



Effect of citric acid in low nutrient diet on growth and bone mineral metabolism of broiler

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Abstract

The study was conducted to observe the performance of broiler in low level of dietary nutrients but using citric acid (CA) as feed additive. A total of 240 day old straight run broiler chicks (COBB 500) were randomly distributed into eight groups, with three replicate cages having 10 birds in each. Control diet (Group 1) was formulated with corn-soybean based ingredients contained 22.7% CP and 3213 kcal ME/kg. Other dietary Groups 2, 3, 4, 5, 6, 7 and 8 were control+0.5% CA, control+4% low protein and energy, control+4% low protein and energy with 0.5% CA, control+8% low protein and energy, control+8% low protein and energy with 0.5% CA, control+12% low protein and energy and 12% low protein and energy with 0.5% CA, respectively. At the age of 31 days 8% lowering the protein and energy could compensate by the addition of citric acid (group 6). There was no significant difference of total feed intake among the groups. As live weight 8% lowering the protein and energy could compensate the feed efficiency when addition of CA. Tibia ash was determined in first four groups that were 44.2, 49.5, 46.8 and 47.1% in group 1, 2, 3, and 4, respectively. The mortality was 0.0% during the whole experimental period. The cost of production (BDT/kg broiler) was lowest in group 8, that means lowering protein and energy reduces feed cost. It may be concluded that diets containing low protein and energy up to 8% but addition of 0.5% citric acid would compensate the performance of broiler, but 12% reduction of energy and nutrients is cost effective.

Keywords: broiler, citric acid, low protein, low energy, performance

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Introduction

Continuous use of antibiotic in broiler diet as growth promoter may results the presence of antibiotic residues in meat. As a consequence, development of drug-resistant bacteria or other microbes in human body (Starr and Reynolds, 1951). Several alternatives such as organic acids, prebiotics, herb and herbal products, enzymes and essential oils have been recently used in poultry. Among those, organic acids (OAs) and probiotics are important alternative to antibiotics exclusively used as a growth promoter and for improvement of the feed conversion rate in farm animals (Esteive *et al.*, 1997). Organic acid in poultry diet effectively reduce production of toxin component by bacteria and a change the morphology of the intestinal wall and reduce the colonization of (Langhout, 2000). Dietary addition of organic acids (CA/acetic acid/lactic acid) improved feed conversion of broiler compared to those of un-supplemented diet (Abdel-Fattah *et al.*, 2008; Nezhadet *al.*, 2007).

Several studies reported that, addition of citric acid in broiler diet improved weight gain (Nezhad

et al., 2007), increased feed consumption rate (Moghadam *et al.*, 2006), and feed conversion efficiency (Abdel-Fattah *et al.*, 2008), increased retention of phosphorus and deposition of tibia ash. It also decreased pH of caecal digesta, crop, gizzard and intestine (Andryset *al.*, 2003, Denil *et al.* 2003). It reduces microbial load, increased dressing yield and showed better immune response in broilers (Atapattu and Nelligawatta, 2005, Gunal *et al.*, 2006, Rahmani and Speer, 2005). Addition of CA in low nutrient diet was found effective in compensating deficiency of nutrients (Das *et al.*, 2009). Chowdhury *et al.*, (2009) stated that CA is safe for human and can be used as growth promoter in broiler. Considering the facts the hypothesis of the study was that addition of CA will compensate the depressed performance due to lowering the nutrients in the diet.

Materials and Methods

The experiment was conducted in the poultry rearing unit of Sahjalal Animal Nutrition Field Laboratory, Bangladesh Agricultural University, Mymensingh for a period of 31 days using of 240 day old straight run broiler chicks (COBB 500).

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Table 1: Formulation of diet (kg/100kg) in different dietary treatments

