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Review Article
Roles of Fructooligosaccharides and Phytase in Broiler Chickens: Review

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Abstract
Supplementing prebiotics and enzymes into poultry diets are among the most effective strategies in order to improve nutrient utilization, growth performance, intestinal development, immune system, intestinal microbiome and gut health. Fructooligosaccharides (FOS) is one of the most common prebiotics used in poultry. It has been reported that dietary FOS supplementation in broilers improved body weight gain, feed conversion and carcass yield. It could also enhance intestinal development, improve immune responses and increase short chain fatty acid fermentation of the broilers. Furthermore, *Salmonella* infection has been reduced by FOS supplementation into broiler diets. Phytase supplementation is one of the successful enzyme application in poultry. Phytase supplementation has increased body weight gain, Ca and P utilization and bone development in broilers. The combination of prebiotics and phytase, based on the modes of action of each component has shown potential benefit in poultry. Prebiotics is capable of increasing gut fermentation, producing short chain fatty acid and reducing gut pH. It has been hypothesized that prebiotics supplementation could create an acidic environment, which is favorable for phytase, increasing phytase activity and P utilization in the intestine. Therefore, the combination of prebiotics and enzyme could be a potential strategy to improve gut health and nutrient utilization in poultry.

Key words: Phytase, fructooligosaccharides, prebiotics, enzymes, broiler

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INTRODUCTION

Maximizing feed nutrient utilization and identifying effective infeed antibiotics growth promoters in poultry have been major issues for the poultry industry[1-5]. Dietary supplementation of prebiotics and enzymes may be one of the solutions for the poultry industry in order to optimize feed nutrient digestibility/absorption/utilization and maintain effective growth without antibiotics growth promoters. Prebiotics and enzymes are important feed additives to promote growth performance, maintain gut health and healthy immune system and enhance feed nutrient utilization in poultry[1-3]. Prebiotics are non-digestible multicarbohydrates and fermented in the lower intestine for beneficial effects on the hosts, such as lowering gut pH, producing organic acids (acetic acid, propionic acid, butyric acid and lactic acid), stimulating the host's immune system, changing gut microbial community and reducing pathogen colonization[1,2,6,7]. Although the mechanisms of prebiotics have been studies for a long time, detailed mechanisms of prebiotics still need to be further elucidated. One of the most effective dietary enzymes which have been used for poultry nutrition is phytase. Dietary phytase hydrolyzes phytic acid (phytate), which cannot be hydrolyzed in the intestine of poultry, to improve P utilization and reduce P excretion in the manure[4,5,8,9]. Supplementation of dietary phytase can reduce feed cost and environment pollution because P is one of the most expensive feed ingredient in poultry and excess P land application can cause P run off and contaminate ground and surface water[3,6,10-12]. Since the activity of dietary phytase is maximized in acidic pH (7-2.5)[4,9], fermentable prebiotics supplementation may provide better gut environment for phytase to hydrolyze phytic acid in the gut. It has been identified the potential benefits from the combination of dietary prebiotics and enzymes in poultry nutrition[13]. Thus, effective use of these feed additives can reduce production cost and increase profit for the poultry industry. In this review, we discuss overviews of prebiotics and enzymes, effects of fructooligosaccharides (FOS) and phytase and potential benefits for combination of prebiotics and enzymes.

PREBIOTICS

An overview of prebiotics: Prebiotics are defined as non-digestible food ingredients that stimulate the growth of beneficial micro-organisms in the intestine, in ways claimed to be beneficial to health[2]. To be classified as a prebiotic the compound has to be (1) Neither hydrolysable nor absorbable in the stomach or the small intestine, (2) A selective substrate for beneficial bacteria to be colonized in the large intestine, (3) Able to alter the gastrointestinal microbiota in favor of a healthier composition and (4) Able to induce luminal or systemic effects that are beneficial to the host health[1,2]. Prebiotic products are predominantly oligosaccharides, which include fructooligosaccharides (FOS, oligofructose and inulin), mannan-oligosaccharide (MOS), gluco-oligosaccharides (GOS), transgalacto-oligosaccharides (TOS), xylo-oligosaccharides, soybean galacto-oligosaccharides and lactulose[1]. Other sources of prebiotics include indigestible polysaccharides, certain proteins, peptides and lipids such as ethers and esters[6,2].

