Indian Journal of Animal Sciences **80** (8): 813–816, August 2010

Effects of dietary citric acid, probiotic and their combination on the performance, tibia ash and non-specific immune status of broiler

M KHATUN[1], K M S ISLAM[2], M A R HOWLEDER[3], M N HAQUE[4], R CHOWDHURY[5] and M R KARIM[6]

Bangladesh Agricultural University, Mymensingh 2202 Bangladesh

Received: 24 September 2009; Accepted: 6 April 2010

Key words: Broiler, Citric acid, Immunity, Probiotic, Tibia ash

A probiotic is a live microbial feed supplement, which beneficially affects the host by improving its intestinal balance (Ahmad 2006). The probiotic microorganisms improved metabolism of the broilers in various ways. The probiotic organism reside in intestinal tract and their metabolites can act as immunomodulatory agent by activating specific and non-specific host immune responses in chicks, which help in prevention and control of various infectious diseases (Koenen *et al.* 2004) and reduce mortality (Hamid and Aijazuddin 2001). Organic acid along with probiotics used in several experiments reported that organic acid group showed better weight gain, feed conversion and good carcass quality than control and others (Muzaffer *et al*. 2003). This experiment was conducted to study the effects of CA, probiotics and their combination on the growth, meat yield, bone mineralization and immune response of broilers.

Chicks and treatments: Chicks (160) were randomly divided into four groups with four replications, each replicate having 10 broilers. The dietary treatments were control, 0.5% CA, 0.001% probiotic (protexin) and their combination which contained both 0.5% CA and 0.001% probiotic. The protexin contains bacteria (*Lactobacillus plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, Enterococcus faecium*), mold *(Aspergilus oryzae, Candida pintolopesii)* and others *(Â. glucan gum*) in different concentrates. Experimental diets were formulated (Table 1) as per the NRC requirements (NRC 1994).

Management practices: The birds were handled carefully to avoid any cause of pain and injury considering welfare of animal. The birds were reared in cages by providing a floor space 0.91 m^2 for 10 birds. Feeds and water were supplied to all broilers *ad lib*. Records were kept on feed intake, live weight, water consumption, mortality per replication. Birds were weighed on weekly basis and at the end of the trial one bird from each replication was selected to measure the meat yield. The shanks were collected to determine bone ash and the major lymphoid organs (bursa of Fabricius, caecal tonsil and ileum) and tissues were collected to determine the distribution of immunocompetent cells.

Chemical and statistical analysis: Proximate analysis of feed was performed following AOAC (AOAC 1990). Data were monitored and calculated by Excel program and analyzed using the SPSS (11.5) program for one way analysis of variance (ANOVA) and Duncan's Multiple Range Test was done to know difference among treatment means at 5% level of significance (Steel and Torrie 1980).

Table 1. Ingredient composition of basal diet

Ingredients	Amount (kg/100kg)	Chemical composition	Amount (g/100g)
Maize	50.00	Dry matter	88.39
Soybean meal	40.00	Crude protein	22.91
Rice polish	4.30	Crude fiber	4.33
Soybean oil	2.00	Ether extract	2.58
Salt	0.25	Nitrogen free extract	60.03
Di-calcium phosphate	1.50	Ash	10.67
Oyster shell	1.0	ME (MJ/kg DM)**	3279
Vitamin premix[a]	0.25	Ca*	0.99
Trace element premix[b]	0.125	Total P*	0.73
DL-Methionine	0.15		
Choline chloride	0.05		

**Calculated according to Wiseman (1987).

*Calculated according to NRC (1994).

[a]2.5 g vitamin premix: Vitamin A, 13.500 IU; vitamin D_3, 1.500 I.U; a-DL-tocopherolacetate, 50 mg; menadion, 2 mg; riboflavin, 5 mg; pyridoxin, 4 mg; cobalamine, 15 µg; folsaure, 200 µg; nicotenic acid, 60 µg;Ca-pantothenate, 30 mg; choline, 750 mg; ascorbic acid, 150 mg.

