SHORT COMMUNICATION

Effect of citric acid and microbial phytase on small intestinal morphology in broiler chicken

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Abstract

An experiment was carried out to investigate the effects of citric acid (CA) (0, 3 and 6%) and microbial phytase (MP) (0, 500 and 1000 U/kg) on morphology of different segments of small intestine (duodenum, jejunum and ileum) in broiler chickens fed on corn and soybean meal based diets. The effect of 9 experimental treatments (3×3 factorial design) were assessed using 270 7-d-old Ross 308 male broiler chicks in a randomized complete block design in three replicates of 10 birds each. The mean villi length (VL), crypt depth (CD) and goblet cell number (GCN) in duodenum, jejunum and ileum were significantly greater for the birds fed on acidified diets compared to the control birds at day 42 of age (P<0.01). Inclusion of 3% CA in diet significantly decreased the epithelial thickness (ET) in duodenum, jejunum and ileum (P<0.01). The birds received diets with 1000 U/kg of MP showed significant increase in CD (P<0.01) and GCN in jejunum (P<0.05), and significant decrease in VL:CD ratio and ET in the duodenum (P<0.01), jejunum (P<0.05) and ileum (P<0.01) segments. No variable of interest were affected by CA×MP interaction. It was concluded that CA and MP independently exhibit positive impact on morphometry of small intestine, toward facilitating the nutrient absorption and reducing the metabolic demands of the intestinal tract in broiler chickens.

Introduction

Phosphorus (P) is an essential mineral for metabolism and skeletal development in broiler chicken. However, 60% to 70% of the total P provided in the typical broiler diet ingredients such as corn and soybean is bound to phytic acid (Sohail and Roland, 1999). Phytic acid acts as an anti-nutritional factor due to its capability in binding with starch, proteins and minerals, such as P, Zn, Fe, Ca and Mg (Ravindran et al., 2001). Supplementation of phytase is expected to improve the nutritive value of feedstuffs and reduce the negative effects such as atrophy of the intestinal villi, enlarged digestive organs and increased size of gastro intestinal tract (Ravindran et al., 2001). Inclusion of organic acids or their salts is broiler diets is a convenient approach to overcome microbial proliferation in the feed and consequently to preserve the microbial balance in the gastrointestinal tract. In addition, by modifying intestinal pH, organic acids also improve the solubility of the feed ingredients, digestion and absorption of the nutrients (Nourmohammadi et al., 2012). Several researchers reported that application of diet acidifiers (such as CA) improve nutrient utilization (Nourmohammadi et al., 2012), lower intestinal pH (Nourmohammadi et al., 2011b), reduce population of pathogenic bacteria and increase population of non-pathogenic bacteria (Gunal et al., 2006). Citric acid (CA) is the most common organic acid which used in the broiler diets to improve health and growth of the birds. The dietary CA may improve MP activity in the intestinal tract. It was shown that the MP activity is correlated with concentration of H+ ion and free cations (Ravindran et al., 2011a, 2012). Therefore, inclusion of MP in broiler diets in accompany with CA may increase digestibility of the minerals chelated in phytic acid (Nourmohammadi et al., 2011a).

To the best of our knowledge experimental results on the same issue are scarce in the literature at hand. The present study was conducted to evaluate the effects of simultaneous supplementation of CA and MP on small intestinal morphology in broiler chickens.

Materials and methods

A total of two hundred-seventy Ross 308 male broiler chicks were randomly allotted to three cage replicates of 10 birds for each of nine dietary treatments such that each cage had a similar initial weight and weight distribution. Randomization of experimental cages was performed in according to a randomized complete block design (RCBD) in which each cage replicate was repeated one time in each location. All chicks were fed a typical commercial broiler starter ration for the first 6 days prior to the start of the experiment. On day 7, the chicks were weighed, wing-banded and allocated to different treatments. Feed and water were provided ad libitum and a continuous lighting schedule was used all through the experimental period. A basal diet was formulated based on corn and soybean meal for grower and finisher periods, according to NRC (1994) recommendations (Table 1). Diets were isoenergetic and iso-proteinous and provided in mash form. The dietary treatment in a 3×3 factorial fashion were: T1, basal diet; T2, basal diet+500 U/kg of MP; T3, basal diet+1000 U/kg of MP; T4, basal diet+3% CA; T5, basal diet+3% CA+500 U/kg of MP; T6, basal diet+3% CA+1000 U/kg of MP; T7, basal diet+6% CA; T8, basal diet+6% CA+500 U/kg of MP; and T9, basal diet+6% CA+1000 U/kg of MP. CA was supplied as monohydrate with 99.5% purity and MP source (Natuphos® 500, BASF, Hopf., Mt. Live, NJ, USA) had 10,000 active phytase unit per gram.

