

Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers

R. Chowdhury,* K. M. S. Islam,*¹ M. J. Khan,* M. R. Karim,† M. N. Haque,*
M. Khatun,‡ and G. M. Pesti§

*Department of Animal Nutrition, †Department of Anatomy and Histology,
and ‡Department of Poultry Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh;
and §Department of Poultry Science, University of Georgia, Athens 30602-2772

ABSTRACT The aim of this study was to determine the effect of the supplementation of an organic acid (citric acid), antibiotic growth promoter (avilamycin), and their combination for a period of 35 d on the growth, feed efficiency, carcass yield, tibia ash, and immune status of broilers. One hundred sixty 1-d-old broiler chicks (Hubbard Classic) were randomly distributed into 4 groups with 4 replicate cages having 10 birds in each. A corn-soybean-based diet was used as the basal diet (control). The basal diet was supplemented with an organic acid (citric acid, 0.5%), an antibiotic growth promoter (avilamycin, 0.001%), and their combination in other groups. The highest BW was attained in citric acid-fed chicks (1,318 g), which was significantly ($P < 0.05$) higher than control chicks (1,094 g) or avilamycin-fed chicks (1,217 g). The combination-fed chicks showed similar weight (1,246 g) as citric acid- or avilamycin-fed chicks ($P > 0.05$). Total feed intake was higher in citric acid-fed chicks compared with antibiotic-supplemented chicks. The addition of citric acid

improved feed conversion efficiency (g of weight gain/kg of feed intake) significantly ($P < 0.05$) compared with control chicks or its combination with avilamycin. Higher carcass weights were found in chicks fed the combination diet. Supplementation of citric acid increased tibia ash percentage significantly ($P < 0.05$) compared with controls. Addition of citric acid reduced the pH of the formulated diets. An improvement of immune status was detected by densely populated immunocompetent cells in the lamina propria and submucosa of cecal tonsils and ileum and also in the cortex and medulla of bursa follicles in citric acid-supplemented chicks. Supplementation of citric acid at 0.5% in the diet had positive effects on growth, feed intake, feed efficiency, carcass yield, bone ash, and immune status of broilers. Therefore, citric acid might be a useful additive instead of antibiotic growth promoters such as avilamycin, considering performance and health status of broilers.

Key words: broiler, citric acid, avilamycin, performance, immunity

2009 Poultry Science 88:1616–1622
doi:10.3382/ps.2009-00119

INTRODUCTION

Growth promoters have been used extensively in animal feeds and water all over the world, especially in the poultry and pig industries (Charles and Duke, 1978). Antibiotics as growth promoters in food animal production have been used since 1946 throughout the world. They are used in poultry production to improve performance, for therapeutic and prophylactic to stabilize (Jones and Rickett, 2003), and are thought to stabilize the intestinal microbial flora and to prevent some specific intestinal pathogens (Waldroup et al., 1995). It

was established that antibiotic use in poultry rations improved growth rate, feed conversion, and reduced mortality (Miles et al., 2006; Pfaller, 2006). Avilamycin is an antibiotic established for use as a growth promoter in poultry, especially broilers, for improvement of performance and feed efficiency (Valadirova et al., 1997). At the same time, its use at subtherapeutic levels in poultry diets decreases the microbial burden in the gastrointestinal tract and reduces the susceptibility to disease.

Continuous use of antibiotics in food animals is suspected to result in antibiotic resistance (Roy et al., 2002). An association between the use of avilamycin for growth promotion and the occurrence of avilamycin-resistant *Enterococcus faecium* on broiler farms was reported by Katsunuma et al. (2007). They also reported that introducing a withdrawal period could minimize

©2009 Poultry Science Association Inc.

Received March 9, 2009.

Accepted April 13, 2009.

¹Corresponding author: kmsislam1@yahoo.com

the risk of avilamycin-resistant *E. faecium* becoming carcass contaminants. Evidence exists that antibiotic-resistant bacteria can be transmitted from animals to humans (Greko, 2001) and their dissemination in the human food chain is considered a hazard (Roe and Pillai, 2003). As a result, alternatives to antibiotic growth promoters are needed due to their recent withdrawal, especially in the EU (2003). For this reason, feed manufacturers and animal growers have been actively looking for efficacious alternatives to antibiotic growth promoters.