[b]1.25 g trace element premix: Fe, 125.00 mg; Cu, 10,00 mg; Mn; Se, 0.25 mg, Co, 0.20 mg.

Present address: [1]Masters Student,[2]Associate Professor (email: kmsislam1@yahoo.com), [3]Professor, Department of Poultry Science, [4,5]Masters Student, Department of Animal Nutrition, [6]Assistant Professor, Department of Anatomy and Histology.

Table 2. Effect of dietary CA, probiotic and their combination on productive performance of broiler at 35 days of age

Parameter	Dietary treatment				
	Control (0.5%)	Citric acid (0.001%)	Probiotic (0.5%)	Citric acid + probiotic (0.001%)	Significance
Initial body weight (g/ broiler)	54±0.6	56±0.8	55±0.2	56±0.8	NS
Final live weight (g/broiler)	1338^{b} ±17.9	1487^{c}±15.1	1363^{b}±15.5	1272^{a}±18.5	*
Body weight gain (g/broiler)	1284^{b} ±17.6	1431^{c}±15.7	1307^{b}±15.3	1216^{a}±17.9	*
Feed consumption (g/broiler)	2927^{a}±66.5	3074^{a}±34.2	3004^{a}±55.7	3338^{b}±21.0	*
Feed conversion ratio (feed/weight gain)	2.2^{a}±0.07	2.1^{a}±0.04	2.3^{a}±0.06	2.7^{b} ±0.05	*
Water consumption from 29–35days (ml/broiler)	1872^{a}±36.5	2157^{b}±96.9	2003^{ab}±74.8	1926^{a}±38.2	*
Dressing yield (%)	49.8±3.10	50.1±0.66	53.2±1.38	51.2±0.61	NS
Tibia ash%	55.3^{a}±0.89	60.0^{c}±0.74	57.1^{ab}±0.52	58.4^{bc}±0.21	*
Cost (British pound)/kg live weight	0.65^{a}±2.000	00.63^{a}±1.101	0.65^{a}±1.672	0.80^{b} ±1.554	*

Values of different variables under different programme indicates average ± SE; ^{abc,} means with dissimilar superscripts in row are significantly different; *, P<0.05; NS, P>0.05.

