

# Synergistic Effects of Phytase, Carbohydrases and Citric Acid on Growth Performance and Nutrient Utilization in Broilers Fed Energy-Restricted Diets

Muhammad Imran<sup>1</sup>, Ali Usman<sup>1</sup>, Kamran Ahmad Nasir<sup>1</sup>, Ammara Ameer<sup>2</sup> and Muhammad Mahboob Ali Hamid<sup>1\*</sup>

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](mailto:dr.mmahboob@uaf.edu.pk)

## ABSTRACT

Increased feed prices have increased pressure on nutritional approaches to support broilers despite low dietary energy density. Nevertheless, reducing dietary energy usually jeopardizes the rate of growth, feed ratio and the use of nutrients. Enzyme supplementation- especially phytase (3000 FTU/kg),  $\beta$ -mannanase (5000 IU/g), xylanase-15000 IU/g at 100 g/ton), organic acids (citric acid 5 g/kg) have proven to mitigate the adverse effects of low-energy diets. These supplements improve the digestibility of nutrients, improve gut morphology, minimize anti-nutritional effects and make the gastrointestinal environment healthier. This review presents the individual and synergistic action of these supplements and their potential use to counteract energy reduced performance losses in broilers fed on energy-restricted diets. It is indicated that incorporation of phytase, carbohydrases, citric acid positively impacts body weight gain and feed conversion ratio (FCR) and increases the bioavailability of energy-yielding nutrients which results into more profitable and sustainable broiler production.

**Keywords:** Phytase, Carbohydrases, Citric Acid, growth performance

**To cite this article:** Imran M, A Usman, KA Nasir, A Ameer & MMA Hamid. Synergistic Effects of Phytase, Carbohydrases and Citric Acid on Growth Performance and Nutrient Utilization in Broilers Fed Energy-Restricted Diets. *Biological Times*. 2025. December 4(12): 5-6.

## Introduction

Plant-based feed materials are vital in the production of Broiler systems all over the world, with corn, soybean meal, wheat, and their by-products making over 90% of the many commercial diet formulations. Although these ingredients have desirable amino acids and energy content, they also have high amounts of anti-nutritional elements, especially phytate and non-starch polysaccharides (NSPs), which greatly hinder the absorption of nutrients and the effectiveness of digestion. Phytate is still among the most salient anti-nutritional compounds in plant based feedstuffs; phytic acid, a form of phosphorus storage complex in 40-60% of total phosphorus, results in strong protein-mineral complexes that are resistant to digestion in the monogastric gastrointestinal tract [1, 2]. This decreases the bioavailability of calcium, zinc, iron, magnesium, amino acids and lipids causing low growth and low feed efficiency [3]. NSPs including arabinoxylans and b-mannans also reduce nutrient utilization by raising the viscosity of the digesta and reducing the interactions of enzymes with their substrates in the gut lumen along with phytate. Their presence entraps nutrients within complex structural frameworks, retards digestion, changes intestinal pH and, finally, decreases availability of metabolizable energy. As a result, broilers which are fed diets that are high in NSPs, tend to have high ratios of feed intake to mass gain, inefficient mass gain, and a disturbed gut morphology [10-12]. Considering these limitations, dietary energy restriction has become a cost-effective approach although it is known to result in significant performance costs when poorly implemented. A potential solution to these shortcomings is the use of exogenous enzymes, including phytase, b-mannanase, and xylanase, and organic acids, like citric acid. Phytases release phosphorus and other nutrients through the degradation of phytate, carbohydrases through the degradation of NSPs and makes the gut less viscous to extract energy, and citric acid through the release of minerals by increasing the gut pH, decreasing gut pH, and improving the balance of microbes. It has been shown that interplay of these additives give synergistic effects which enhance growth performance, nutrient digestibility, immune activity and general intestinal health when present in low-energy feeding regimes [4-7].

