

Effects of Protease and *Bacillus subtilis* Supplementation on the Growth Performance of Broiler Chickens Fed Low-Protein Diets

Ali Usman¹, Kamran Ahmad Nasir¹, Abdullah Nisar¹, Ammara Ameer² and Muhammad Mahboob Ali Hamid^{1*}

1. Institute of Animal and Dairy Sciences, Faculty of Animal Husbandry, University of Agriculture, Faisalabad, Pakistan.
2. Department of Food Science & Technology, Government College Women University Faisalabad, Pakistan

*Corresponding Author: dr.mmahboob@uaf.edu.pk

ABSTRACT

Protein is an essential but expensive ingredient in broiler feeds, and the poultry industry still tries to find a way of balancing between cost-effectiveness and the desirable performance of chickens. One of the potential solutions to reducing feed costs is a reduction in dietary crude protein (CP), but these reductions tend to limit growth, feed efficiency and carcass quality by reducing the capacity to supply amino acids and their digestion. Dietary supplements which include exogenous protease enzymes and probiotics, especially *Bacillus subtilis*, have attracted significant concern in recent years due to their ability to improve nutrient utilization and general gut functionality. These additives have the ability to reduce the biological limitations of low-protein feeding by enhancing protein hydrolysis, stabilizing intestinal microbiota and enhancing mucosal health. There has been emerging evidence that when they are used together, they generate synergistic effects of improvement of weight gain, feed ratio, and nutrient digestibility. All these responses contribute to a more cost-effective, biologically efficient, and environmentally sustainable way of producing broilers.

Keywords: *Bacillus subtilis*, broiler, supplementation, performance

To cite this article: Usman A, KA Nasir, A Nisar, A Ameer & MMA Hamid. Effects of Protease and *Bacillus subtilis* Supplementation on the Growth Performance of Broiler Chickens Fed Low-Protein Diets. *Biological Times*. 2025. December 4(12): 3-4.

Introduction

There is a growing demand of animal protein in the world and poultry meat is one of the fastest developing industries in the livestock industry. Recent estimates indicated that meat production is likely to grow to 382 million tonnes by the year 2032, with poultry taking over 20 million tonnes and its production is projected to rise at a rate of 1.3 per annum [1]. Nutritional and genetic selection methods now allow broiler chickens to grow at an impressive rate, which has further promoted the need to be accurate in nutritional formulations to fulfil metabolic and physiological requirements [2]. The high protein feed components like soybean meal and fish meal continue to be significant production factors. In spite of the fact that reducing crude protein is a cost effective approach, biological impacts, such as decreased amino acids, limited digestive activity, as well as higher metabolic load, often lead to poor growth and reduced feed efficiency [3]. In order to offset these effects, nutritionists are increasingly relying on feed additives that regulate the digestive mechanisms and gut physiology. Exogenous protease enzymes amplify proteolysis by cleaving the peptide bonds that cannot be broken by endogenous proteases such as *Bacillus subtilis* which is a resilient sporing probiotic that amplifies microbial balance, immunity and nutrient delivery to the gastrointestinal tract [4,5]. Notably, the biological activities of protease and *B. subtilis* are not independent of each other. Their complementary processes, which are enzyme-mediated protein degradation and microbe-mediated gut stabilization, can be used in harmony temporarily and physiologically. Protease activity enhances release and absorption of amino acids in the initial digestion and *B. subtilis* balances and sustains mucosal integrity in the long-term. The combination of these effects is particularly useful when it comes to low-protein diets, in which it is crucial to maximize each unit of dietary nitrogen in order to maintain growth [6].

Functional Role of Protease in Broiler Nutrition

Digestive Efficiency

Protease is an early enzyme, which is usually activated in the proventriculus and gizzard where the pH levels favor the enzymes. Protease lowers the burden on the pancreatic secretions by increasing the disintegration of plant and animal protein fractions, and promotes more effective intestinal absorption. Literature has continuously reported increased ileal crude protein digestibility, availability of amino acids and general nitrogen retention with inclusion of protease in broiler diets [7,8]. These consequences enable the nutritionists to develop diets that are low in crude protein without affecting performance.

Performance outcomes

Studies show that protease supplementation alters growth patterns. Whereby birds on low-protein diets still have the ability to grow body weight gain rates that are similar to those of birds on classical formulations

[9]. Increased feed conversion ratio (FCR) is a sign of increased efficiency in the digestive process and lower metabolic costs due to the loss of nitrogen. The more protein is available sooner in the digestive process, the better muscle is synthesized which leads to continued growth despite protein-restricted conditions.