Several alternatives to antibiotic growth promoters have been proposed such as organic acids, probiotics, herbs and herbal products, enzymes, and essential oils. Among these, the organic acids (citric acid, acetic acid, propionic acid, formic acid) and probiotics are the most promising alternatives for poultry (Gunal et al., 2006). Their supplementation in the diet of broilers enhanced nutrient utilization, growth, and feed efficiency (Jin et al., 1998; Denil et al., 2003). The use of citric acid creates an acidic environment (pH 3.5 to 4.0) in the gut that favors the development of lactobacilli and inhibits the replication of *Escherichia coli*, *Salmonella*, and other gram-negative bacteria. It works by activation and functions of proteolytic enzymes, stimulates feed consumption, reduces the production of ammonia and other growth-depressing microbial metabolites, favors mineral absorption, and lowers the incidence of sub-clinical infections. Several studies support the statement that the addition of citric acid to broiler rations improved weight gain (Afsharmanesh and Pourreza, 2005; Nezhad et al., 2007), increased feed consumption (Moghadam et al., 2006), and improved feed efficiency (Abdel-Fattah et al., 2008). Its addition, its use increased retention of phosphorus (Brenes et al., 2003; Liem et al., 2008), tibia ash (Rafacz-Livingston et al., 2005; Martinez-Amezcuca et al., 2006), and toe ash (Atapattu and Nelligaswatta, 2005) in broiler chicks. It also decreased pH of cecal digesta, crop and gizzard (Andrys et al., 2003), and intestine (Denli et al., 2003) in broiler chicks. In addition, improved immune responses by broilers (Rahmani and Speer, 2005; Abdel-Fattah et al., 2008). Considering the above statements, this study was designed to determine the effect of citric acid, the antibiotic growth promoter avilamycin, and their combination on the performance, tibia ash percentage, and immune status of broiler chickens.

MATERIALS AND METHODS

One hundred sixty healthy 1-d-old broiler chicks (Hubbard Classic) were randomly distributed to 16 cages containing 10 broilers in each, and 4 cages were allocated to each of the 4 treatment groups. The chicks were handled carefully to avoid any pain or injury. A corn-soybean-based feed was used as the basal diet for the control group (Table 1). The basal diet was further supplemented with citric acid (0.5%); avilamycin, an antibiotic growth promoter (0.001%); and a combi-

nation of these, respectively. The corn-soybean meal-based mash diet was formulated as recommended by NRC (1994).

The cages, in an open-air trial house, were of steel wire construction and had a surface area of 0.91 m² (120 cm × 76 cm) per cage. Droppings were allowed to drop on the sawdust that was spread below the cages. Electric light (neon bulbs) illuminated the trial house over 24 h. All of the birds were reared in identical conditions. Chicks were given ad libitum access to diets and water during the trial. Birds were weighed initially and at 7-d intervals. The trial house was cleaned frequently and the birds were observed for any types of clinical signs. Dead birds were recorded, necropsied, and histopathology was conducted. At the ages of 9 and 17 d, the birds were vaccinated against infectious bursal disease, and on d 3 and 19, the birds were vaccinated against Newcastle disease, all via eye drop as per recommendation of the manufacturer.

Four birds from each group (one from each replicate cage) were randomly killed at the end of the trial (d 35). After complete bleeding, head, legs, feathers, viscera, and skin were removed to determine carcass weight. The tibias of killed broilers were boiled to remove the tissues and cartilage caps. Ground dried tibia samples were extracted to remove the fat. Then, the extracted samples were burned on an electric heater and finally placed in a muffle furnace at 600°C for 5 h (Hall et al.,

Table 1. Ingredients and chemical composition of the experimental diet

Item	Amount (%)
Ingredient	
Ground corn	50.00
Soybean meal	40.00
Rice polish	4.30
Soybean oil	2.00
Salt	0.25
Dicalcium phosphate	1.50
Oyster shell	1.375
Vitamin premix ¹	0.25
Trace element premix ²	0.125
DL-Methionine	0.15
Choline chloride 60%	0.05
Chemical components — (g/100 g of air dry sample) —	
DM	88.46
CP	22.71
Crude fiber	4.23
Ether extract	2.48
Nitrogen-free extract	48.45
Ash	10.59
Calcium ³	0.98
Total phosphorus ³	0.74
ME ⁴ (kcal/kg of DM)	3,278

¹2.5 g of vitamin premix: vitamin A, 13,500 IU; vitamin D₃, 1,500 IU; α-DL-tocopherol acetate, 50 mg; menadione, 2 mg; thiamine, 3 mg; riboflavin, 5 mg; pyridoxine, 4 mg; cobalamine, 15 µg; folic acid, 200 µg; nicotinic acid, 60 µg; Ca-pantothenate, 30 mg; choline, 750 mg; ascorbic acid, 150 mg.