## Enzymatic Additives and Their Functions

### Phytase

One of the most widely used feed enzymes in the monogastric nutrition is phytase, which has a significant role in relieving anti-nutritional impact of phytate. Since phytate is poorly digested by poultry because the poultry lacks endogenous phytase, its presence reduces the bioavailability of phosphorus and causes complexations with proteins and minerals, making them unable to be absorbed. The phosphate groups that have been conjugated into the inositol rings are cleaved off by the exogenous phytase gradually releasing inorganic phosphorus, myo-inositol, amino acids, lipids

and trace minerals that had previously been united into phytate complex (Riley, 2002). This action of the enzyme makes it unnecessary to supplement phosphorus inorganically which not only lowers the cost of feed but also decreases the amount of phosphorus released to the environment. In addition to mineral liberation, phytase has further digestive properties that include better protein digestibility, better intestinal mucosal health, and less gut inflammation which in combination, lead to higher growth performance and feed efficiency [9]. Phytase activity lowers the viscosity of digesta and decreases fermentation efficiency of the undigested phytate complexes by the intestinal microbes, enhancing the intestinal gut absorption of nutrients and creating a more stable microbial environment. The presence in low-energy diets shows significant changes in metabolizable energy since a decrease in phytate-mediated nutrient binding will enable the animal to obtain the nutrient-dense compounds that are otherwise not accessible. These are the physiological advantages that cause phytase to be a necessary part of standard and energy-limited broiler diets, where nutrient usage optimization would grow in significance.

### $\beta$ -Mannanase and Xylanase as Carbohydrase

The prevalence of inos NSPs including b-mannan and arabinoxylan are common with common feed materials that include cereal and legumes, but poultry do not have sufficient innate enzymes to break them down. The impacts of these NSPs are numerous and they affect the gut viscosity, diffusion of nutrients, getting access to enzymes and alteration of microbial communities in the intestine. Corn-soybean diets With 400-450 kcal/kg of digestible energy, up to 400-450 kcal/kg is unavailable because of the encapsulation of NSPs and the formation of viscous digesta [10]. B-mannanase and xylanase are the most important carbohydrases that attack these NSPs. Xylanase cleaves the b-1,4 bonds in arabinoxylans to generate low-viscosity xylo-oligosaccharides, b-mannanase cleaves b-mannan to mannan-oligosaccharides [11]. They lower the viscosity of the digesta, maximize nutrient release and improve passage rate, giving more access to substrates by endogenous enzymes. Better digestion of NSPs provides more energy is available on a given feedstuff hence favoring growth in energy-diluted diets [12]. Besides enhancing the digestibility of nutrients, the two enzymes are prebiotics. Short-chain oligosaccharides increase the beneficial microbial populations including *Lactobacillus* spp. and *Bifidobacterium* spp. which improve intestinal immunity and gut barrier function to save energy that would otherwise be used in unwarranted immune responses [13,14]. Carbohydrases therefore provide both digestive and metabolic benefits, which allows feed formulators to reduce dietary energy without compromising performance.

### Citric Acid and Its Role in Nutrition

Citric acid (CTA) is commonly known as one of the most effective organic acids that are utilized in the poultry feed because of its capacity to reduce

the gastrointestinal pH, enhance the availability of minerals, and prevent the pathogenic microorganisms. When citric acid enters the crop and the upper small intestine, it lowers the pH enough to prevent the toxicity of the acid-sensitive microorganisms, like *Escherichia coli*, allowing the intestine to experience a more favorable microenvironment in nutrient absorption [15]. Decreased PH further increases the activation of pepsin and the solubility of minerals like calcium and phosphorus that are essential in the development of bones and metabolism. Phytase also synergizes with citric acid. Citric acid ensures that the phytate hydrolysis is as effective as possible by decreasing the pH to the optimal pH at which phytase can work, which increases the release and uptake of nutrients [6]. A combination of phytase and citric acid has improved villus height and crypt depth thus showing enhanced ability to uptake nutrients and regenerate the epithelial lining of the small intestine [7]. These modifications help in better digestion of proteins, fats and minerals particularly in the diets with lower energy density. Citric acid is also a metabolic intermediate in the tricarboxylic acid cycle which may be useful in generating energy at cellular level. Enzymes and Citric Acid synergistic effects occur when combined. Although the supplementation of phytase, carbohydrases or citric acid separately enhances the use of nutrients, simultaneous addition of all the three has exhibited significantly superior effects owing to the complementary characteristics of their action mechanism. Their incorporation generates a multi-layered system to enhance digestion and gut performance and energy liberation of plant-based feed materials.