Bacillus subtilis: A Robust and Functional Probiotic Gut Health and Microbiota

In contrast to most of the lactic acid bacteria, the *Bacillus subtilis* are able to withstand feed processing and gastric acidity because of its ability to form spores [10]. After entering the intestine, the spores germinate and actively regulate the microbiota through the production of antimicrobial peptides, competitive metabolites and digestive enzymes. Such measures inhibit the growth of non-beneficial bacteria, minimize the inflammatory condition of the intestines and stabilize the condition of the microbiological communities at the essential developmental phases [11].

Nutrient Absorption

Biological enhancements of intestinal morphology, that has increased villus length, reduced crypt depth, and enhanced epithelial differentiation, correspond into augmented absorption area and enhanced nutrient uptake [12]. These structural improvements not only enhance the utilization of proteins and energy but also cause lower physiological cost of intestinal tissue maintenance, thus adding to the better feed efficiency.

Immune Support

The *Bacillus subtilis* has a close interaction with the gut-associated lymphoid tissue (GALT), which activates the immune responses including induction of cytokines, secretion of mucosal antibodies, and activation of phagocytic cells. The modifications enhance the resistance of the bird to enteric pathogens, decreases subclinical infections and a stable physiological condition that supports efficient growth [13].

Combined Effects on Broiler Growth

Combination of protease and *Bacillus subtilis* is used at various levels of digestion and gut physiology. Protease enhances the release of nutrients following consumption and *B. subtilis* helps the continuity of microbial and immune stability. Their combined action promotes the availability of amino acids, lowers the gut inflammations and increases the efficiency of entire metabolism [9].

Body Weight Gain

Birds fed on both additives tend to gain higher body weights because of better digestion of proteins and better assimilation of nutrients. Improved levels of amino acids favor muscle accretion in the production cycle [6].

Feed Conversion Ratio

The condition of the gut and digestive efficiency of various organisms will improve synergistically, decreasing the weight gain on a required feed intake. This adds up to more lucrative production systems.

Economic and Environmental Impact

Maintaining growth on diets with reduced protein does not only help in minimizing the cost of feeds, but also minimizes the amount of nitrogen excretion, which reduces ammonia emissions and environmental pollution a major factor to be considered by intensive poultry farms.

Experimental Insights

In a study conducted on 192 broiler chicks under five dietary treatments which lasted 35 days, the growth of the broilers, their FCR, and digestible crude protein and essential amino acids were found to be superior in birds receiving both protease and *B. subtilis*. These results highlight the time-based complement in the enzyme and microbial activity in the maximization of the digestive process

Practical Applications

The joint use of protease enzymes and *Bacillus subtilis* in the real-life poultry production have multiple benefits which directly contribute to the performance of the flock, profitability and sustainability. Among the largest applications is the reduction of feed costs, where a safe reduction in the amount of dietary crude protein by nutritionists can reduce the costs of growth and feed ratio. Protein digestibility and amino acid absorption can be increased by reformulating diets using protease supplementation to create a more economical nutritional plan that meets the protein and amino acid requirements of the rapidly growing broilers. Meanwhile, *Bacillus subtilis* reinforces the integrity of the gut and balances microbial populations, which leads to the improved assimilation of nutrients and decreases the level of enteric disorders. This turn reduces reliance on antibiotic treatments and leads to the health and welfare of flocks. Also, the enhanced nitrogen use of such additives decreases the nitrogen release and ammonia emission, which makes the production systems more eco-responsible. The second important practical advantage is higher flexibility in the formulation of feeds since it is possible to utilize alternative or less costly sources of proteins without compromising the performance of birds due to the higher efficiency of the digestive system. Together, these uses enable protease and *Bacillus subtilis* to be useful products in maximizing nutritional efficiency of broilers in contemporary economic and environmental limitations.

