability and methods of detection of feed enzymes in complete diets," 2001.
- [22] B. A. Slominski, "Recent advances in research on enzymes for poultry diets," *Poult. Sci.*, vol. 90, no. 9, pp. 2013-2023, 2011.
- [23] A. Brenes, W. Guenter, R. R. Marquardt, and B. A. Rotter, "Effect of β -glucanase/pentosanase enzyme supplementation on the performance of chickens and laying hens fed wheat, barley, naked oats and rye diets," *Can. J. Anim. Sci.*, vol. 73, no. 4, pp. 941-951, 1993.
- [24] M. Choct, R. J. Hughes, R. P. Trimble, K. Angkanaporn, and G. Annison, "Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy," *J. Nutr.*, vol. 125, no. 3, pp. 485-492, 1995.
- [25] M. Almirall, M. Francesch, A. M. Perez-Vendrell, J. Brufau, and E. Esteve-Garcia, "The differences in intestinal viscosity produced by barley and β -glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks," *J. Nutr.*, vol. 125, no. 4, pp. 947-955, 1995.
- [26] Y. Zhou, Z. Jiang, D. Lv, and T. Wang, "Improved energy-utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolizable energy levels," *Poult. Sci.*, vol. 88, no. 2, pp. 316-322, 2009.
- [27] I. Zanella, N. K. Sakomura, F. G. Silversides, A. Figueirido, and M. Pack, "Effect of enzyme supplementation of broiler diets based on corn and soybeans," *Poult. Sci.*, vol. 78, no. 4, pp. 561-568, 1999.
- [28] K. Oloffs, H. Jeroch, and F. J. Schöner, "The efficiency of non-starch-polysaccharide hydrolysing enzymes on nutrient digestibility and gross energy convertibility of barley/rye and wheat/rye diets for laying hens," 1999.
- [29] D. J. Langhout, J. B. Schutte, C. Geerse, A. K. Kies, J. De Jong, and M. W. A. Verstegen, "Effects on chick performance and nutrient digestibility of an endo - xylanase added to a wheat - and rye - based diet in relation to fat source," *Br. Poult. Sci.*, vol. 38, no. 5, pp. 557-563, 1997.
- [30] S. Kaczmarek, M. Bochenek, D. Józefiak, and A. Rutkowski, "Effect of enzyme supplementation of diets based on maize or hominy feed on performance and nutrient digestibility in broilers," *J. Anim. Feed Sci.*, vol. 18, no. 2, pp. 113-123, 2009.
- [31] Y. Liu and P. Geraert, "Versatility takes enzymes beyond simple blending," *Feed Tech*, vol. 7, no. 8, pp. 25-27, 2003.
- [32] T. S. Sarvestani, N. Dabiri, M. J. Agah, and H. Norollahi, "Effect of pellet and mash diets associated with biozyme enzyme on broilers performance," *Int. J. Poult. Sci.*, vol. 5, no. 5, pp. 485-490, 2006.
- [33] J. J. Wang, J. D. Garlich, and J. C. H. Shih, "Beneficial effects of versazyme, a keratinase feed additive, on body weight, feed conversion, and breast yield of broiler chickens," *J. Appl. Poult. Res.*, vol. 15, no. 4, pp. 544-550, 2006.

- [34] M. R. Bedford, "Exogenous enzymes in monogastric nutrition—their current value and future benefits," *Anim. Feed Sci. Technol.*, vol. 86, no. 1-2, pp. 1-13, 2000.
- [35] D. Ziggers, "NSP-enzymes in corn-soybean rations," *Feed Tech*, vol. 10, pp. 30-31, 2006.
- [36] A. Serena, H. Jørgensen, and K. E. Bach Knudsen, "Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber," *J. Anim. Sci.*, vol. 87, no. 1, pp. 136-147, 2009.