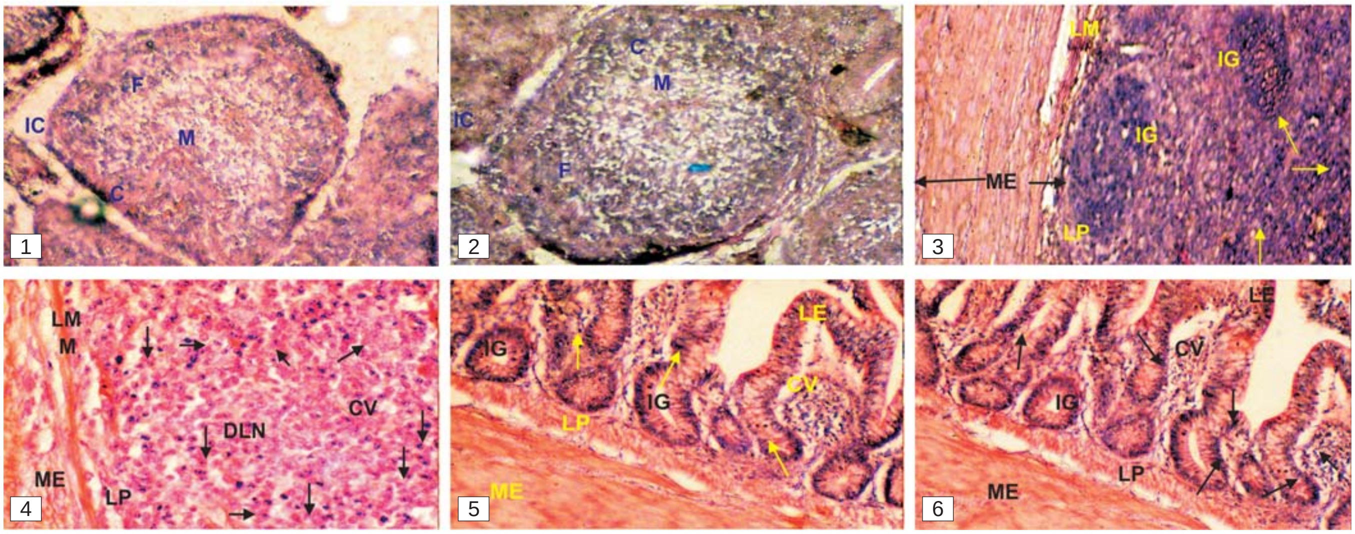
Growth performance and feed intake and feed conversion: The body weight gain recorded significant increase on CA than control. Body weight on probiotic diet was comparable with control whereas CA+ probiotic diet results significantly decreased body weight. Dietary acidification perhaps improved the gut health by decreasing pH of GI-tract and thereby inhibiting harmful intestinal bacteria and reducing toxic effects (Thompson and Hinton 1997). Thus it helped to improve (P<0.05) live weight. Such an assumption has been supported by histological evidence given in this study. Moreover, it has been supported by numerous previous studies of Abdel-Fattah *et al.* (2008) and Nezhad *et al.* (2007). The beneficial microorganisms present in the probiotic may also increase acidic condition of GI tract and ultimately improve the growth performance of broiler. Numerically increased of live weight gain (P>0.05) for addition of probiotic compared to control (Table 2), agreed with Vicente *et al.* (2007). The negative effect for combination of probiotic and CA on body weight was probably due to their antagonism. Increasing acidic condition for adding CA probably reduced the activity of the beneficial microorganisms present in the probiotic. Thus the effect of both supplements became neutralized and ultimately reduce the enzymatic activity and performance.

The combination of CA and probiotic enhanced (P<0.05) feed intake promisingly and CA stood in second position in this context, followed by probiotic and both were comparable with control group. The best feed conversion was attained by CA fed group but it was comparable with control. The poor FCR was noted on combination group (P<0.05) than control. Insignificant effect (P>0.05) of CA on the feed intake and feed conversion agreed with findings Moghadam *et al.* (2006). Lack of effect of CA (P>0.05) probiotic on feed intake and feed conversion catalogued is in harmony with result of Ladukar *et al.* (2001). The poor feed conversion efficiency observed in the combination group may possibly be attained to their antagonism.

Water consumption and meat yield: The water consumption (ml) per broiler significantly (P<0.05) increased on CA than control and also on other groups. Water consumption on probiotic, control and combination groups were comparable. No significant difference was observed in the dressing yield (without skin) in different dietary treatments; showed similarity with others like Abdel-Fattah *et al.* (2008) and Nezhad *et al.* (2007).

Tibia ash: The highest (8% more than control) tibia ash per cent was observed on CA group (P<0.05%), whereas 3% and 5% higher tibia ash was observed in probiotic and combination group than control. Significantly higher (P<0.05) blood Ca and P than control possibly be due to the increased absorption of such minerals from the gut into the blood stream, improving the utilization of Ca and P for provision of organic acids (Abdo 2004). Complex formation of acidic anion with Ca, P, Mg and Zn results in an improved digestibility of these minerals (Edwards and Baker 1999) and ultimately increase tibia ash deposition. Increased (P<0.05) tibia ash on 0.5% CA diet strongly agreed to other studies (Liem *et al.* 2008 and Boling *et al.* 2001). Probiotic addition related numerically increased tibia ash observed could not be compared paucity of published information.