#### Synergistic Effects of Enzymes and Citric Acid

While individual supplementation of phytase, carbohydrases, or citric acid improves nutrient utilization, the combined inclusion of all three has demonstrated markedly enhanced effects due to complementarity in their modes of action. Their integration creates a multi-layered approach to improve digestion, gut health, and energy release from plant-based feed ingredients.

#### Nutrient Digestibility

Phytase releases phytate bound minerals and nutrients, xylanase and  $\beta$ -mannanase hydrolyze structural NSPs to more fermentable substances, and citric acid increases solubility and reduces pH to maximize enzyme activity. Such a combination makes sure that nutrients are not only liberated out of chemical complexes, but they are absorbed optimally by the intestinal mucosa. This synergetic effect enhances ileal digestibility of amino acids, fatty acids and complex carbohydrates enhancing the effective metabolizable energy of the diet. Research indicates that diets with phytase, carbohydrases, and citric acid are equivalent or even better than traditional-energy diets in terms of their digestible energy despite a lower formulated energy [4].

#### Feed Conversion Ratio

FCR, which is the greatest measure of economic efficiency, is also augmented by the increase of nutrient digestibility and gut functionality. When all additives are used (phytase +  $\beta$ -mannanase + xylanase + citric acid), the lowest FCR values are usually observed when the environment is reduced-energy. Better performance is achieved because of a number of factors includes low viscosity of the digesta facilitates absorption of nutrients, reduced PH boosts the activity of the enzymes, breakdown of the anti-nutritional factors liberates nutrients and enhanced gut morphology enhances absorptive surface.

Furthermore, the low immune stimulation saves metabolic energy. These enhancements enable broilers to change the feed into body mass more effectively.

#### Body Weight Gain

The increase in weight is especially sensitive to the energy intake of the diet, yet the loss of performance can be reduced with the aid of enzyme-acid combinations supplementation. Birds fed all additives consistently perform better than those on low energy diets not supplemented and usually gain weight in the same manner as the positive controls. Improved extraction of nutrients in the feeds promote quicker muscle deposition, whereas better gut health is used in the feeding of livestock to enable them to feed regularly. When citric acid and enzymes are added, the load of intestinal pathogens is also decreased, which guarantees the minimum competition of nutrients between the host and pathogenic microorganisms.

#### Practical Applications

The advantages of enriching energy-reduced diets with integrating phytase, carbohydrases, and citric acid have the following practical implications to commercial poultry feed:

**Reduced Feed Costs:** Reduced energy in the diet enables the reduction of the consumption of costly components like fats and oils.

**Better Environmental Sustainability:** Improved phosphorus and nitrogen digestibility leads to the reduction of nutrient leakage and thus less environmental pollution is caused.

**Enhanced Gut Integrity:** The pathogenic pressure is decreased and the villus morphology is enhanced which leads to healthier gut and resilience.

**Better Flexibility of Ingredients:** With enzyme supplementation, alternative feedstuffs can be included much more including wheat, barley, or by-products.

**Increased Nutrient Utilization Effectiveness:** The increased metabolizable energy and protein efficiency is a result of improved digestion of NSPs and phytate-bound nutrients.

**Consistency in Performance:** Such additives assist in sustaining growth, as well as FCR in times of changes in the quality of ingredients. The cumulative benefits of all of these are great economic and biological value, so the enzyme acid supplementation is becoming a part of the current broiler nutrition programs.

#### Conclusion

Increasing demand to lower the cost of feed in poultry production has demonstrated more interest in the low energy density diets. These diets can however affect performance of broilers negatively unless they are supplemented with specific nutritional interventions. The synergistic approach of phytase,  $\beta$ -mannanase, xylanase, and citric acid is another successful strategy of overcoming the constraints of phytate and NSPs, as well as poor gut conditions. Inclusion increases the digestibility of nutrients, improves the morphology of the intestine, stabilizes the microbial population, and increases the efficiency of feed utilization- ultimately increases growth performance similar to broilers fed high-energy diets. With feeds still being an ingredient that is sporadic in its price and supply, enzyme and organic acid supplementation will continue to be at the center of sustainable and profitable broiler feeding. The future studies must be aimed at finding the best dosage combination, assessing the interaction with new feed ingredients, and the possibility of precision nutrition by combining enzyme technologies and real-time nutrient monitoring.