Ingredients	1	2	3	4	5	6	7	8
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA	8% LP and LE	8% LP and LE + 0.5 % CA	12% LP and LE	12% LP and LE + 0.5 % CA
Maize	44.00	44.00	45.00	45.00	46.00	46.00	47.00	47.00
Wheat	5.40	5.40	7.30	7.30	9.70	9.70	10.30	10.30
Rice polish	2.75	2.75	3.75	3.75	5.75	5.75	7.75	7.75
Meat & bone	8.50	8.50	6.00	6.00	4.00	4.00	1.00	1.00
Soybean meal	27.75	27.75	28.25	28.25	27.25	27.25	28.55	28.55
Soybean oil	7.00	7.00	5.00	5.00	2.50	2.50	0.50	0.50
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.20	0.20	0.30	0.30	0.40	0.40	0.50	0.50
DCP	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vit-min premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Starch	0.50	0.00	0.50	0.00	0.50	0.00	0.50	0.00
Citric Acid	0.00	0.50	0.00	0.50	0.00	0.50	0.00	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Vitamin mineral premix: Vitamin A, 4,800,000 I.U/kg; Vitamin D₃, 1,000,000 I.U/kg; Vitamin-E 8,000 mg/kg, Vitamin-K₃ 1600 mg/kg, Vitamin-B₁ 600 mg/kg, Vitamin-B₂ 2000 mg/kg, Vitamin-B₃ 1600 mg/kg, Vitamin-B₆ 1600 mg/kg, Vitamin B₁₂ 4 mg/kg, Vitamin-PP 12,000 mg/kg, Biotin 20 mg/kg, Iron 9600 mg/kg, Copper 2400 mg/kg, Manganese 19,200 mg/kg, Cobalt 120 mg/kg, Zinc 16,000 mg/kg, Iodine 240 mg/kg, Selenium 80 mg/kg, Antioxidant 4000 mg/kg, Lysine 1.2%, Methionin 2%. Source: NOVAVIT-L (NOVA Nutrition, Belgium. CA, Citric acid ; LP, Low protein ; LE, Low energy.

Experimental design

Birds were randomly divided into 8 treatment groups having 3 replicate in each (10 birds each replicate). The eight dietary groups were 1-Control (CP-22.74%; ME-3213kcal/kg), 2-Control+0.5%CA, 3-4%LP and LE(CP-22%;ME-3097kcal/kg), 4-4% LP and LE+0.5%CA, 5-8% LP and LE(CP-21.04%;ME-2962kcal/kg), 6-8% LP and LE + 0.5%CA, 7-12% LP and LE(CP-20.32%; ME-2843kcal/kg), 8-12% LP and LE+0.5 %CA. Formulation of different diets and their chemical composition are given in the Table 1 and 2, respectively.

Management practices

Fresh dried rice husk was spread on the floor under the cages at a depth of 4cm and managed properly. After arrival of chicks in the experimental house, they were supplied 5.0% glucose solution to minimize transportation stress. For the control of temperature and light, a 100 watt electric bulb was used for each cage. Electric light was provided in the trial house for 24 hours. Feeds and water were supplied to all broilers on *ad libitum*. Birds from three replicate

cages from each treatment were separately vaccinated against ND at 4th day of age, Gumboro disease vaccine at 11th day of age and no vaccine at all respectively. Booster dose of vaccine for ND was again administered at 20th day of age in the first replication of each treatment.

Broilers were weighted in a group at the beginning of the trial and then every week at the age of day 11, 17, 24 and 31. Feed offered were recorded when supplied in cages and refusal at the end of each week also recorded. Due to the fact that there is no death of bird occur during the experiment period so there is no data was recorded for dead bird. Tibia ash (%) was determined by following method described in AOAC (1990)

The major inputs were cost for day old chick, feed, citric acid and other management. Live broiler which was sold per kg live weight was the output. The profit was then calculated by subtracting return (price*total live weight of broiler) from total cost.

Table 2: Chemical composition of different diets (g/100g) in different dietary treatments

Ingredients	1	2	3	4	5	6	7	8
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA	8% LP and LE	8% LP and LE + 0.5 % CA	12% LP and LE	12% LP and LE + 0.5 % CA
Dry Matter (%)	89.4	89.4	88.9	88.9	88.5	88.5	88.0	88.0
Crude Fiber (%)	3.67	3.67	3.66	3.66	3.79	3.79	3.89	3.89
Crude Protein (%)	22.74	22.74	22	22	21.04	21.04	20.32	20.32
Calcium (%)	1.46	1.46	1.36	1.36	1.28	1.28	1.15	1.15
Phosphorus (%)	0.71	0.71	0.68	0.68	0.67	0.67	0.64	0.64
Lysine (%)	1.40	1.40	1.45	1.45	1.47	1.47	1.53	1.53
Metheonine (%)	0.73	0.73	0.72	0.72	0.71	0.71	0.70	0.70
Metabolizable Energy (kcal/kg)	3213	3213	3097	3097	2962	2962	2843	2843

CA, Citric acid; LP, Low protein; LE, Low energy.

Results

Statistical analysis

All data were analyzed by using statistical SPSS.11 program for one way analysis of variance (ANOVA) and Duncans Multiple Range Test (Duncan, 1955) was done to know the differences among the treatment means at 5.0% level of significance (Steel and Torrie, 1980).

