THREONINE SUPPLEMENTATION ON GUT HISTOMORPHOMETRY AND MUCIN BIOLOGY OF BROILERS

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Abstract

Threonine is one of the important limiting amino acids (after methionine and lysine) in broiler diets. Adequate threonine level is mandatory to obtain optimal growth of broilers. Threonine takes part in some important metabolic processes (uric acid formation and protein synthesis). It represents 30% of the total amino acid content of mucin. Threonine plays an immense role in gastrointestinal health and thus threonine optimization may protect the intestine from microbial infections. It is presumed that threonine level of healthy diet is not sufficient when the animal experiences a gut challenge either by microbial invasion and/or added with environmental extremes. Till date no guideline has been framed for optimizing the requirement of threonine considering the role of mucin in gut health of poultry. Hence, optimizing the supplemental threonine level in commercial broiler chicken for better performance and gut fitness is utmost necessary.

Key words: Crypt depth, Goblet cell, Mucin, Threonine, Villus height

Introduction

Threonine is essential in providing precise nutrition to poultry birds as they are not competent enough of synthesizing this amino acid *de novo*, thus it is nutritionally essential. L-threonine (98.5%) is normally mixed to broiler diet to make amino acid balance in the nutrition status of the birds. Threonine helps in synthesis of uric acid, pancreatic enzyme, protein, collagen, elastin and antibody production (Sá *et al.*, 2007). Adequate threonine level is mandatory to obtain optimal growth of birds (Kerr *et al.*, 1999). Many researchers put forward their findings on the threonine requirements of poultry for growth performance (Rosa *et al.*, 2001; Ahmadi and Golian, 2010), but meagre information is reported on the intestinal histomorphometry of broilers. Threonine plays an immense role to offer protection from bacterial

infections in gastrointestinal tract. Hence, level of supplemental threonine requirement in the broiler diets must be optimised. The goblet cells of the intestine secrete mucins. This mucin is mainly glycoproteins in nature, principal component of which is threonine constituting around 30% of total amino acids. Mucins are synthesized and secreted by goblet cells distributed along the villi of the small intestine. Threonine is the main essential amino acid which is utilized by the goblet cells of the intestine thereby helping in the synthesis of mucins (Uni *et al.*, 2003). The small intestine is protected by the mucin which mainly protect acidic chyme, various digestive enzymes and pathogens, filtering nutrient in the gastrointestinal tract, nutrient digestion and absorption (Horn *et al.*, 2009; Jiang *et al.*, 2013).

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Optimisation of threonine requirement in intestinal histomorphometry

Threonine supplementation helps in formation of tall villus that enhances the absorptive efficiency due to the increased absorptive surface area of mature epithelia (Hampson, 1986). An improved digestibility coefficient is observed due to higher villi heights which increase the activity of the digestive enzymes secreted from their tips. Increases in dietary threonine levels provide adequate threonine which has special importance as an essential amino acid, because, compared with other amino acids, it has the highest metabolism in the portal-drained viscera (Schaart *et al.*, 2005). Villus height in broilers fed on threonine-deficient diets decreased in comparison with groups receiving adequate threonine, however, threonine supplementation (at 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1% standard ileal digestible threonine with two level of protein 16 and 19%) increased ($p < 0.01$) villus height, and the crypt depth and reduced epithelial thickness and goblet cell number (Zaefarian *et al.*, 2008). Villi height, crypt depth and villi surface increased as dietary threonine increased from 0.80% to 0.87% and decreased in 0.94% and 1.01% threonine (Heshmat *et al.*, 2011). There was a tendency for an increase in goblet cell density as dietary threonine increased from 0.80 to 0.87% but it was not significant. Villus surface area was decreased as the threonine levels increased from 0.87% to 1.01% threonine. There was a tendency for a decrease in crypt depth when dietary threonine increased from 0.80% to 1.01%. Though *Saccharomyces cerevisiae* supplementation did not affect broiler intestinal morphology, however, supplemental threonine improved intestinal morphology by increasing crypt depth and villi height and villi width of ileum and jejunum (Rezeipour *et al.*, 2012). Dietary threonine supplementation, especially 110% level of Ross 308 specification, had increased villus height: crypt depth ratio significantly (Abbasi *et al.*, 2013). Dietary

L-threonine was evaluated at 0.0%, 0.5%, 0.75% and 1.0% level on gastrointestinal tract development in broiler chickens during summer where 0.75% L-threonine improved the intestinal morphology of broilers under hot conditions (Shirzadegan *et al.*, 2015).

