Significance of enzymes as gut health solution

Preeti Mohanty
CJ BIO India

Abstract

Gut health is one of the foremost subjects in the existing poultry production. The rapid rise in the global human population has increased the demand for animal protein for human nutrition, which consequently led to the intensive production of broiler chickens to meet the demand for food, causing unintended gut health problems and performance impairment in broiler chickens. The use of exogenous enzymes to improve performance is now a well settled concept in modern poultry nutrition and this theory is still establishing new facts with the ongoing studies. Feed enzymes offer vital functions to improve the efficiency of feed utilization and, consequently, growth performance. The flexibility of using alternative feed ingredients has increased by the targeted use of a specific enzyme which allows animals to extract more nutrients from the feed. Furthermore, poultry lack the specific enzymes able to break down certain components e.g. cellulose, arabinoxylan and phytate. Poorly digested feeds lead to digestive problems ultimately affecting growth performance and claiming higher cost for producers, and also increase the negative impacts on the environment. With exogenous enzyme supplementation, birds can utilize nutrients thus contribute to the gut health and diminish environmental impact.

Introduction

Gut is considered as the natural habitat for a large and dynamic community of microbes that regulate not only intestinal, but also systemic functions of the host (Oakley et al., 2016). It also acts as the major barrier that separates the largest interface between the external environment and the internal milieu while still allowing molecules to be absorbed or secreted (Mwangi et al., 2010). Thus, the gut and its integral components are functionally dependent for the proper physiological development of the host.

Gut health can be defined as the ability of the gut to perform normal physiological functions and to maintain homeostasis and thus supporting its ability to withstand infections and non-infectious stressors (Kogut et al., 2017). This definition explains the underlying components of gut health i.e. effective digestion and absorption of food, a stable gut microbial population, structure and function of the gut barrier, and effective function of the immune system, all of which play a critical role in gut physiology, the productivity of the animal and its well-being. Over the past two decades, this topic has gained even more interest in poultry production due to increasing demands for economic efficiency, animal welfare, food safety, reduction in environmental impacts, and a ban on or avoidance of antibiotic growth promoters (AGPs) use (Morgan, 2017). The exogenous enzymes are capable of reducing the variability in feed ingredients and enhance the feed digestibility availing more nutrients for absorption and thus reduce digesta viscosity. Recently the enzyme culture has gained immense value in poultry industry and this has led to development of more potential enzyme combinations to target specific substrates in feed and also complement to endogenous enzymes. In this article, the role of exogenous enzymes emphasizing particularly xylanase, protease and phytase on gut health in poultry is mainly depicted.

Factors responsible for gut health impairment

The common aspects affecting broiler gut health are stress, exogenous infection, diet and water etc. Recently, with the advancement of exogenous enzyme study, more studies have been conducted on the impairment factors of the intestinal health of broilers focusing on phytic acid and non-starch polysaccharides (NSPs).

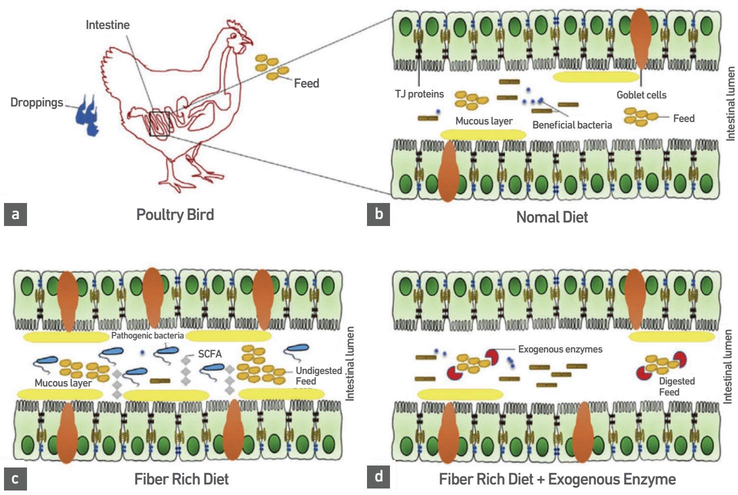
Phytic acid is a natural antioxidant found in the form of salts and is present in cereals, vegetables, nuts, and natural oils (Silva and Bracarense, 2016). Phytic acid forms insoluble salts with minerals, including phosphorus, calcium, zinc, magnesium, and copper. Phytic acid increases the mucin (MUC) excretion and endogenous nutrient losses, which are hazardous to intestinal health (Onyango et al., 2009). Phytic acids also forms complexes with proteins and other nutrients (Yu et al., 2012). According to Selle et al. (2012), the possible mechanism of action is that, at an acidic pH, a binary protein-phytate complex is formed where phytate can bind to the α-NH2 groups and side chains of the basic amino acids arginine (Arg), histidine (His) and lysine (Lys).