²1.25 g of trace element premix: Fe, 125.00 mg; Zn, 100.00 mg; Cu, 10.00 mg; Mn, 50.00 mg; Se, 0.25 mg; Co, 0.20 mg.

³According to NRC (1994).

⁴Calculated using the formula of Wiseman (1987).

Table 2. Effect of citric acid (CA), avilamycin (AV), and their combination on growth, feed intake, and feed conversion in broiler chicks fed corn-soybean diet¹

Days of test	Treatment groups			
	Nonmedicated	CA (0.5%)	AV (0.001%)	CA + AV (0.5% + 0.001%)
BW (g)				
Initial	55 ± 0.2	55 ± 0.2	55 ± 0.2	55 ± 0.4
28	716 ± 30.7 ^b	855 ± 37.3 ^a	859 ± 42.6 ^a	852 ± 34.6 ^a
35	1,094 ± 31.2 ^c	1,318 ± 32.7 ^a	1,217 ± 38.7 ^b	1,246 ± 4.2 ^{ab}
Weight gain (g)				
1 to 28	661 ± 30.6 ^b	799 ± 36.9 ^a	803 ± 42.6 ^a	796 ± 34.3 ^a
1 to 35	1,038 ± 30.7 ^c	1,262 ± 32.7 ^a	1,161 ^b ± 38.5	1,190 ± 4.3 ^{ab}
Feed intake (g)				
1 to 28	1,347 ± 90.1 ^b	1,472 ± 49.0 ^{ab}	1,493 ± 18.8 ^{ab}	1,576 ± 34.68 ^a
1 to 35	2,341 ± 93.1 ^b	2,528 ± 57.3 ^{ab}	2,451 ± 31.1 ^{ab}	2,625 ± 50.6 ^a
FCE ² (g of live weight gain/kg of feed intake)				
1 to 28	493 ± 14.6	543 ± 16.3	537 ± 22.5	504 ± 16.6
1 to 35	445 ± 19.7 ^b	499 ± 6.6 ^a	473 ± 10.5 ^{ab}	453 ± 8.0 ^b
Feed conversion ratio				
d 35	2.2 ± 0.10 ^a	2.0 ± 0.02 ^b	2.1 ± 0.04 ^{ab}	2.2 ± 0.03 ^a

^{a-c}Mean values within the same row with different superscripts are significantly different ($P < 0.05$).

¹Mean ± SE (n = 40).

²FCE = feed conversion efficiency.

2003) and ash weight of each sample was expressed as a percentage of dry-extracted bone weight.

For the immunological studies, 5 birds from the control and 5 from the citric acid-supplemented group were killed by cervical dislocation at d 35 to know the density of immunocompetent cells. The lymphoid organs (cecal tonsil, ileum, bursa of Fabricius) were collected and fixed in the Bouin's fluid (Gridley, 1960) for 24 h. The tissues were dehydrated in ascending-grade alcohol (50, 70, 80, 95% and absolute), cleaned in xylene, embedded in paraffin, and finally the sections were cut at 6- μ m thickness using a Sliding Microtome (MIC509, Euromax, Tokyo, Japan). Then, the sections were stained with the standard hematoxylin and eosin method (Gridley, 1960). Distribution of immunocompetent cells in the major lymphoid organ and tissues (mucosa and submucosal layer) was observed randomly on 20 microscopic fields of each slide by using a light microscope with low ($\times 10$) and high ($\times 40$) magnification. The measurements of histological structure and lymphatic tissues were performed by the calibrated stage micrometer. The frequency of immunocompetent cells was counted in 5 microscopic fields (each field was 0.05

mm²) per slide selected randomly by using an ocular grid under $\times 40$ magnification.

Initially, the raw data were organized using Microsoft Excel (Microsoft Corp., Redmond, WA) and the data were analyzed using SPSS 11.5 (SPSS Inc., Chicago, IL). All data were analyzed by 1-way ANOVA and statistical difference among the treatment means was determined using Duncan's new multiple range test with 5% probability (Duncan, 1955).

