SPECIALTY

Effects of proteases on broilers fed diets with low crude protein levels

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Abstract

Feeding low crude protein (CP) diets to broilers provide several opportunities for the current challenges in poultry production. It allows flexibility for animal nutritionists to optimize the usage of other available raw materials that will reduce feed costs and environmental pollution without negatively affecting the growth performance of broilers. However, reducing CP content and NEAA composition of the diets with the use of other raw materials could negatively impact growth performance and intestinal morphology in poultry. Exogenous addition of proteases may compensate for the possible negative effects of feeding very low CP levels on the intestinal architecture of broilers resulting in an enhanced CP, amino acids (AA), energy, and fat digestibility which are translated to an increased meat deposition and/or similar growth performance with diets containing standard CP levels.

Introduction

With the increasing prices of feed ingredients, especially on protein sources, lowering the dietary crude protein (CP) level is one of the many approaches being considered to reduce the inclusion rate of expensive raw materials to poultry feed formulations. Supplementation of crystalline amino acids (AAs) is recommended to fulfill the requirements for essential amino acids (EAA). Additionally, lowering dietary CP levels reduces feed cost and environmental pollution and allows the inclusion of alternative raw materials, but requires understanding to maintain the optimum performance of the animals and digestive functions. In some cases feeding low CP diets in broilers does not yield a positive response. In this regard, Sumanasekara et al. (2020) suggest the addition of protease on diets with a 5% reduction in CP to improve protein digestibility without adversely affecting the growth performance of broilers with lower feed costs and maximum economic benefits.

In broilers, dietary CP level reduction may range from 1 to 2%, or up to 4% in some cases (Wang et al., 2020), based on the breeder nutrient specification guideline for crude protein. In order to meet the individual AA requirements of the animals, reducing CP levels will increase the dietary crystalline AAs inclusion. With the hope to also increase the AA availability from intact ingredients on the feed formulations, such as in corn and soybean meal, the addition of dietary proteases becomes an interesting approach to most animal nutritionists. Its incorporation into diets with low CP levels permits the nutritionists to formulate feeds without negatively affecting the animals' growth rate and overall farm productivity. Though proteases produced endogenously are generally considered sufficient to optimize feed utilization, a significant volume of protein, by around 18 to 20%, still passes through the GIT without absolutely being digested (Angel et al., 2011; Freitas et al., 2011; Jabber et al., 2021; Kamel et al., 2015; Mohammadigheisar & Kim, 2018; Ndazigaruye et al., 2019; Sumanasekara et al., 2020; Vieira et al., 2016).

These undigested proteins, especially from intact protein sources, represent an opportunity for the use of supplemental dietary proteases to improve nutrient digestibility in poultry. These enzymes are necessary to digest vegetable protein sources, especially in the early stages of poultry as they produce limited amounts of endogenous enzymes during this period (Jiang et al., 2020; Sumanasekara et al., 2020). The use of supplemental proteases may also potentially lower the production cost, total excreta nitrogen (N), and N excretion in the environment (Amer et al., 2021; Kamel et al., 2015; Mohammadigheisar & Kim, 2018; Sumanasekara et al., 2020).

According to Angel et al. (2011), Freitas et al. (2011), Shahir et al. (2016), and Vieira et al. (2016), there are several factors affecting the protein digestibility of vegetable feedstuffs, such as genetic variability within an ingredient and heat treatments or processing which contributes to the most effects. Genetic variability within the ingredient includes the magnitude of antinutritional factors (ANFs), protease inhibitors, non-starch polysaccharides, phytate, and lectins present in poultry diets (Jiang et al., 2020; Ndazigaruye et al., 2019; Riaz et al., 2020; Sumanasekara et al., 2020; Wang et al., 2020). With the supplementation of proteases, it supports the endogenous peptidases by enhancing protein digestibility and hydrolyzing proteinaceous ANFs such as antigenic proteins and trypsin inhibitors (Cardinal et al., 2019; Ding et al., 2016; Law et al., 2018). Moreover, the dietary addition of proteases has positive effects on AA digestibility attributed to its ability to target protease inhibitors and the cereal component of the feed formulation (Mohammadigheisar & Kim, 2018).