Bifidobacteria in humans, dietary prebiotic supplementations have demonstrated positive effects on promoting beneficial gut micro-organisms (especially on the stimulation of endogenous bifidobacteria), modulating lipid metabolism via fermentation and reducing gastrointestinal pH[2,14]. The use of prebiotics in animal production, as a possible alternative to Antibiotic Growth Promoters (AGPs) has also exhibited the capability of modulating the gut microbial communities. Prebiotics contribute to the establishment of beneficial microbial community with an increased number of bifidobacteria and/or lactobacilli[1]. In poultry, prebiotics are able to modulate the immune cells in the gut-associated lymphoid tissue (GALT) due to the lactic action that stimulates the innate and adaptive immune activity[6,7]. It has also been shown that dietary prebiotics supplementation reduced the population of *Clostridium perfringens*, *Escherichia coli* and *Salmonella* spp. in the large intestine and cecum of the chickens[15].

FRUCTOOLIGOSACCHARIDES (FOS)

An overview of FOS: The fructooligosaccharides (FOS) are one of the most popular prebiotic supplements available, consisting of several (on average 5) fructosyl residues that are linked by α, β (2, 1) glycosidic bond to a terminal glucose moiety[15,16]. This structure is different from oligofructose, which may only contain fructose molecules. The β (2, 1) glycosidic bond is resistant to be broken down by endogenous digestive enzymes of the monogastric animals and thus, becomes available for intestinal microbiota fermentation, which in turn result in increased bifidobacteria population, lowered gut pH, production of short-chain fatty acids (SCFA) as well as suppression of putrefactive substances[15-17]. The fermentation of FOS is faster than that of other fructans such as inulin, which has a Degree of Polymerization (DP) of 10-60, whereas FOS's DP ranges from 3-7[18,19].

Fructooligosaccharides can be naturally extracted from plant sources such as chicory root, onion, asparagus, beet, edible burdock, wheat, bananas and cane sugar[16,20]. They can also be commercially produced from sucrose by transfructosylation of *A. niger* enzyme or from inulin by enzymatic hydrolysis[16]. The FOS compound consists of a glucose monomer (G) linked by α-1, 2 bound to two or more β-2,1-linked fructosyl units (F), forming 1-kestose (GF_2), nystose (GF_3) and I^F-β-fructofuranosylnystose (GF_4).

Applications of FOS supplementation in broiler chickens: Several studies have been conducted in previous years to investigate the effect of dietary FOS supplementation on growth performance, nutrient utilization, intestinal morphology, gut microbiota, immune response and *Salmonella* immunity in broiler chickens.

Positive effects on growth performance parameters were reported by Yusrizal and Chen[21] that FOS supplementation has improved Body Weight Gain (BWG), feed conversion and carcass weight of female broiler chickens. Similar results related to increased BWG and improved Feed Conversion Ratio (FCR) were observed by Bailey *et al.*[22] and Xu *et al.*[23]. Variation in FOS inclusion levels may affect the growth rate and performance parameters of the bird[24]. It has been reported that excessive FOS (1%) may cause diarrhea and generate carbon dioxide and hydrogen gases due to intensive fermentation in the gastrointestinal (GI) tract, thus decreasing the production performance[14,25].

The gut morphology is an important indicator on digestive tract health and bird performance. Stress factors in the intestine can result in the changes of intestinal mucosa such as shortening of villus and deepening of crypts[15]. It is commonly believed that increasing in the villus height and decreasing in the crypts depth can positively affect the digestive and absorptive functions of the birds, due to an enlarged absorptive area and reduced tissue turnover rate in the GI tract[15,23]. Xu *et al.*[23] reported that FOS exhibited positive effects on intestinal morphology in broilers. About 0.4% of FOS supplementation significantly increased ($p<0.05$) ileal villus height, jejunal and ileal microvillus height and villus height to crypt depth ratio, while decreased crypt depth in the jejunum and ileum. Similarly, Shang *et al.*[26] reported that villus height, crypt depth and total mucosal thickness were significantly increased in the ileum of broiler chickens fed 0.5% of FOS supplementation. The beneficial changes in the intestinal mucosa structures are most likely due to the ability of FOS to create a favorable gut microbial environment[15].

Recent studies with dietary FOS supplementation have also been shown to improve intestinal microbiota of broiler chickens by stimulating the growth of beneficial bacteria such as bifidobacteria and lactobacilli, while limiting the growth of pathogenic bacteria, such as *Salmonella* spp. and *Escherichia coli*[15,23,27]. A number of *in vivo* studies have demonstrated that the intensive growth of beneficial bacteria suppresses the activities of the potential hazardous bacterial species and reduces the production of toxic substances, such as ammonia and phenols, thereby improving the overall health of the animals[15,21-23,28]. Furthermore, the supplementation of FOS in poultry diet increases gut fermentation, SCFA production and enzymes activities, which results in acidification and reducing pH in the GI tract[15,23,29].