RESULTS

Analysis of variance revealed that BW, BW gain, feed intake, feed conversion efficiency, water consumption, carcass weight, and tibia ash (Tables 2 and 3) were affected significantly ($P < 0.05$) by supplementing citric acid, antibiotic avilamycin, and their combination to the basal diet.

Growth, Feed Intake, and Feed Conversion

Birds of all groups had a similar live weight (55 ± 0.2) during the beginning, but at the age of d 28, sup-

Table 3. Effect of citric acid (CA), avilamycin (AV), and their combination on water consumption and carcass weight of broilers fed corn-soybean diet¹

Parameters	Treatment groups			
	Nonmedicated	CA (0.5%)	AV (0.001%)	CA + AV (0.5% + 0.001%)
Water intake (mL)/broiler (29 to 35 d)	1,615 ± 44.6 ^b	1,902 ± 94.6 ^a	1,729 ± 66.7 ^{ab}	1,861 ± 54.9 ^a
Water intake (mL)/bird per kilogram of feed (29 to 35 d)	1,624 ± 32.9 ^b	1,801 ± 51.6 ^a	1,801 ± 68.0 ^a	1,774 ± 59.1 ^{ab}
Carcass weight				
Live weight (g)	925 ± 52.0	987 ± 55.4	1,062 ± 92.2	1,067 ± 78.8
Carcass weight ² (% live weight)	51.5 ± 1.19 ^b	55.2 ± 0.75 ^{ab}	53.7 ± 1.54 ^{ab}	56.0 ± 1.58 ^a

^{a,b}Mean values within the same row with different superscripts are significantly different ($P < 0.05$).

¹Mean ± SE (n = 40).

²Without skin.

plemented groups showed higher weight than control. At the end of the trial (d 35), citric acid resulted in higher ($P < 0.05$) weight than avilamycin and control, but their combination showed similar growth to the citric acid and avilamycin group ($P > 0.05$). Cumulative weight gain of broilers was higher in supplemented groups than control; for example, citric acid supplementation caused better weight gain than avilamycin during d 35 ($P < 0.05$).

Supplementation of citric acid and avilamycin individually increased feed intake numerically, but their combination caused significantly higher feed intake ($P < 0.05$) at the age of either 28 and 35 d.

In general, significant ($P < 0.05$) differences were observed in feed conversion efficiency especially during the end of the trial (d 35). The highest efficiency was observed in citric acid-fed chicks (499) and the lowest in the control (445) and combination groups (453). Avilamycin-supplemented chicks showed similar efficiency (473) in the control as well as citric acid supplemented groups.

Water Consumption, Carcass Weight, the pH of Feed, and Mortality

Water consumption was higher ($P < 0.05$) in the citric acid and combination group compared with the control, with the avilamycin treatment being intermediate (Table 3). Water intake per kilogram of feed was also higher in the citric acid and avilamycin group than control, but the combination group was similar to control. The combination of citric acid and avilamycin-supplemented chicks had higher carcass weights (% of live weight) than control ($P < 0.05$). Supplementation of 0.5% citric acid in the diet resulted in lower dietary pH (6.62) compared with the basal (7.38) and avilamycin (7.15), whereas the pH of the combination-supple-

mented diet was 6.55 (data not shown). One bird from the control and one from the avilamycin-supplemented group died during trial, which is below the accepted level of modern broiler raising.

Tibia Ash and Immune Status

The citric acid-fed chicks showed the highest tibia ash percentage (Figure 1), which was significantly ($P < 0.05$) higher than control but similar to avilamycin and its combination group ($P > 0.05$). Citric acid, avilamycin, and their combination increased tibia ash 10, 5, and 3%, respectively, compared with control. The mean size of bursal follicles was larger in the citric acid-treated group ($492 \times 269 \mu\text{m}$) than control ($462 \times 241 \mu\text{m}$; Figure 2, panels C and D). Similarly, the mean size of cecal tonsil ($279 \times 201 \mu\text{m}$) and ileum ($242 \times 212 \mu\text{m}$) of the citric acid-treated group was larger than the size of cecal tonsil ($258 \times 187 \mu\text{m}$) and ileum ($209 \times 191 \mu\text{m}$) of the control group, respectively (Figure 2, panels A to F). The lamina propria and submucosa of cecal tonsil and ileum were more densely populated with lymphocytes (399 and 268 per 0.05-mm^2 area) in the citric acid-supplemented group than control (352 and 194 per 0.05-mm^2 area; Figure 2, panels A to F).