Opportunities of proteases in low CP diets

Several studies are showing that feeding low crude protein levels to poultry with the supplementation of crystalline AAs maintained the growth performance of the animals with reduced N excretion in the environment (Wang et al., 2020). However, as more dietary CP levels were reduced, the growth performance of poultry decreased (Law et al., 2018; Wang et al., 2020). Application of extremely low CP levels on poultry diets, by more than 2.5 to 3%, showed negative impacts on growth performance, carcass traits, intestinal morphology of the villi, and profitability (Amer et al., 2021; Jabber et al., 2021; Law et al., 2018; Law et al., 2019). Lowering the dietary CP levels provides an opportunity to use alternative feed ingredients which contains antinutritional factors that can contribute to the low digestibility of feedstuffs and impaired intestinal health of the animals.

According to Wang et al. (2020), 10 to 43% of excess or undigested proteins consumed by broilers from the diet can be fermented by the resident micro-organisms in the ileum and cecum to produce a huge diversity of end products, including short-chain fatty acids (SCFA), branch-chain fatty acids (BCFAs), amines, phenols, indoles, thiols, CO_2, H_2, and H_2S, many of which have toxic characteristics. Hence, reducing the dietary CP may improve the overall intestinal health of poultry. Formation of BCFAs, such as isobutyrate, 2-methyl butyrate, and isovalerate in the cecum, can synthesize from the degradation of Val, Ile, and Leu in the GIT of the animals (Ndazigaruye et al., 2019; Wang et al., 2020). This is possible when cecal bacteria proteolytically ferment the undigested proteins (Amer et al., 2021; Cardinal et al., 2019; Ndazigaruye et al., 2019). Supplementation of exogenous protease may improve the overall utilization of dietary CP and amino acid levels contribute to the shifting of substrates available in the intestine for digestion in the upper section of the small intestine and reduce the amount of undigested protein that pass through the large intestine for bacterial fermentation the intestine for bacterial growth (Amer et al., 2021; Wang et al., 2020).

The development and morphologic parameters, including villus height (VH), crypt depth (CD), and/or VH:CD measured at the intestinal level, are widely used as a standard to evaluate the intestinal health of poultry (Wang et al., 2022). The maturation of the small intestine is important to optimize broiler growth because the digestion and absorption rates of nutrients are directly affected by cell proliferation and differentiation (Kamel et al., 2015). Low protein diets and the presence of undigested protein may damage ileal morphology and decrease serum-free EAA, especially the free branched-chain AAs (BCAA) concentration (Amer et al., 2021; Wang et al, 2020). The degree of damage or negative impact on intestinal development and function depends on the level of CP reduction and the quality of protein in the diet (Kamel et al., 2015; Law et al., 2018; Wang et al., 2020). Ding et al. (2016), Law et al. (2018), and Wang et al. (2020) reported that the VH, CD, and VH:CD of the duodenum, jejunum, and ileum were reduced with decreased levels of dietary CP. VH is closely associated with nutrient absorption and normally this is reduced in chicks fed with unbalanced diets (Kamel et al., 2015; Ndazigaruye et al., 2019). With dietary protease addition, the amount of undigested protein and ANFs, which are potentially affecting the disruption of nutrient assimilation will be reduced as well as the possible negative impacts on intestinal morphology.

The negative impact of feeding diets with very low CP levels may also be associated with the reduction in the levels of non-essential AA (NEAA) such as glycine (Gly), glutamic acid (Glu) and proline (Pro) which represents a major proportion of gut epithelium, intestinal architecture and digestive secretions and mucins (Amer et al., 2021; Jiang et al., 2020; Law et al., 2018; Wang et al., 2020). Similarly, the serum concentration of free AAs like arginine (Arg), isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), valine (Val) and Pro decreased as the dietary CP levels were reduced (Wang et al., 2020). Arg is an EAA for poultry and when metabolized by the body produces important molecules such as nitric oxide (NO), polyamines, and creatine (Wang et al., 2020). Protease tends to release intact nutrients from feed ingredients to optimize its usage for growth and other metabolic functions. According to Wang et. al. (2020), protease supplementation tends to increase the levels of NEAAs in the diets to slow down the catabolism of EAAs to provide the needed substrates for their synthesis when low CP diets are fed to animals.