Non-starch polysaccharides, together with resistant starch and lignin called the dietary fiber, are found in plants especially in the endospermic cell wall of multiple kinds of seeds (Lovegrove et al., 2017). NSPs can be divided into soluble and insoluble fractions. Soluble NSPs when fed in bulk amount increase the viscosity of intestinal contents by making viscous gels which decrease the rate of diffusion of endogenous digestive enzymes and substrates with hampered interaction at the mucosal surface (Raza et al., 2019). This increased viscosity also induces thickening of the mucous layer in the intestine (Hedemann et al., 2009) hampering the digestion and absorption of nutrients in the intestinal tract. It has been estimated that 400-450 kcal of digestible energy per kg of feed remains undigested due to the NSP contents present in corn-soybean meal diets (Cowieson, 2010). On the other hand, insoluble NSP present in the cell wall entrap starch, protein and other nutrients inside called "cage effect" and hinder the access of endogenous enzymes to digestible nutrients (Bedford and Partridge, 2010).

Exogenous enzymes as gut health solutions

Carbohydrases

The major barriers of the intestinal tract are mucus layer and tight junctions (TJ) of the epithelium as illustrated in Figure. 1b. Intestinal morphology (villus height, crypt depth and epithelial turnover rate) changes in response to exogenous agents, for example, presence or absence of food and pathological conditions (Gomide Junior et al., 2004). Deeper crypts indicate faster tissue turnover as they contain stem cells and considered villus factories (Awad et al., 2009). Intestinal mucins/mucous are high molecular weight glycoproteins secreted by goblet cells. In chickens, mucin-2 is observed to be extensively expressed in goblet cells of colon and small intestine (Smirnov et al., 2005). NSP have been shown to increase mucin secretion (Tanabe et al., 2006) as illustrated in Figure. 1c. Therefore, NSP lessen the digestion and absorption of nutrients through its physicochemical effect in the intestinal tract. As a result of high fiber diets, undigested/unabsorbed nutrients change in microbial populations in the gut (Bird et al., 2007; Choct et al., 1999; Mathlouthi et al., 2002). Langhout (2000) observed that dietary NSP considerably decrease beneficial bacteria while increases intestinal populations of pathogenic bacteria. Exogenous enzymes improve digestion in the small intestine and reduce the amount of substrate availability for putrefactive and starch utilizing bacteria in the large intestine. Also enzymes help in the disease prevention by to reducing digesta viscosity (Pluske et al., 1997) as illustrated in Figure. 1d. Xylanase and glucanase supplementation in barley, wheat, oats, and rye based diets significantly raised caecal butyrate and acetate concentrations, but such effect was absent in hull-less varieties of barley and oats (Jozefiak et al., 2006). Degradation and solubilisation of NSP by feed enzyme increases available substrates (oligosaccharides or mono-saccharides) for microbial fermentation in the cecum (Cadogan & Choct, 2015), and results in decreased VFA/SCFA production in the ileum suggesting decreased fermentation whereas caecal fermentation markedly increased. The increment in caecal fermentation resulted an influx of xylo-oligosaccharides (XOS) which produces VFA/SCFA and energy from indigestible substrates and often leads to a healthier microflora (lactic acid bacteria, LAB) (Jia et al., 2009). Therefore, the NSP fraction supplemented with EFE represents another potential energy reservoir to increase the performance of broilers if rendered fermentable.