Cost of Production

Production costs (Table 4) were calculated considering costs of chicks, feed, and test substances. The addition of citric acid and avilamycin to the diet created a progressive increase in feed cost in all of the treatment groups except the control. Production cost per kilogram of live weight of broilers was lowest in citric acid-fed chicks followed by avilamycin, control, and combination-fed chicks, respectively.

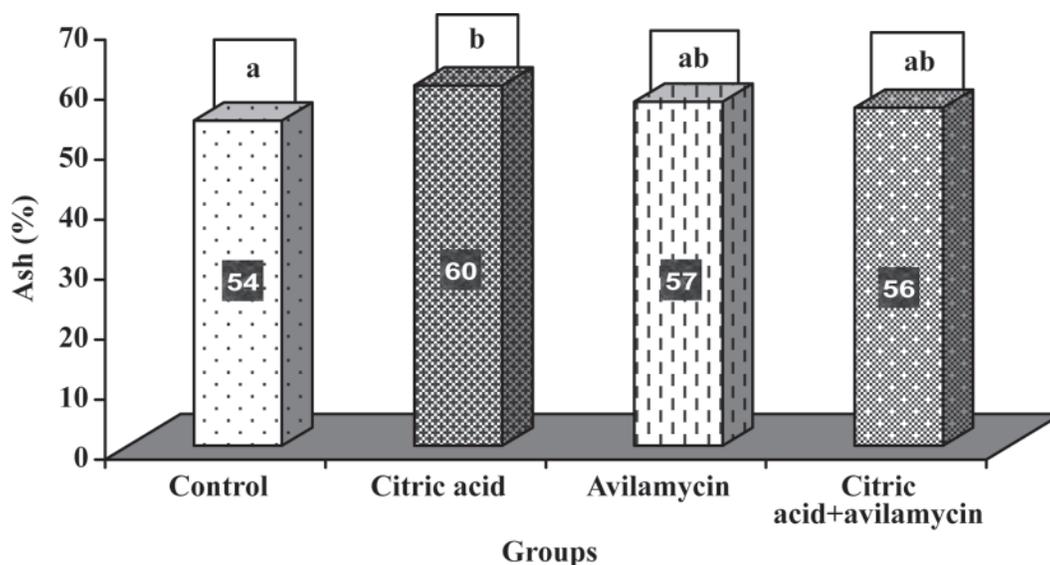


Figure 1. Effect of citric acid, avilamycin, and their combination on percentage of tibia ash of chicks ($n = 40$) at the age of 35 d fed corn-soybean diet. Different letters in bars indicate significant difference ($P < 0.05$) among means.

DISCUSSION

The growth increased by supplementing citric acid, avilamycin, or their combination during different stages of growth in comparison to the control group, and the citric acid-fed broilers showed the best performance. The increased growth response observed from 0.5% citric acid in this study is in agreement with the results reported by Abdel-Fattah et al. (2008), who found that the addition of dietary citric acid, acetic acid, or lactic acid improved live BW of broiler chicks as compared with those fed unsupplemented diets. Similar results were found by other researchers (Moghadam et al., 2006; Nezhad et al., 2007). Feed intake was significant-

ric acid in this study is in agreement with the results reported by Abdel-Fattah et al. (2008), who found that the addition of dietary citric acid, acetic acid, or lactic acid improved live BW of broiler chicks as compared with those fed unsupplemented diets. Similar results were found by other researchers (Moghadam et al., 2006; Nezhad et al., 2007). Feed intake was significant-