Optimisation of threonine requirement in intestinal goblet cells and mucin production

Threonine concentration in the small intestine helps in synthesis of mucosal and mucin proteins, which demonstrates the importance of dietary threonine supply for gut metabolism (Nichols and Bertolo, 2008). Increasing supplementary threonine improved a significant increase in goblet cell numbers lead to production of more mucin which in turn provides protection of intestinal absorptive area. In broilers, the goblet cell density decreased when dietary treatments contained approximately 40, 70 and 100% (based on NRC, 1994) of threonine requirement (Horn *et al.*, 2009). However, there had a tendency for increase ($p = 0.09$) in goblet cell density (cells/micrometer of villus height) as dietary threonine increased in ducklings from 0.33 to 0.82%. Due to an imbalance intake of supplemental threonine, the rate of fractional protein synthesis of small intestinal mucosal protein and mucin were decreased (Wang *et al.*, 2006).

Role of threonine in preventing intestinal colonization of *Salmonella* and *Coccidia*

Increase in threonine:lysine ratio did not affect villus width, crypt depth and villus height: villus width ratio in *Salmonella* infected chicken (Valizade *et al.*, 2014a). However, villus height: villus width ratio showed a non-significant trend to increase with increase in threonine: lysine ratio. Villus height increased in adequate and excess threonine: lysine ratio diets compared with threonine deficient diet ($p < 0.001$). Villus height: crypt depth ratio increased in excess threonine: lysine ratio diet compare with other treatments ($p < 0.05$). Increase in threonine: lysine ratio resulted in an increase ($p < 0.01$) of

villus height, crypt depth, villus height: villus width ratio and goblet cell density in both challenged and unchallenged group of *Salmonella* (Valizade *et al.*, 2014b). In Coccidiosis of broiler birds, the intestinal morphological parameters of infected chicken in response to increase in threonine: lysine ratio did not alter (Kidd and Kerr, 1996; Wils-Plotz *et al.*, 2013).

Threonine for better gut health

Birds fed with high dietary threonine have higher jejunal villus height, villus width and crypt depth which might be due to better utilization of threonine in the gastro intestinal tract. Threonine might have maintained the intestinal strength and vigour in addition to providing energy for normal intestinal functions (Zaefarian *et al.*, 2008). A high protein turnover is required for basal metabolism and development of small intestine. Secondly, if we compare threonine with other amino acids, it has the highest metabolism in the portal-drained viscera (Schaart *et al.*, 2005). The small intestine utilises around 30 to 50% of threonine along with arginine, proline, isoleucine, valine, leucine, methionine, lysine, phenylalanine, glycine and serine (Wu, 1998). Increase in supplemental threonine levels might have provided sufficient concentrations for the high turnover of mucosal tissues. Birds fed with supplemental threonine will have taller villus height which is positively related to villus surface area and the expansion of surface area that occurs with villus growth has been used to explain the increased absorptive capacity (Mitchell and Carlisle, 1995).

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Threonine for optimum mucin production

An attribution to increase in absorptive surface of the intestinal villi indicates relatively higher mean goblet cell number/villus of birds fed with higher supplemental threonine. Increased surface area of small intestine due to supplemental threonine is to be protected from the acidity of ingesta, digestive enzymes and harmful microbes (Horn *et al.*, 2009). Hence, in order to protect the increased villus surface area, a good number of goblet cells might have been synthesized. A high production of mucin observed due to a significant increase in number of goblet cells and supplemental threonine also plays an important role in improvement of intestinal absorptive area (Nichols and Bertolo, 2008). On increased levels of threonine supplementation, the goblet cell numbers of small intestine have increased proportionately to the improved villus surface area, irrespective of experimental groups.

Conclusion

The beneficial effect of supplementation of threonine in terms of increased absorptive intestinal surface area along with goblet cell abundance is indicative for more production of mucin in the lumen, which may be correlated for better gut health of supplemented birds. Hence, that requirement of threonine in commercial chicken's diet needs to be reviewed critically. Supplementation of 10% more threonine over and above the recommended level may influence the overall performance of birds as well as its gut health and mucosal immunity.

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