Considerations for Implementation

To be successful, the implementation of protease and *Bacillus subtilis* in the diets of broilers involves paying close attention to a number of nutritional and management factors. The right dosage of every additive is vital to be stated, and the best amounts of them are usually based on the age of the birds, the nutrient content of the food stuff, and the production goals in general. The next issue as well is to make sure that these supplements are stable and viable throughout the processing of feed, especially those subject to pelletizing where elevated temperatures can inactivate enzymes or destroy probiotic viability. The choice of strains of *Bacillus subtilis* that have shown effectiveness, strong spore-forming capacity and does not interfere with the established diet are essential in enhancing the benefits

associated with gut health and immunomodulation. Producers are also required to consistently check on the flock performance, feed consumption and health indications to ensure that the additives are producing desired results and to make relevant adjustments to the diets where necessary. The combination of acceptable dosage, compatibility of ingredients, stability in processing, and monitoring of performance of protease and *Bacillus subtilis* all mean that these products can provide the full biological and economic benefits they have to claim when they are incorporated into commercial broiler feeding programs.

Conclusion

Pre-treatment of protease enzymes with *Bacillus subtilis* is a biologically viable approach to improving nutrient utilization, intestinal status and growth performance in broilers especially during low crude protein situations. Their synergistic functions, which are rapid enzyme hydrolysis and maintenance of gut, facilitate economical, efficient, and environmentally friendly production of poultry. The further developments in their applications in the contemporary poultry nutrition will be possible through continued research on strain specificity, interactions between enzymes and microbes, and long-term physiological effects.

References

- [1] Food and Agriculture Organization. OECD-FAO Agricultural Outlook 2023-2032. 2023.
- [2] Barbarelli S, Maertens L, Claes E, Derave W, De Smet S. Differences in muscle histidine-containing dipeptides in broilers. *Sci Food Agric*. 2019;99:5680-6.
- [3] Attia Y, Al-Khalaf H, Abd El-Hamid H, Al-Harthi M, El-Shafey A. Effect of different levels of multienzymes on immune response, blood hematology and biochemistry, antioxidants status and organs histology of broiler chicks fed standard and low-density diets. *Front Vet Sci*. 2020;6:510.
- [4] Alagawany M, Abd El-Hack ME, Farag MR, Sachan S, Karthik K, Dhama K. The use of probiotics as ecofriendly alternatives for antibiotics in poultry nutrition. *Environ Sci Pollut Res*. 2018;25:10611-8.
- [5] Hafeez A, Pfützner A, Mader A, Männer K. Influence of protease supplementation in broiler diets on nutrient digestibility, bone traits and performance. *Poult Sci*. 2021;100:1012-9.
- [6] Vieira SL, Stefanello C, Cemin HS. Lowering the dietary protein levels by the use of synthetic amino acids and the use of a mono component protease. *Anim Feed Sci Technol*. 2016;221:262-6.
- [7] Velazquez-De Lucio BS, Hernandez-Dominguez EM, Villa-Garcia M, Diaz-Godinez G, Mandujano-Gonzalez V, Mendoza-Mendoza B, Alvarez-Cervantes J. Exogenous enzymes as zootechnical additives in animal feed. *Catalysts*. 2021;11:851.
- [8] Borda-Molina D, Zuber T, Siegert W, Camarinha-Silva A, Feuerstein D, Rodehutschord M. Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens. *Poult Sci*. 2019;98:2906-18.
- [9] Jabbar A, Tahir M, Khan RU, Ahmad N. Interactive effect of exogenous protease and step-wise increment in dietary crude protein on growth and digestibility indices in broiler chickens during the starter phase. *Trop Anim Health Prod*. 2021;53:23.
- [10] Goo D, Choi J, Ko H, Choppa VSR, Liu G, Lillehoj HS, Kim WK. Effects of Eimeria maxima infection doses on growth performance and gut health in dual infection model of necrotic enteritis in broiler chickens. *Front Physiol*. 2023;14:85-7.
- [11] Chen X, Chen W, Ci W, Zheng Y, Han X, Huang J, Zhu J. Effects of dietary supplementation with LactoBacillus acidophilus and Bacillus subtilis on mucosal immunity and intestinal barrier are associated with its modulation of gut metabolites and microbiota in late-phase laying hens. *Probiotics Antimicrob Proteins*. 2023;15:912-24.
- [12] Jager R, Purpura M, Farmer S. Probiotic Bacillus coagulans GBI-30, 6086 improves protein absorption and utilization. *Probiotics Antimicrob Proteins*. 2018;10:611-5.
- [13] Memon FU, Yang Y, Leghari IH, Soliman AM. Transcriptome analysis revealed ameliorative effects of Bacillus based probiotic on immunity, gut barrier system and metabolism of chicken under an experimentally induced Eimeria tenella infection. *Genes*. 2021;12:536.