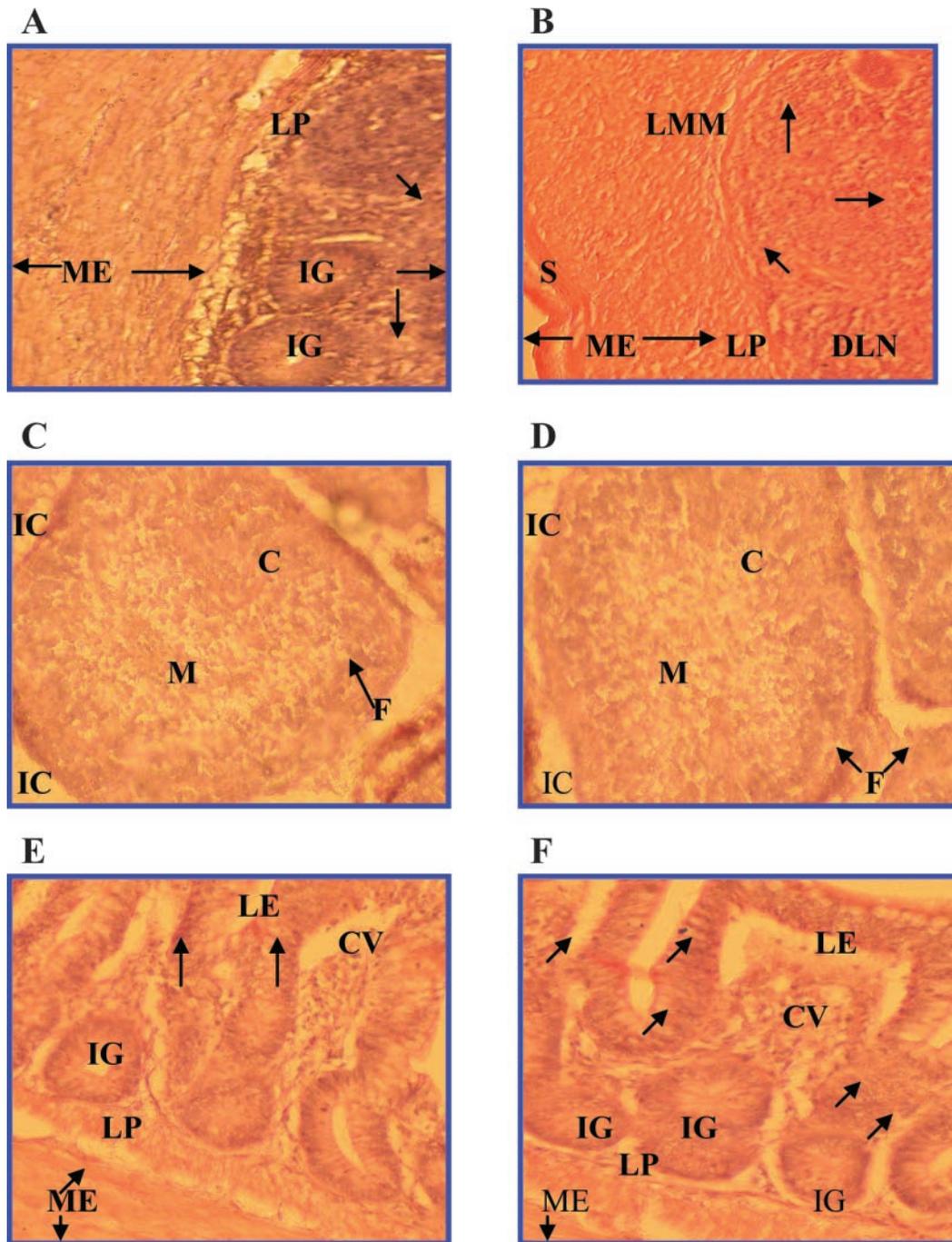


Figure 2. (A) The section of cecal tonsil of control chicks showing sparsely distributed lymphocytes (arrow), lamina propria (LP), muscularis externa (ME), and intestinal gland (IG). (B) The section of cecal tonsil of citric acid-supplemented chicks showing lymphocytes (arrow), LP, lamina muscularis mucosa (LMM), disorganized lymphatic nodule (DLN), ME, and serosa (S). (C) The section of bursa of Fabricius of control chicks showing bursa follicle (F) composed of cortex (C) and medulla (M) and interfollicular connective tissue (IC). (D) The section of bursa of Fabricius of citric acid-supplemented chicks showing large F and distribution of the lymphocyte cells in the C and M and IC. (E) The section of ileum of control chicks showing distribution of immunocompetent cells (arrow), core of the villi (CV), LP, lamina epithelium (LE), IG, and ME. (F) The section of ileum of citric acid-supplemented chicks showing distribution of immunocompetent cells (arrow), CV, LP, IG, lamina epithelium (LE), and ME.

Table 4. Analysis of cost [in US dollars (USD)¹] of broilers supplemented citric acid (CA), avilamycin (AV), and combination in feed

Cost (35-d trial)	Treatment groups			
	Nonmedicated	CA (0.5%)	AV (0.001%)	CA + AV (0.5% + 0.001%)
Cost/kg feed ²	0.37	0.39	0.38	0.40
Cost (feed/broiler)	0.87	0.97	0.91	1.02
Cost (feed + chick)/broiler	1.06	1.16	1.10	1.21
Cost (feed + chick)/kg of live weight	0.96	0.88	0.91	0.97

¹1 USD = 69 Bangladeshi taka.