The main reason for the decline in NEAA supply when reducing dietary CP levels is because the animals' body sources their NEAA requirement from the CP component of the diet. Glu is the most catabolized AA to provide energy in the gut mucosa (Wang et al., 2020). Increased Glu catabolism indicates poor intestinal development and function. Most AAs undergo catabolism in the intestinal mucosa and there is an increased activity of the glutamic-oxaloacetic transaminase activity in the ileum, which is an indication of Glu catabolism (Wang et al., 2020). The increase in the catabolism of intestinal mucosal AAs is how the intestine maintains its architecture and function when animals are fed with low CP diets (Law et al., 2018; Wang et al., 2020). Feeding diets with protease supplementation may improve the digestibility of feedstuffs and speed up the availability of essential nutrients required for several metabolic activities to function at their best state, which is beneficial for animals' growth and reproduction.

Moreover, the system and local ileal inflammatory response of poultry when fed with low CP diets may be elevated, ileal barrier function may change and the cecal total SCFAs concentration is reduced (Wang et al., 2020). CP content of the diet and its digestibility affect the development and volume of microbial metabolites, such as ammonia N, and volatile fatty acids, resulting from the hindgut fermentation (Wang et al., 2020). Kamel et al. (2015) explained that lowering the CP content of the diet is also associated with a decline in fecal microflora and the number of total aerobic mesophilic bacteria and *E. coli* in broilers' excreta. Protease may potentially help alleviate and reduce microbial gut infection by influencing the mucous layers of the intestine.

SCFA forms the gut microflora and helps maintain the intestinal barrier function, especially, butyrate, which boosts the intestinal cells and enhances intestinal integrity by easing inflammation, improving tight junctions, and accumulating mucus (Cardinal et al., 2019; Wang et al., 2020). Tight junctions serve as an important barrier in the epithelial defense that protects the birds from translocating pathogens and allergens and keeps their productivity (Wang et al., 2020). Deterioration of tight junctions increases intestinal permeability resulting in high serum endotoxin levels. The endotoxins will enter the bloodstream and activate toll-like receptor 4 which is located at the surface of immune cells, leading to the release of proinflammatory cytokines, such as IL-6 and TNF-α. IL-6 performs a significant role in host defense due to its wide range of immune and hematopoietic activities (Simpson et al., 1997). Establishing an effective gut microflora is possible by enhancing the digestibility of nutrients from different feedstuffs for absorption and utilization of the animals, needed for innate defense processes in the body.

Addition of supplemental proteases to low CP diets

Proteases have several functions including the regulation, localization, and activity of many proteins, modulation of protein-protein interactions, production of new bioactive molecules, contributing to the processing of cellular information, and generating, transducing, and amplifying molecular signals (Mohammadigheisar & Kim, 2018). Protease supplementation on diets with 10% lower CP levels considerably compensated for the performance losses of broilers resulting in BWG and FCR that were not significantly different from birds fed with standard CP levels, as stated in the study of Angel et al. (2011) and Cardinal et al. (2019). Similarly, Jabber et al. (2021), Kamel et al. (2015) and Vieira et al. (2016) mentioned a 1% improvement in CP digestibility when protease was added on a low CP diet, with 7% CP reduction, fed to animals.

Like any other enzymes, proteases must build an efficient enzyme-substrate complex. The improvement that was observed with the addition of supplemental proteases is dependent on the protein ingredients used in the feed formulations, because AA composition is ingredient-dependent, similar to endogenous proteases where peptide bond specificity directly influences the degree of protein hydrolysis and the number of peptides and AA that will be released and made available for utilization (Angel et al., 2011; Freitas et al., 2011; Law et al., 2018; Vieira et al., 2016). For example, trypsin, a serine protease produced by the pancreas will preferentially cleave AA bonds that have Lys and Arg (Freitas et al., 2011; Vieira et al., 2016). Therefore, the effects of CP and AA digestibility on growth may be related to physical and chemical factors of each individual ingredients including granule size and ANFs (Cardinal et al., 2019).