Intestinal histology measurements were done according to the method of Yu et al. (1998). At the end of the feeding trial, three broilers per replicate (nine birds per treatment), representative of the mean body weight, were selected and killed by rupture of carotid artery and jugular vein. Sample sec-
lations (3 cm in length) were taken from the descending duodenum, the middle region of the jejunum, and the ileum region; rinsed with 0.01 M PBS (pH 7.2); and placed into 10% buffered neutral formaldehyde solution (pH 7.2 to 7.4). Then, all samples were gradually dehydrated, sectioned at 6 μm, and stained with hematoxylin and eosin. Villus length, crypt depth, villus width and the thickness of epithelium were measured at 100x magnification using computer software (Sigma Scan, Jandel Scientific, San Rafael, CA, USA). The ratio of villus length to crypt depth was calculated. Neutral mucin was detected by staining the provided sections with periodic acid-Schiff (PAS) reagent (McManus, 1948). The slides holding the fixed tissue sections were deparaffinized, rehydrated, incubated with 5 g/L of periodic acid solution for 15 min, washed, and finally incubated with Schiff’s reagent (1 g of basic fuchsin, 200 mL of distilled water, 20 mL of 1 mol/L HCl, 6 g of sodium pyrosulfite) for 30 min. The sections were then washed in distilled water, dehydrated and mounted. Goblet cells were counted from 5-μm sections stained with periodic acid-Schiff reagent (Armed Forces Institute of Pathology, 1992). Briefly, tissues were deparaffinized and hydrated, oxidized in periodic acid (5 g/L) for 5 min, rinsed in distilled water, and placed in Coleman’s Schiff reagent (Sigma Chemical Co.) for 30 min. After a 15-min rinse in lukewarm tap water, tissues were counterstained in hematoxylin, rinsed, dehydrated, and mounted. Positively stained periodic acid-Schiff cells were enumerated on 10 villi per sample, and the means were considered for statistical analysis.

The data obtained was statistically assessed by the analysis of variance (ANOVA) using General Linear Model (GLM) procedure of SAS (2006) software. Initial design was randomized complete block design; however, since the effect of block on studied parameters was insignificant, this item was not considered in the final model. Cage was used as the experimental unit. Tukey-Kramer test was used to test the significance of the difference between means. For the different statistical tests, significance was declared at P<0.05.

Results and discussion

The results of ANOVA for the effects of dietary CA and MP levels on intestinal morphometry measurements are summarized in Table 2. Addition of 1000 unit of dietary MP in to the diet significantly increased goblet cell number (GCN) in the jejunum (P<0.05) and crypt depth (CD) in the duodenum (P<0.01), in contrast, it significantly decreased VL:CD ratio as well as epithelial thickness (ET) in the duodenum (P<0.01), jejunum (P<0.05) and ileum (P<0.01). The CA-supplemented diets significantly increased (P<0.01) VL, CD and GCN in all the segments of small intestine. Moreover, in the mean duodenal villus width (VW) and VL:CD ratio in the duodenum and jejunum were significantly greater in the bird fed with acidified diets (P<0.01). However, dietary CA at the concentration of 3% significantly decreased (P<0.01) the ET in all the segments of small intestine. The greater duodenal, jejunal and ileal VL were observed in the birds fed on diets supplemented with CA. These results are in agreement with the earlier workers (Pelicano et al., 2005) who reported increased VL in duodenum and jejunum of broiler chicken fed on diets with organic acidifiers. It has been shown that organic acids reduce the growth of many pathogenic or nonpathogenic bacteria in gut lumen (Pluske et al., 1996). Acidified diets also decrease the intestinal colonization and delay the infectious processes, and the inflammatory reactions at the intestinal mucosa, which increases the VL and functions of secretion, digestion and absorption of nutrients by the mucosa (Pelicano et al., 2005). Since jejunum is recognized as the major site of absorption in the small intestine, the increase in VL could represent an attempt to increase intestinal surface area to maximize absorption once digesta are passing through the villus.