²Cost of test substances included.

ly increased due to the addition of citric acid and its combination with avilamycin. Moghadam et al. (2006) found that the effects of citric acid on feed consumption of broiler chicks were significant and similar results were found by Atapattu and Nelligaswatta (2005). However, this observation was not found by the findings of Nezhad et al. (2007), who reported that there was no significant effect on feed intake in broiler chicks fed a diet supplemented with citric acid. The addition of citric acid to the diet improved feed efficiency ($P < 0.05$). This result is in agreement with the results of Nezhad et al. (2007), who reported that the addition of citric acid to a broiler diet improved feed efficiency, and the results on the beneficial effects of citric acid on feed conversion has been reported by several researchers (Andrys et al., 2003; Afsharmanesh and Pourreza, 2005; Abdel-Fattah et al., 2008).

In the present study, the addition of citric acid decreased the pH of the diets, which is similar to the findings of Radcliffe et al. (1998). Significant ($P < 0.05$) differences were observed in terms of carcass weight (%) of broilers receiving different supplements, which is in agreement with the findings of Atapattu and Nelligaswatta (2005), who reported that the addition of citric acid to a rice by-products-based diet increased the carcass weight of broilers.

Citric acid has been evaluated numerous times for its efficacy in improving growth performance; however, very little work with citric acid has investigated its efficacy for improving immune response and mineral utilization by broiler chicks. The citric acid treatment significantly ($P < 0.05$) increased tibia ash, indicating improved utilization of phosphorus. This finding is in an agreement with the result of Liem et al. (2008), who reported that the addition of citric, malic, and fumaric acids increased the percentage of tibia ash, but only the effect of citric acid was significant. Similar results were reported by Rafacz-Livingston et al. (2005), Martinez-Amezcuca et al. (2006), and Nezhad et al. (2007).

A higher density of immunocompetent cells indicates that the broilers were healthy and possessed higher immune status to fight against enteric pathogens and infectious diseases; thus, the addition of citric acid to the diet increased the immune status of the broilers (Abdel-Fattah et al., 2008; Khan et al., 2008).

In conclusion, this study demonstrated that citric acid as a feed additive (instead of the antibiotic growth promoter avilamycin) enhanced the growth performance of broiler chicks, increased bone ash deposition, and produced healthy broilers that possessed a higher immune status to fight against enteric pathogens and infectious diseases.

REFERENCES

- Abdel-Fattah, S. A., M. H. El-Sanhoury, N. M. El-Mednay, and F. Abdul-Azeem. 2008. Thyroid activity of broiler chicks fed supplemental organic acids. *Int. J. Poult. Sci.* 7:215–222.
- Afsharmanesh, M., and J. Pourreza. 2005. Effect of calcium, citric acid, ascorbic acid, vitamin D₃ on the efficacy of microbial phytase in broiler starters fed wheat-based diets on performance, bone mineralization and ileal digestibility. *Int. J. Poult. Sci.* 4:418–424.
- Andrys, R., D. Klecker, L. Zeman, and E. Marecek. 2003. The effect of changed pH values of feed in isophosphoric diets on chicken broiler performance. *Czech J. Anim. Sci.* 48:197–206.
- Atapattu, N. S. B. M., and C. J. Nelligaswatta. 2005. Effects of citric acid on the performance and utilization of phosphorous and crude protein in broiler chickens fed rice byproducts based diets. *Int. J. Poult. Sci.* 4:990–993.
- Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro, and C. Bravo. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Anim. Feed Sci. Technol.* 110:201–219.
- Charles, O. W., and S. Duke. 1978. The response of laying hens to dietary fermentation products and probiotic-antibiotic combinations. *Poult. Sci.* 57(Suppl. 4):1125. (Abstr.)
- Denil, M., F. Okan, and K. Celik. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pak. J. Nutr.* 2:89–91.
- Duncan, D. B. 1955. Multiple range test and multiple F test. *Biometrics* 11:1–42.
- EU. 2003. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September (2003). *Off. J. L* 268:29–43.
- Greko, C. 2001. Safety aspects on non-use of antimicrobials as growth promoters. Pages 219–230 in *Gut Environment of Pigs*. A. Piva, K. E. Bach Knudsen, and J. E. Lindberg, ed. Nottingham University Press, Nottingham, UK.
- Gridley, M. F. 1960. Pages 28–29 and 82–83 in *Manual of Histologic and Special Staining Technique*. McGraw-Hill Book Company, New York, NY.
- Gunal, M., G. Yayli, O. Kaya, N. Karahan, and O. Sulak. 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *Int. J. Poult. Sci.* 5:149–155.
- Hall, L. E., R. B. Shirley, R. I. Bakalli, S. E. Aggrey, G. M. Pesti, and H. M. Edwards Jr. 2003. Power of two methods for the estimation of bone ash of broilers. *Poult. Sci.* 82:414–418.