- [37] U. Anwar *et al.*, "Impact of exogenous xylanase and phytase, individually or in combination, on performance, digesta viscosity and carcass characteristics in broiler birds fed wheat-based diets," *Animals*, vol. 13, no. 2, p. 278, 2023.
- [38] O. A. Olukosi and O. Adeola, "Whole body nutrient accretion, growth performance and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal based diets," *J. Poult. Sci.*, vol. 45, no. 3, pp. 192-198, 2008.
- [39] J. I. Bromfield, L. C. Hoffman, D. Horyanto, and E. A. Soumeh, "Enhancing growth performance, organ development, meat quality, and bone mineralisation of broiler chickens through multi-enzyme super-dosing in reduced energy diets," *Animals*, vol. 11, no. 10, p. 2791, 2021.
- [40] R. Gružauskas *et al.*, "Effects of enzymes, organic acids mixture and prebiotics on productivity of broiler chickens and sensory attributes of the meat," 2007.
- [41] D. Jóźefiak, A. Rutkowski, B. B. Jensen, and R. M. Engberg, "Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation on growth performance of broiler chickens and fermentation in the gastrointestinal tract," *Anim. Feed Sci. Technol.*, vol. 132, no. 1-2, pp. 79-93, 2007.
- [42] H. A. H. Zakaria, M. A. R. Jalal, and M. A. A. Ishma, "The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens," *Int. J. Poult. Sci.*, vol. 9, no. 2, pp. 126-133, 2010.
- [43] G. Tassidis, "Effect of Enzyme Supplementation on Broiler Chicken: A Literature Review," 2023.
- [44] S. P. Macelline, P. V. Chrystal, S. Y. Liu, and P. H. Selle, "The dynamic conversion of dietary protein and amino acids into chicken-meat protein," *Animals*, vol. 11, no. 8, p. 2288, 2021.
- [45] X. M. Ding *et al.*, "Effects of dietary crude protein levels and exogenous protease on performance, nutrient digestibility, trypsin activity and intestinal morphology in broilers," *Livest. Sci.*, vol. 193, pp. 26-31, 2016.
- [46] S. A. Amer *et al.*, "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.*, vol. 17, pp. 1-16, 2021.
- [47] S. Swiatkiewicz, A. Arczewska - Wlosek, and D. Jozefiak, "The nutrition of poultry as a factor affecting litter quality and foot pad dermatitis-an updated review," *J. Anim. Physiol. Anim. Nutr. (Berl.)*, vol. 101, no. 5, pp. e14-e20, 2017.
- [48] J. M. Lourenco, S. C. Nunn, E. J. Lee, C. R. Dove, T. R. Callaway, and M. J. Azain, "Effect of supplemental protease on growth performance and excreta microbiome of broiler chicks," *Microorganisms*, vol. 8, no. 4, p. 475, 2020.
- [49] M. Karami, A. Karimi, A. Sadeghi, J. Zentek, and F. Goodarzi Boroojeni, "Evaluation of interactive effects of phytase and benzoic acid supplementation on performance, nutrients digestibility, tibia mineralisation, gut morphology and serum traits in male broiler chickens," *Ital. J. Anim. Sci.*, vol. 19, no. 1, pp. 1428-1438, 2020.
- [50] J. Broch *et al.*, "Phytase and phytate interactions on broilers' diet at 21 days of age," *J. Appl. Poult. Res.*, vol. 29, no. 1, pp. 240-250, 2020.
- [51] V. H. C. Moita, M. E. Duarte, and S. W. Kim, "Supplemental effects of phytase on modulation of mucosa-associated microbiota in the jejunum and the impacts on nutrient digestibility, intestinal morphology, and bone parameters in broiler chickens," *Animals*, vol. 11, no. 12, p. 3351, 2021.
- [52] E. G. Kiarie, S. Steelman, M. Martinez, and K. Livingston, "Significance of single β -mannanase supplementation on performance and energy utilization in broiler chickens, laying hens, turkeys, sows, and nursery-finish pigs: a meta-analysis and systematic review," *Transl. Anim. Sci.*, vol. 5, no. 4, p. txab160, 2021.