Moreover, increased jejunal VL could hypothetically be caused by reduced cell turnover and migration, resulting in the retention of epithelial cells on the villi (Holt et al., 1986). In this study, VL and CD were stimulated by dietary CA and MP suggesting an enhanced rate of nutrient absorption and a reduced rate of enterocyte cell migration from the crypt to the villus. The VL:CD ratio is an indicator of the likely digestive capacity of the small intestine (Xu et al., 2003). An increase in this ratio corresponds to an increase in digestion and absorption (Montagne et al., 2003). The increased VL and VL:CD ratio might be associated with the increased numbers of beneficial bacteria (lactobacilli) in gut lumen (Gariga et al., 1996). The increased VL:CD ratio provides intestinal circumstances in favor of digestion, absorptive and hydrolysis potential, as well as requiring fewer nutrients to be directed towards intestinal maintenance (Pluske et al., 1996).

The reduction of ET of the small intestine improves digestion and absorption of nutrients by the pillar generative cell (Pelicano et al., 2005). However, the thickening of mucus layer on the intestinal mucosa con-

### Table 1. Composition of the basal diet in grower and finisher periods.

| Ingredients, g/kg | Grower, 7 to 21 d | Finisher, 22 to 42 d |
|------------------|------------------|---------------------|
| Corn             | 570.0            | 586.0               |
| Soybean meal     | 331.0            | 300.0               |
| Fish meal        | 34.0             | 35.0                |
| Soybean oil      | 20.0             | 35.0                |
| Dicalcium phosphate | 15.5          | 11.0               |
| Oyster shell     | 10.3             | 11.8                |
| DL-Methionine    | 0.1              | 0.1                 |
| Common salt      | 2.6              | 2.6                 |
| Sand             | 6.5              | 8.5                 |
| Trace minerals mix | 5.0            | 5.0                 |
| Vitamins mix     | 5.0              | 5.0                 |
| Calculated composition |           |                     |
| Metabolizable energy, kcal/kg | 2910 | 3030               |
| Crude protein, % | 20.10            | 19.00               |
| Calcium, %       | 0.95             | 0.90                |
| Total phosphorus, % | 1.23     | 1.06               |
| Non-phytate phosphorus, % | 0.45 | 0.36               |
| Methionine, %    | 0.50             | 0.38                |
| Lysine, %        | 1.10             | 1.00                |

*Mineral mix supplied / kg diet: Ma, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg. **Vitamins mix supplied / kg diet: vitamin A, 18,000 IU; vitamin D₃, 4000 IU; vitamin E, 36 mg; vitamin K₃, 4 mg; vitamin B₁₂, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.
tributes to the reduced digestive efficiency and nutrient absorption. Thinner intestinal epithelium enhances nutrient absorption and reduces the metabolic demands of the gastrointestinal system (Visek, 1978). Increased GCN in all the segments of intestine is attributed to higher mucin production and endogenous protein secretion. Silva and Smithard (2002) suggested that the absorption of nutrients may be impeded by an increase in the thickness of the epithelium in the small intestine. A fewer GCN in the epithelium of intestine may be impeded by an increase in the thickness of the epithelium in the small intestine. The effects induced by either MP or CA could facilitate the nutrient absorption and growth performance in broiler chickens. Supplementation of diet with dietary inclusion of MP at concentration of 1000 U/kg improves the duodenal ET and CD in broiler chicken. Supplementation of diet with CA at 3% concentration exhibited greater impact in morphology of all the segments of small intestine. The results of this study revealed that dietary inclusion of MP at concentration of 1000 U/kg improves the duodenal ET and CD in broiler chicken. Supplementation of diet with CA at 3% concentration exhibited greater impact in morphology of all the segments of small intestine. The effects induced by either MP or CA could facilitate the nutrient absorption and growth performance in broiler chicken. The morphology of small intestine was not affected by MP × CA interaction.