- Jin, L. Z., Y. W. Ho, N. Abdulrahim, and S. Jaludin. 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diet containing *Lactobacillus* cultures. *Poult. Sci.* 77:1259–1265.
- Jones, F. T., and S. C. Rickett. 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. *Poult. Sci.* 82:613–617.
- Katsunuma, Y., M. Hanazumi, H. Fujisaki, H. Minato, Y. Hashimoto, and C. Yonemochi. 2007. Influence of avilamycin administration and its subsequent withdrawal on emergence and disappearance of antimicrobial resistance in enterococci in the intestine of broiler chickens. *J. Appl. Microbiol.* 102:1159–1166.
- Khan, M. Z. I., S. H. Akter, M. N. Islam, M. R. Karim, M. R. Islam, and Y. Kon. 2008. The effect of selenium and vitamin E on the lymphocytes and immunoglobulin-containing plasma cells in the lymphoid organ and mucosa-associated tissue of broiler chick. *Anat. Histol. Embryol.* 37:52–59.
- Liem, A., G. M. Pesti, and H. M. Edwards Jr. 2008. The effect of several organic acids on phytase phosphorus hydrolysis in broiler chicks. *Poult. Sci.* 87:689–693.
- Martinez-Amezcuca, C., C. M. Parsons, and D. H. Baker. 2006. Effect of microbial phytase and citric acid on phosphorus bioavailability, apparent metabolizable energy, and amino acid digestibility in distillers dried grains with soluble in chicks. *Poult. Sci.* 85:470–475.
- Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poult. Sci.* 85:476–485.
- Moghadam, A. N., J. Pourreza, and A. H. Samie. 2006. Effect of different levels of citric acid on calcium and phosphorus efficiencies in broiler chicks. *Pak. J. Biol. Sci.* 9:1250–1256.
- Nezhad, Y. E., M. Shivazad, M. Nazeeradi, and M. M. S. Babak. 2007. Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. *J. Fac. Vet. Med. Univ. Tehran.* 61:407–413.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. National Academy Press, Washington, DC.
- Pfaller, M. A. 2006. Flavophospholipol use in animals: Positive implications for antimicrobial resistance based on its microbiologic properties. *Diagn. Microbiol. Infect. Dis.* 56:115–121.
- Radcliffe, J. S., Z. Zhang, and E. T. Kornegay. 1998. The effect of microbial phytase, citric acid and their interaction in a corn-soybean meal based diet for weaning pigs. *J. Anim. Sci.* 76:1880–1886.
- Rafacz-Livingston, K. A., C. Martinez-Amezcuca, C. M. Parsons, D. H. Baker, and J. Snow. 2005. Citric acid improves phytate phosphorus utilization in crossbred and commercial broiler chicks. *Poult. Sci.* 84:1370–1375.
- Rahmani, H. R., and W. Speer. 2005. Natural additives influence the performance and humoral immunity of broilers. *Int. J. Poult. Sci.* 4:713–717.
- Roe, M. T., and S. D. Pillai. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. *Poult. Sci.* 82:622–626.
- Roy, P., A. S. Dhillon, L. H. Lauerman, D. M. Schaberg, D. Bandli, and S. Johnson. 2002. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. *Avian Dis.* 46:17–24.
- Valadirova, L., S. Sourdjiisda, and T. Dilow. 1997. Nutritive effect of avilamycin supplementation to chicken broiler mixes. *Zhivotnov'Dni-Nauki* 34:3–4, 49–52.
- Waldroup, A., S. Kaniawati, and A. Mauromoustakos. 1995. Performance characteristics and microbiological aspects of broilers fed diets supplemented with organic acids. *J. Food Prot.* 58:482–489.
- Wiseman, J. 1987. *Feeding of Non-Ruminant Livestock*. Butterworths and Co. Ltd., London, UK.