- [53] A. M. Grieve, S. Cervantes-Pahm, and M. A. Martinez, "The impact of β -mannanase enzyme on the intestinal health of poultry under commercial conditions," in *27th ANNUAL AUSTRALIAN POULTRY SCIENCE SYMPOSIUM*, 2016, p. 52.
- [54] R. J. Arsenault, J. T. Lee, R. Latham, B. Carter, and M. H. Kogut, "Changes in immune and metabolic gut response in broilers fed β -mannanase in β -mannan-containing diets," *Poult. Sci.*, vol. 96, no. 12, pp. 4307-4316, 2017.
- [55] J. Liu, N. Wang, Y. Liu, Y. Jin, and M. Ma, "The antimicrobial spectrum of lysozyme broadened by reductive modification," *Poult. Sci.*, vol. 97, no. 11, pp. 3992-3999, 2018.
- [56] M. A. Abdel-Latif, A. H. El-Far, A. R. Elbestawy, R. Ghanem, S. A. Mousa, and H. S. Abd El-Hamid, "Exogenous dietary lysozyme improves the growth performance and gut microbiota in broiler chickens targeting the antioxidant and non-specific immunity mRNA expression," *PLoS One*, vol. 12, no. 10, p. e0185153, 2017.
- [57] G. Brugaletta *et al.*, "A multi-omics approach to elucidate the mechanisms of action of a dietary muramidase administered to broiler chickens," *Sci. Rep.*, vol. 12, no. 1, p. 5559, 2022.
- [58] K. Ganar, M. Das, S. Sinha, and S. Kumar, "Newcastle disease virus: current status and our understanding," *Virus Res.*, vol. 184, pp. 71-81, 2014.

- [59] M. I. El-Katcha, M. A. Soltan, H. F. El-Kaney, and E. R. Karwarie, "Growth performance, blood parameters, immune response and carcass traits of broiler chicks fed on graded levels of wheat instead of corn without or with enzyme supplementation," *Alexandria J. Vet. Sci.*, vol. 40, no. 1, pp. 95-111, 2014.
- [60] R. A. Dalloul and H. S. Lillehoj, "Poultry coccidiosis: recent advancements in control measures and vaccine development," *Expert Rev. Vaccines*, vol. 5, no. 1, pp. 143-163, 2006.
- [61] E. G. Kiarie, H. Leung, R. Akbari Moghaddam Kakhki, R. Patterson, and J. R. Barta, "Utility of feed enzymes and yeast derivatives in ameliorating deleterious effects of coccidiosis on intestinal health and function in broiler chickens," *Front. Vet. Sci.*, vol. 6, p. 473, 2019.
- [62] P. I. P. Ponte, L. M. A. Ferreira, M. A. C. Soares, L. T. Gama, and C. Fontes, "Xylanase inhibitors affect the action of exogenous enzymes used to supplement *Triticum durum*-based diets for broiler chicks," *J. Appl. Poult. Res.*, vol. 13, no. 4, pp. 660-666, 2004.
- [63] H. U. Zamani, T. C. Loh, H. L. Foo, A. A. Samsudin, and M. I. Alshelmani, "Effects of feeding palm kernel cake with crude enzyme supplementation on growth performance and meat quality of broiler chicken," *Int. J. Microbiol. Biotechnol.*, vol. 2, pp. 22-28, 2017.
- [64] F. L. Law, I. Zulkifli, A. F. Soleimani, J. B. Liang, and E. A. Awad, "The effects of low-protein diets and protease supplementation on broiler chickens in a hot and humid tropical environment," *Asian-Australasian J. Anim. Sci.*, vol. 31, no. 8, p. 1291, 2017.
- [65] V. Ravindran, "Feed enzymes: The science, practice, and metabolic realities," *J. Appl. Poult. Res.*, vol. 22, no. 3, pp. 628-636, 2013.