Fibers, EFE and intestinal health. (a) poultry bird, (b) intestinal lumen presenting normal goblet cells, TJ proteins, mucous layer, feed, beneficial cells and enterocytes, (c) intestinal lumen presenting highly viscous environment with increased mucous, undigested feed, competition of host and microbiota for SCFA in small intestine, (d) intestinal lumen presenting carbohydrases, normal mucous, beneficial bacteria and digested feed. (Adapted from Raza et al., 2019)

Figure 1. **Effect of exogenous enzyme supplementation on gut health in poultry (Raza et al., 2019)**

Xylanase is a non-starch polysaccharide (NSP) degrading enzyme which cleaves the internal β-xylosidic glycosidic linakges of linear xylan chains to xylo-oligosaccharides (Jompengmuengbout et al., 2009), resulting in a mixture of arabinose-substituted xylo-oligosaccharides (arabinoxylan-oligosaccharides, AXOS) and non-substituted xylo-oligosaccharides. As an energy source, probiotics (beneficial bacteria like Lactococcus, Lactobacillus and bifidobacterium) have significantly higher XOS utilization efficiency than pathogenic bacteria, especially bifidobacterium which is comparable to glucose in XOS utilization efficiency. Secondly, SCFAs are mainly produced by beneficial microorganism and, thirdly, SCFAs can improve pH values in gut and contribute to a suitable environment for beneficial microbes which prefer acidic environment, also serves as an energy source for intestinal epithelial cells. So, the XOS can be utilised more efficiently and it also potentiates the activity of endogenous digestive enzyme and reduces the availability of indigestible substrates for microbial growth and as a result digesta viscosity is decreased leading to reduced microbial populations in the upper tract and there is reduced loss of endogenous amino acids through modifications to pancreatic amylase and mucin secretion (Cowieson and Bedford, 2009). The prebiotic effects of XOS also include optimisation of colon function, alter the amount and ratio of SCFAs and thus providing more energy, augmenting mineral absorption, immune stimulation and increased ileal villus length (Kiarie et al. 2014). Also, researches have shown that xylanase supplementation can improve chicken immunity (Gao et al., 2007), reduce the detrimental effect of Salmonella typhimurium infection (Vandeplas et al., 2009; Amerah et al., 2012), or alleviate the intestinal mucosal barrier impairment of broiler chickens challenged by Clostridium perfringens (Liu et al., 2012).

Soybean meal (SBM) is a primary source of vegetable protein that contains 3% soluble NSP and 16% insoluble NSP (Irish and Balnave, 1993), consisting mainly of mannans and galactomannans (Slominski, 2011). Beta-mannan (β-mannan), also referred to as beta-galactomannan (βGAL), is a polysaccharide that has repeating units of mannose containing galactose and/or glucose (Hsiao et al., 2006). Although βGAL content of SBM is in relatively low concentrations, it is a concern for nutritionists due to the presence of anti-nutritive properties (Arsenault et al., 2017). ß-mannan has a molecular structure similar to some pathogens, which may trigger immune stimulation. Acemannan (ß-1,4-acetylated mannan) induced the activation of macrophages via increasing the nitric oxide synthase level at transcription level as reported by Ramamoorthy et al. (1996). Karaca et al. (1995) reported that nitric oxide acts as a cytostatic effector in the removal of viral replication and is proposed to be toxic for tumor cells (Karupiah et al., 1993). The response of this complex to ß-mannan containing compounds could lead to losses in dietary energy utilization. Supplementation of ß-mannanase improved the utilization of dietary energy in corn-soya diet in broiler chickens (Li et al., 2010) as well as layers (Wu et al., 2005; Saeed et al., 2019).