#### References

- [1] Naghsbbandi, M.P. and H. Moghimi. 2020. Stabilization of phytase on multi-walled carbon nanotubes via covalent immobilization. In: *Methods in Enzymology*. 630:431-451.
- [2] Dersjant-Li, Y., A. Awati, H. Schulze and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of the Science of Food and Agriculture*. 95:878-896.
- [3] Gonzalez-Uarquin, F., M. Rodehutscoord and K. Huber. 2020. Myo-inositol: its metabolism and potential implications for poultry nutrition—a review. *Poultry Science* 99(2):893-905.
- [4] Roofchaei, A.V. Rezaeipour, S. Vatandour and F. Zaeferian. 2019. Influence of dietary carbohydrases, individually or in combination with phytase or an acidifier, on performance, gut morphology and microbial population in broiler chickens fed a wheat-based diet. *Animal Nutrition*. 5(1):63-67.
- [5] Ramigani, V.R., J.V. Ramana, D. Rao Srinivasa, S. Shakila and J. Suresh. 2015. Effect of dietary supplementation of organic acids on digestibility of nutrients and serum biochemical profile of broiler chicken. *Indian Journal of Animal Nutrition*. 32:300-304.
- [6] Nourmohammadi, R. and N. Afzali. 2013. Effect of citric acid and microbial phytase on small intestinal morphology in broiler chicken. *Italian Journal Animal Science*. 12(1):7.
- [7] Fikry, A.M., A.I. Attia, I.E. Ismail, M. Alagawany and F.M. Reda. 2021. Dietary citric acid enhances growth performance, nutrient digestibility, intestinal microbiota, antioxidant status, and immunity of Japanese quails. *Poultry Science* 100(9):101326.
- [8] Sousa, J.D., L.F. Albino, R.G. Vaz, K.F. Rodrigues, G.F. Da Silva, L.N. Renno, V.R. Barros and I.N. Kaneko. 2015. The effect of dietary phytase on broiler performance and digestive, bone, and blood biochemistry characteristics. *Revista Brasileira de Ciencia Avicola*. 17(1):69-76.
- [9] Li, W., R. Angel, P.W. Plumstead and H. Enting. 2021. Effects of limestone particle size, phytate, calcium source, and phytase on standardized ileal calcium and phosphorus digestibility in broilers. *Poultry Science* 100(2):900-909.
- [10] Jasek, A., R.E. Latham, A. Mañón, S. Llamas-Moya, R. Adhikari, R. Poureslami and J.T. Lee. 2018. Impact of a multicarbohydrase containing  $\alpha$ -galactosidase and xylanase on ileal digestible energy, crude protein digestibility, and ileal amino acid digestibility in broiler chickens. *Poultry Science* 97(9):3149-3155.
- [11] Raza, A., S. Bashir and R. Tabassum. 2019. An update on carbohydrases, growth performance and intestinal health of poultry. *Heliyon*. 10:12-13.
- [12] Choct, M. 1997. Feed non-starch polysaccharides: chemical structures and nutritional significance. *Feed Milling International*. 19(1):13-26.
- [13] Munyaka, P.M., N.K. Nandha, E. Kiarie, C.M. Nyachoti and E. Khafipour. 2016. Impact of combined  $\beta$ -glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poultry Science* 95(3):528-540.
- [14] Kim, M.C., J.H. Kim, F.M. Pitargue, D.Y. Koo, H.S. Choi and D.Y. Kil. 2017. Effect of dietary  $\beta$ -mannanase on productive performance, egg quality, and utilization of dietary energy and nutrients in aged laying hens raised under hot climatic conditions. *Asian-Australasian Journal of Animal Sciences*. 30(10):1450.
- [15] Ramigani, V.R., J.V. Ramana, D. Rao Srinivasa, S. Shakila and J. Suresh. 2015. Effect of dietary supplementation of organic acids on digestibility of nutrients and serum biochemical profile of broiler chicken. *Indian Journal of Animal Nutrition*. 32:300-304.