Fructooligosaccharide supplementations have demonstrated positive effects toward the immune responses of the chickens by promoting the growth of lactic acid producing bacteria[23]. Janardhana *et al.*[7] supplemented 5 g kg^{-1} of FOS in addition to the basal broiler chicken diet and observed higher titers of plasma immunoglobulin (I g), M ($p<0.01$) and Ig G ($p<0.01$) than the control group. The FOS treated birds also had reduced number of B cells and depressed mitogen responses of lymphocytes in the cecal tonsil ($p<0.05$), without detrimental effects on performance, which is likely due to the SCFA fermentation and a combination of toll-like receptor mediated responses through their interaction with the gut micro-organisms and microbial products. Emami *et al.*[30] investigated FOS as an alternative to virginiamycin on immune response of male broilers and discovered that the primary antibody titers against sheep red blood cell were higher in the FOS fed treatment. Kim *et al.*[28] reported that the H:L ratio and the basophil leukocytes were significantly higher in 0.5% FOS groups than treatments with other prebiotics.

Salmonella spp. infection is a major cause of food-borne illness in human. Effective control of salmonellosis in meat-type chicken production is essential to ensure poultry food safety[31]. The FOS supplementation has been reported to have anti-*Salmonella* activity and it is mostly due to the shift of intestinal microbiota and the production of short-chain fatty acids[32]. Bailey *et al.*[22] reported that treatments with FOS showed a fourfold reduction of *Salmonella* in chicken ceca. Shang *et al.*[26] intraperitoneally injected *Salmonella* enteritidis lipopolysaccharides (LPS) into broiler chickens and observed a significant increase of specific IgY to *Salmonella* LPS level in the FOS supplemented group. Alterations of leukocytes compositions, such as reduced heterophils and increased monocytes count along with elevated expressions of certain cytokine genes were also reported in the FOS supplemented broilers. In general, feeding FOS in broiler diet may result in improved immunity against *Salmonella* and reduced *Salmonella* colonization.

PHYTASE

An overview of phytase: Phytases are types of phosphatase enzymes that can be found naturally in plants and microoganisms, such as fungi and bacteria[9]. Depending on the activity profile and the optimum pH for catalysis, phytase enzymes can be further classified as acid, neutral and alkaline phosphatases[33]. The majority of the phytases (myo-inositol hexakisphosphate phosphohydrolase) are acid phosphatases from fungal sources and belong to a subfamily of the high molecular weight Histidine Acid Phosphatases (HAPs). The HAPs have a two-step mechanism to hydrolyze phosphomonoesters bond from phytic acid (myo-inositol 1, 2, 3, 4, 5 and 6-hexakis dihydrogen phosphate) and release phytate phosphorous (P)[33,34]. Some of the commercial HAPs include *Aspergillus terreus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *Emericella nidulans*, *Myceliophthora thermophila* and *Saccharomyces cerevisae*[4,9]. Specific enzymatic activities of these fungal phytase are closely related to the environmental pH and the temperature. The optimum pH ranges from 2.5-7.0, with most of the phytase enzymes achieving their maximum activity at under pH 5.0[4,9]. The optimum temperature is ranging from 40-60°C with an acceptable temperature at 41°C for highest phytase activities[4]. These conditions are close to the ideal physical condition in the GI tract of the animals, thus dietary supplementation of phytase would result in a high rate for hydrolyzing phytic acid from the animal feeds[4].

Monogastric animals, such as poultry and swine are unable to utilize phytic acid (phytate) due to minimum phytase activity in the brush broader membrane of their digestive tracts and since phytate-P cannot be absorbed[5,8,9]. However, the majority of poultry and swine feeds are of plant origin, in which around 50-80% of total P are presented as phytate-P. Therefore, phytase has been supplemented in animal diets to liberate phytate bond P molecule and prevent the formation of insoluble Ca-phytate complexes[35,36,9]. Supplementation with phytase has been proven to be an effective method to increase the P availability in seed-based animal feed and also to improve P digestibility in the animals[3,5,9]. It further reduces the excessive P level from animal waste that may lead to environmental pollution[10,37].