Phytase

Phytic acid interferes with normal digestive process resulting into significant quantities of starch and protein enter the large intestine, stimulating putrefactive bacteria and reducing gut health. Due to the current scenario of volatile feed prices more unconventional feed sources are introduced in poultry feed thus diets have become more complex and variable. Thus, the amount and availability of undigested substrates, has increased, which has a direct impact on microbiota composition and populations of non-beneficial bacteria in the intestine. The use of exogenous microbial phytase to release phytate-P for absorption is almost ubiquitous in poultry feed, thus reducing the environmental impact of poultry production and requirements for costly dietary inorganic phosphorus supplementation. However, there is an additional bonus of phytase application in that it potentially has a direct impact on microbiota and hence improves gut health (Morgan et al., 2017). Phytase increases protein digestibility and reduces endogenous losses, which limits protein supply to the hind gut. Dahiya et al. (2007) and Drew et al. (2004) observed that undigested protein substrates act as predisposing factors for dysbacteriosis, particularly necrotic enteritis (NE), suggesting that phytase could possibly alleviate the prevalence and severity of NE. According to Lumpkins et al. (2009), phytase reduced intestinal mucin mRNA abundance in broiler chickens; as Clostridium thrives on mucin, reduced mucin concentrations could correspond with a reduction in C. perfringens. Smulikowska et al. (2010) stated that phytase increased caecal acetate, which is an indicator of microbial activity modulation, and Ptak et al. (2015) observed that phytase supplementation increased total ileal bacterial counts, Lactobacillus spp., Enterococcus spp. and total SCFA, DL-lactate and acetic acid concentrations.

Proteases

Protease is an important factor in protein digestion as it hydrolyses the less digestible proteins in animal feeds and break them down into more usable peptides. Improving dietary protein digestibility with a specific protease inclusion can reduce feed cost by allowing the use of lower crude protein feedstuffs with lesser quality amino acids, effectively lowering protein and digestible amino acids levels required from the feedstuffs up to 10%. Recent researches in poultry and swine have shown that supplementing diets with a protease enzyme support gut health and optimise animal performance compared to non-protease supplemented diets. Understanding the anti-nutritional effect of indigestible proteins in the hindgut of the animal helps to explain this effect. Unconventional protein sources, such as Rapeseed meal (RSM), Cottonseed Meal (CSM) and Corn Distillers Dried Grains with Solubles (DDGS) due to the nutrient variability can lower quality protein levels and reduced digestibility. Supplementing exogenous protease enables poultry that lack adequate levels of endogenous enzymes to digest proteins in the diet, which reduces the flow of undigested protein and other anti-nutritionals entering the large intestine. Glycinin and β-conglycinin are two main anti-nutritional factors. These allergens can cause intestinal allergies in piglets, causing irreparable damages. Protease, especially alkaline protease, has shown excellent effects on hydrolysing soybean allergens (Yin et al., 2021). This indigestible protein serves as a fermentation substrate for undesirable bacterial strains, such as Escherichia coli, Clostridium perfringens(C. perfringens), Salmonella and Campylobacter in the gut. The proteins will be used by these harmful bacteria as nutrients. These pathogenic bacteria can then increase in population, shifting the balance of intestinal microflora against beneficial strains. Pathogenic bacteria can also produce toxic components such as bacteriotoxins, but also fermentation metabolites like biogenic amines, ammonia and volatile sulphur compounds. All of which can be detrimental to performance by favouring oxidative stress, intestinal inflammation and lesions increasing both mortality and morbidity rates. In case of high protein diet, in hindgut proteins are broken down into ammonia and amines, thus increasing the pH and favouring bacterial growth, including C. perfringens. Protein sources such as fishmeal, meat and bone meal, and soybean meal may increase the chances of necrotic enteritis in poultry. These protein sources contain some amounts of indigestible protein and amino acids that accumulate in the ceca and serve as a substrate for C. perfringens to flourish. Therefore, protease can be an effective solution when looking at necrotic enteritis prevention measures.

Conclusion

Anti-nutritional factors impair the smooth digestion such that significant quantities of undigested starch and/or protein enter the large intestine, stimulating the activity of putrefactive bacteria and pre-disposing the animal to intestinal disorders. As already evident from the studies, the supplementation of exogenous enzymes will improve small intestine digestion and as a result limit substrate availability in the hind gut, therefore mitigating any potential GIT microbial dysfunction. There is still scope to explore the effect of supplementation of enzyme combinations targeting feed composition on gut health and ultimately impacting growth and efficiency.

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