Conclusions

The results of this study revealed that dietary inclusion of MP at concentration of 1000 U/kg improves the duodenal ET and CD in broiler chicken. Supplementation of diet with CA at 3% concentration exhibited greater impact in morphology of all the segments of small intestine. The effects induced by either MP or CA could facilitate the nutrient absorption and growth performance in broiler chicken. The morphology of small intestine was not affected by MP × CA interaction.

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Table 2. Effects of citric acid and microbial phytase on intestinal morphometry of broiler chicks.

|                | MP, U/kg | 0 | 500 | 1000 | CA, % | 0 | 3 | 6 | SEM | MP | CA | MP × CA |
|----------------|----------|---|-----|------|-------|---|---|---|-----|----|----|---------|
| Duodenum       |          |   |     |      |       |   |   |   |     |     |    |         |
| Villus length, μm | 1309     | 1310 | 1307 | 1234 | 1348 | 1343 | 1.3 |     |     |     |    |         |
| Villus width, μm | 118      | 117 | 118 | 110  | 122  | 121  | 0.4 |     |     |     |    |         |
| Crypt depth, μm | 179      | 180a | 182a | 177  | 182  | 183  | 0.6 |     |     |     |    |         |
| Ratio          | 7.30b | 7.27a | 7.17a | 6.96 | 7.41 | 7.37 | 0.02 |     |     |     |    |         |
| Epithelial thickness, μm | 54a | 54a | 52a | 54  | 52  | 54  | 0.22 |     |     |     |    |         |
| Goblet cell number | 10.62 | 10.65 | 10.72 | 9.39 | 11.30 | 11.31 | 0.05 |     |     |     |    |         |
| Jejunum        |          |   |     |      |       |   |   |   |     |     |    |         |
| Villus length, μm | 1170     | 1174 | 1175 | 1117 | 1204 | 1200 | 1.3 |     |     |     |    |         |
| Villus width, μm | 107      | 106 | 104 | 104  | 107  | 106  | 1.5 |     |     |     |    |         |
| Crypt depth, μm | 159      | 160 | 160 | 155  | 162  | 161  | 1.1 |     |     |     |    |         |
| Ratio          | 7.36a | 7.35a | 7.35 | 7.19a | 7.43a | 7.44 | 0.05 |     |     |     |    |         |
| Epithelial thickness, μm | 35a | 35a | 34a | 36  | 32  | 36  | 0.32 |     |     |     |    |         |
| Goblet cell number | 11.32a  | 11.47ab | 11.60a | 10.32b | 11.99 | 12.08b | 0.07 |     |     |     |    |         |
| Ileum          |          |   |     |      |       |   |   |   |     |     |    |         |
| Villus length, μm | 806      | 802 | 805 | 761  | 831  | 820  | 5.6 |     |     |     |    |         |
| Villus width, μm | 65       | 68  | 66  | 66   | 67   | 65   | 1.7 |     |     |     |    |         |
| Crypt depth, μm | 156      | 150 | 154 | 148  | 156  | 157  | 1.8 |     |     |     |    |         |
| Ratio          | 5.17a | 5.32a | 5.23 | 5.15a | 5.32a | 5.25 | 0.06 |     |     |     |    |         |
| Epithelial thickness, μm | 34a | 33a | 32a | 34  | 31  | 33  | 0.28 |     |     |     |    |         |
| Goblet cell number | 10.80 | 11.17 | 11.12 | 9.76 | 11.58 | 11.73 | 0.17 |     |     |     |    |         |

CA, citric acid; MP, microbial phytase. "Mean values within a row with no common superscript differ significantly from each other (P<0.05). °Ratio of villus length to crypt depth, †numbers in area of epithelial cells; ns, not significant.
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