Effects of phytase on phosphrous utilization and bone mineralization in broiler chickens: Similar to other monogastric animals, positive effects such as improvement in hydrolyzing phytate-P, increased P digestibility, improved bone mineralization and reduced P excretion have been observed in poultry with phytase supplementation[5,13]. In the same time, phytase supplementation reduced the addition of inorganic phosphate in poultry rations and thus lowered the production costs[3,11,12,37].

It is generally recognized that in poultry a 0.1% reduction of the available P content can be achieved with phytase supplementation, although as recently reviewed by Slominski[35] approximately 0.05% of phytate-P would only originate from poultry diets following phytase supplementation. Moreover, promising results have been observed on the growth performance of broiler chickens by supplementing phytase. For instance, Simons *et al.*[38] reported that phytase increased bird performance and improved bone mineralization, while El-Sherbiny *et al.*[39] examined broiler diets containing a reduced level of di-calcium phosphate and concluded that the addition of 500 U kg^{-1} phytase enhanced BWG, Feed Intake (FI) and FCR of the birds from 23-40 day of age. Phytase supplementation in P standard broiler diets have been shown to generate equivalent growth performance, whereas significantly increased weight gain (7.6%) and feed efficiency (4.7%) have been observed in birds that fed reduced P and calcium (Ca) diet[5].

Phytase addition has been shown to have positive effects on bone ash content and bone mineralization in broiler chickens fed low available P diet[11,40,41]. El-Sherbiny *et al.*[39] reported that phytase increased dietary Ca and P utilization, reduced Ca and P excretion and improved tibia breaking strength and tibia ash percentage in broiler chickens. Previous studies indicated that the tibia ash percentage and bone breaking strength of birds fed low Ca and available P diet were improved by phytase supplementation, however, the values were not equivalent to that of the control diet[39,42,41]. Angel *et al.*[40] reported that whole body and tibia Bone Mineral Density (BMD) and Bone Mineral Content (BMC) of birds were higher in diets with 0.26% available P and 600 U kg^{-1} of phytase, although lower than those fed the control diet. Chung *et al.*[43] found similar results showing that phytase supplementation improved bird femur and tibia BMD and BMC when compared with birds fed the low-P control diet (available P reduced by 0.1%).

COMBINATION OF FOS AND PHYTASE

As previously described, prebiotic fructooligosaccharides are bioactive substances which can influence on nutrition, immune response, overall health and gut microbiota of the broiler chickens by stimulating microbial fermentation and producing short-chain fatty acid, which consequently acidify the gastrointestinal tract[15,44]. Several studies conducted on mice have demonstrated that FOS counteracted the deleterious effects of phytic acid by improving cecal absorption of minerals and stimulating the hydrolysis of phytate via fermentation by probiotic-like bacteria[45,44]. Phytase enzyme is commonly applied in the poultry industry to hydrolyze phytate-P and improve P utilization. Results have indicated that an acidic gut pH is favourable for mineral solubility as well as for phytase activity[4,9,46]. Shang *et al.*[13] demonstrated that a combination of 0.5% FOS and phytase increased P utilization, in specific, increased apparent P digestibility and P retention in broilers. Therefore, the combination of FOS and phytase could have additive effects on improving growth performance, bone quality and P utilization of broiler chickens. To date, very few studies have evaluated the synergistic effects of different types of prebiotics and enzymes in poultry diet. This review could serve as a reference and thus to encourage more studies to be done in the very near future to advance our knowledge on the roles of prebiotics and enzymes supplementations in poultry.

CONCLUSIONS

Prebiotics and enzymes, including but not limited to FOS and phytases are 2 functional dietary supplementations that can efficiently improve poultry performance and health. Previous studies showed that broiler chicken are able to acquire improved nutrient utilization, better intestinal development, stronger immune system and healthier gut microbiome after receiving FOS or phytase supplementation. Although, supplementation of prebiotics or enzymes alone is proven to be effective and is getting more attention in the poultry production, it is important to realize that supplementing the combination of both prebiotics and enzymes could also be a potential strategy to improve growth performance, nutrient utilization and gut health in poultry.

SIGNIFICANCE STATEMENT

This study reviews dietary supplementation of prebiotic fructooligosaccharides and phytase enzyme on the improvement of growth performance, nutrient utilization, intestinal development, immune system and gut health in broiler chickens. In the meantime, it proposed a potential strategy by supplementing both prebiotics and enzymes in the diet to further improve poultry health and performance.

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