Immunity: The population of immunocompetent cells in the cortex and medulla of bursal follicle, the lamina propria and sub-mucosa of caecal tonsil and ileum of CA supplemented broilers were more in comparison with control. The mean size of the bursal follicles was larger in CA treated group (499 µm × 271 µm) than control (474 µm × 251 µm) (Figs 1, 2). Similarly the mean size of caecal tonsil (282 µm × 205 µm) and ileum (247 µm × 214 µm) of CA treated group was larger than the size of caecal tonsil (260 µm × 189 µm) and ileum (211 µm × 194 µm) of control group respectively (Figs 3, 4). The lamina propria and sub-mucosa of caecal tonsils and ileums were more densely populated with lymphocytes (402 and 270 / 0.05 mm^2 area) in CA



Figs 1–6. **1.** The section of bursa of Fabricius (×40) of control group showing bursa follicle (F) composed of cortex (C) and medulla (M) and interfollicular connective tissue (IC). **2.** The section of bursa of Fabricius (×40) of citric acid supplemented group showing large bursa follicle (F) and distribution of the lymphocyte cells in the cortex (C) and medulla (M), interfollicular connective tissue (IC). **3.** The section of caecal tonsil (×40) of control group showing sparsely distributed lymphocytes (arrow), lamina propia (LP), mucosal externa (ME), intestinal gland (IG). **4.** The section of caecal tonsil (×40) of citric acid supplemented group showing lymphocytes (arrow), lamina propia (LP), lamina muscularis (LMM), disorganised lymphatic nodule (DLN), mucosal externa (ME) and serosa (S). **5.** The section of ileum (×40) of control group showing distribution of immunocompetent cells (arrow), core of the villi (CV), lamina propia (LP), lamina epithelium (LE), intestinal gland (IG) and muscularis externa (ME). **6.** The section of ileum (×40) of citric acid supplemented group showing distribution of immunocompetent cells (arrow), core of the villi (CV), lamina propia (LP), intestinal gland (IG), lamina epithelia (LE) and muscularis externa (ME).

supplemented group than control (354 and 196 / 0.05 mm^2 area) (Figs 5, 6). Higher density of immunocompetent cells indicates that, the broilers were healthy and possess higher immune status to fight against enteric pathogens and infectious diseases, and thus addition of CA through diet increased the immune response of broiler. Several studies concluded that dietary acidification increased digestive enzyme activities of the pancreas and intestinal mucosa (Jang *et al.* 2004) and improved the utilization and digestibility coefficient of protein (Abdo 2004) and resulted in remarkable increase in the small intestine morphology (weight and length). The impact of dietary acidification on small intestine densities (weight/length) has been considered as an indication of the intestinal villi dimension of mucosa layer (Palo *et al.* 1995). Supplementation of acidifier diet also increased the relative weight of lymphoid organs like spleen, bursa and thymus (Katanbaf *et al.* 1989), which act as a natural barrier against different harmful microorganisms (Abdel-Fattah *et al.* 2008).

Production cost: Addition of CA and probiotic created a progressive increase in feed cost in all treatments than control. Cost per kg live weight of broiler was lowest in CA group among the treatment group and control. Probiotic and combination group showed higher cost per kg live weight relative to control (Table 2).

Therefore it can be concluded that supplementation of probiotic and CA may improve the growth performance of broilers and unaffected feed intake, FCR and carcass yield but the CA may show better response than probiotic. The combination of CA and probiotic appears to be ineffective. Bone mineralization may increase by the CA and probiotic supplementation; CA addition might improve immune status of broiler.

SUMMARY

Day-old Hubbard Classic broiler chicks (160) were fed on a basal diet containing 229g CP, 3279 MJ ME/kg, 43g CF, 26g EE, 600g NFE, 106g ash, 9.9g Calcium and 7.3g total phosphorus per kg diet. The basal diets were fortified either with 0.5% citric acid (CA), 0.001%, probiotic or 0.5% CA + 0.001% probiotic to study the inclusion effect of CA and probiotic on growth, meat yield, bone mineralization and immune status of broiler. CA increased live weight by 11% while probiotic increased only 2% than that of control. However, combination treatment reduced live weight. Both CA and probiotic increased bone mineralization. Immune status was best on dietary CA. It was concluded that use of CA in broiler diet may improve growth, tibia ash and immune status in broiler.