Protease supplementation in diets with low CP content increases the serum-free Glu to mitigate the catabolism of EAA, and lower the concentration of serum-free Arg, plasma ET and IL-6, and cecal isovalerate (Wang et al., 2020). As a result, a large proportion of AAs is absorbed along the small intestine, particularly increasing the apparent digestibility of Arg, Ile, Lys, Thr, His, Asp, Cys, Met, Val, and Ser, and making it available for protein deposition because of the vast utilization in the gut mucosa via anabolic and catabolic pathways (Angel et al., 2011; Wang et al., 2020). A reduction in serum-free Arg with protease supplementation on diets with low CP increases the Arg metabolism in the GIT to maintain intestinal health (Wang et al., 2020). In contrast, Law et al. (2018) noted a linear reduction in uric acid with declining CP levels in the diet with no protease supplementation with the assumption that protease supplementation may have released Gly in the digestive system and compensated for the shortage of Gly in low CP diets. According to Ndazigaruye et al. (2019), uric acid concentrations can be used as an indicator of AA utilization in broilers. The performance of broilers fed with low CP diets and protease supplementation generally improves BWG and FE (Ajayi, 2015; Amer et al., 2021; Mahmood et al., 2017; Riaz et al., 2020).

Improved overall growth performance of animals with dietary protease addition is expected to happen through enhanced AA, N, energy, starch and fat digestibility, and protein utilization resulting in an improvement in the carcass and breast meat yield of broilers associated with increased utilization and deposition of protein, especially with graded levels of protease (Ajayi, 2015; Amer et al., 2021; Angel et al., 2011; Freitas et al., 2011; Jabber et al., 2021; Kamel et al., 2015; Law et al., 2018; Mohammadigheisar & Kim, 2018; Rada et al., 2013; Rada et al., 2014; Sarica et al., 2020; Shahir et al., 2016; Wang et al., 2020). Riaz et al. (2020) speculated that the increase in energy came from the improved proteolysis and starch digestibility of vegetable ingredients after disrupting the starch-protein complexes with protease addition.

The improvement in energy and N digestibility and utilization of broilers fed with supplemental proteases may also be attributed to the improvement in VH and VH:CD of the duodenum, since the absorptive epithelium of the villi serves a critical role in the final stage of nutrient assimilation and improved intestinal morphology (Amer et al., 2021; Ding et al., 2016; Kamel et al., 2015; Law et al., 2018). Law et al. (2018) reported a significant increase in jejunal and ileal VH with protease supplementation, irrespective of dietary CP level. Protease supplementation can mitigate the intestinal mucosal injury brought by reducing the CP content of the diet significantly to improve intestinal integrity (Cardinal et al., 2019; Law et al., 2018; Wang et al., 2020). This also agrees with the findings of Kamel et al. (2015) and Rada et al. (2013) that protease addition increases the growth efficiency in the gut and reduces systemic inflammation with an improved crypt-villus ratio. Moreover, proteases influence the mucus layer thickness in the GIT which relieves the effect of a coccidial infection resulting in better growth performance (Angel et al., 2011). These mucins are involved in the diffusion and absorption of nutrients along the GIT and protect the gut from the negative impacts of ANFs (Cardinal et al., 2019).

Feed efficiency (FE) improvement was also observed in the trial of Freitas et al. (2011), Kamel et al. (2015), Jiang et al. (2020), Mohammadigheisar & Kim (2018), and Sumanasekara et al. (2020) with the supplementation of protease even with no improvement on BWG or FI indicating a possible increase in the AA availability for protein accretion. On the contrary, Law et al. (2018), Magsood et al. (2022), Mohammadigheisar & Kim (2018), Rada et al. (2014) and Sarica et al. (2020) reported an improvement in average daily weight gain, final BW and FCR when the low CP diets fed to broilers were supplemented with protease. The improvements are associated with improvement in the intestinal health of the ileum after the addition of protease on diets with low CP levels (Cardinal et al., 2019).

Conclusions

Supplemental proteases may offset the negative effects caused by dietary CP reduction on growth performance and intestinal morphology of broilers. Additionally, proteases also helps to improve the overall digestibility of feedstuffs by breaking the undigested proteins from feed ingredients and releasinge its intact nutrients for the utilization of the animals. This will also eventually lead to an elevated N levels in the body for the synthesis of NEAA in low CP diets fed to animals that are essential for protein synthesis and other biological functions.

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