ACKNOWLEDGEMENTS

The authors are thankful to the scientific staff and acknowledge to laboratory staff of the collaborative departments for excellent technical assistance.

REFERENCES

Abdel-Fattah S A, EI-Sanhoury M H, EI-Mednay N M and Abdul-Azeem F. 2008. Thyroid activity of Broiler chicks fed supplemental organic acids. *International Journal of Poultry Science* **7**: 215–22.

Abdo Zeinb M A. 2004. Efficacy of acetic acid in improving the utilization of low protein-low energy broiler diets. *Egyptian Poultry Science* **24**: 123–41.

Ahmad I. 2006. Effect of probiotic (Protexin) on the broilers Performance. *International Journal of Poultry Science* **5**: 593–97.

AOAC 1990. *Official Method of Analysis.*15 edn. Association of Official Analytical Chemists. Washingoton, DC: American Chemical Society.

Boling-Frankenbach S D, Snow J L, Parsons C M and Baker D H. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. *Poultry Science* **80**: 783–88.

Edwards H M and Baker D H. 1999. Effect of dietary citric acid on zinc bioavailability from soy products using an egg white diets with zinc sulfate hepatahydrate as the stander. *Poultry Science* **1**: 576.

Hamid M S and Aijazuddin Q. 2001. Trail study on the efficiency of protexin (water soluble) on the performance of broilers. *Pakistan Veterinary Journal* **21**: 224–25.

Jang I S, Ko Y H, Yang H Y, Ha J S, Kim JY, Kang S Y, Yoo D H, Nam D S, Kim D H and Lee C Y. 2004. Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens. *Asian-Australian Journal of Animal Science* **17**: 394–00.

Katanbaf M N, Dunnington E A and Siegel P B. 1989. Restricted feeding in early and late-feathering chickens, growth and physiological responses. *Poultry Science* **68**: 344–51.

Koenen M E, Kramer J, vander R, Hulst L, Heres S, Jeurissen H and Boersma W J. 2004. Immunomodulation by probiotic lactobacilli in layer-and meat-type chickens. *British Poultry Science* **45**: 355–66.

Ladukar M D, Mehta M K and Rane A S. 2001. Effect of commercial probiotic preparations on performance of broilers. *Indian Journal of Animal Nutrition* **18**: 357–62.

Liem A, Pesti G M and Edwards H M J. 2008. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. *Poultry Science* **87**: 689–93.

Moghadam A N, Pourreza J and Samie A H. 2006. Effect of different levels of citric acid on calcium and phosphorus efficiencies in broiler chicks. *Pakistan Journal of Biological Science* **9**: 1250–56.

Muzaffer D, Ferda O and Kemal C. 2003. Effect of dietary probiotic, organic acid and autibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan Journal of Nutrition* **2**: 89–91.

Nezhad Y E, Shivazad M, Nazeeradl M and Babak M M S. 2007. Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. *Journal of Veterinary Medicine, University of Tehran* **61**: 407–13.

NRC. 1994. National Research Council. *National Requirements of Poultry.* 9th Rev. edn. National Academy Press, Washington, DC. USA.

Palo P E , Sell J L, Piquer F J, Soto Satanova M F and Vilaseca L. 1995. Effect of early nutrient restriction on broiler chicken. Performance and development of gastrointestinal tract. *Poultry Science* **74**: 88–01.

Steel G D and Torrie J H. 1980. *Principles and Procedure of Statistics.* McGrow Hill Book Company, New York.

Thompson J L and Hinton M. 1997. Antibacterial activity of formic and propionic acids in diets of hens on salmonellas in the crop. *British Poultry Science* **38**: 59–65.

Vicente J L, Avina L, Torres-Rodriguez A, Hargis, B and Tellez G. 2007. Effect of a Lactobacillus Spp-based probiotic culture product on broiler chick performance under commercial conditions. *International Journal of Poultry Science* **6**: 154–56.

Wiseman J. 1987. *Feeding of Non-ruminant Livestock.* pp. 10. Butterworths and Co.Ltd., London.