Growth performance

During 1st, 2nd and 3rd weeks of ages the live weight of birds are significantly different among dietary groups (P<0.05) shown in Table 3. At the end of the trial, except group 5 all the groups showed similar live weight (P<0.05). At the end of the trial, the live weight gain of broiler was 1036, 1047, 955, 997, 925, 992, 959 & 980g in group 1, 2, 3, 4, 5, 6, 7 & 8 respectively follows similar trend like final weight.

Table 3: Live weight (g) and live weight gain of broilers in different dietary groups at different ages

Groups	1	2	3	4	5	6	7	8
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA	8% LP and LE	8% LP and LE + 0.5 % CA	12% LP and LE	12% LP and LE + 0.5 % CA
Live weight (g) in different age								
Initial weight	250 ^a ± 2.53	250 ^a ±3.06	250 ^a ±1.91	250 ^a ±3.62	250 ^a ±3.75	249 ^a ±3.20	251 ^a ±2.50	253 ^a ±.87
17 Days	527 ^d ±3	527 ^d ±3	502 ^{ab} ±6	531 ^d ±8	496 ^a ±5	520 ^{cd} ±9	501 ^{ab} ±6	511 ^{bc} ±8
24 days	894 ^b ±21	897 ^b ±22	842 ^a ±14	874 ^{ab} ±10	839 ^a ±33	859 ^{ab} ±12	838 ^a ±30	857 ^{ab} ±16
31 Days	1285 ^b ±60	1297 ^b ±40	1205 ^{ab} ±54	1246 ^{ab} ±36	1176 ^a ±45	1241 ^{ab} ±43	1210 ^{ab} ±72	1232 ^{ab} ±69
Cumulative weight gain								
11 to 31 days	1036 ^b ±62	1047 ^b ±42	955 ^{ab} ±54	997 ^{ab} ±38	925 ^a ±47	992 ^{ab} ±41	959 ^{ab} ±70	980 ^{ab} ±68

CA, Citric acid; LP, Low protein; LE, Low energy. Value indicate mean ± SD; ^{abc}Means with dissimilar superscripts are significantly different (P<0.05).

Table 4: Feed Intakes (g) of birds in different weeks receiving different dietary treatments

Groups	1	2	3	4	5	6	7	8
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA	8% LP and LE	8% LP and LE + 0.5 % CA	12% LP and LE	12% LP and LE + 0.5 % CA
Feed intake (g)								
11-17 Days	458 ^a ±7	481 ^{bc} ±4	493 ^c ±9	479 ^{abc} ±18	495 ^c ±16	461 ^{ab} ±12	500 ^c ±10	479 ^{abc} ±15
18-24 Days	739 ^c ±15	718 ^{abc} ±14	700 ^{ab} ±11	691 ^{ab} ±16	709 ^{abc} ±7	723 ^{bc} ±13	688 ^{ab} ±40	682 ^a ±18
25-31 Days	876 ^a ±2	868 ^a ±21	876 ^a ±31	866 ^a ±17	865 ^a ±31	856 ^a ±6	894 ^a ±14	894 ^a ±13
Cumulative feed intake (g)								
11 to 31 days	2073 ^a ±12	2068 ^a ±16	2069 ^a ±30	2036 ^a ±4	2069 ^a ±30	2040 ^a ±14	2082 ^a ±36	2055 ^a ±41

CA, Citric acid; LP, Low protein; LE, Low energy. Value indicate mean ± SD; ^{abc}Means with dissimilar superscripts are significantly different ($P < 0.05$).

Table 5: Feed conversion ratio (FCR-Kg Feed intake per Kg lives weight gain) of birds in different weeks receiving different dietary treatments

Groups	1	2	3	4	5	6	7	8
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA	8% LP and LE	8% LP and LE + 0.5 % CA	12% LP and LE	12% LP and LE + 0.5 % CA
FCR (Kg Feed intake per Kg lives weight gain)								
11-17 Days	1.65 ^a ±0.03	1.74 ^{ab} ±0.02	1.95 ^{cd} ±0.02	1.71 ^a ±0.13	2.02 ^d ±0.09	1.70 ^a ±0.05	2.00 ^d ±0.07	1.86 ^{bc} ±0.11
18-24 Days	2.02 ±0.08	1.94 ±0.10	2.06 ±0.06	2.02 ±0.10	2.08 ±0.18	2.13 ±0.07	2.05 ±0.25	1.97 ±0.09
25-31 Days	2.28 ±0.38	2.18 ±0.16	2.44 ±0.29	2.35 ±0.25	2.57 ±0.07	2.26 ±0.22	2.49 ±0.54	2.43 ±0.40
Cumulative FCR (Kg Feed intake per Kg lives weight gain)								
11 to 31 days	2.01 ^a ±0.11	1.98 ^a ±0.08	2.17 ^{ab} ±0.09	2.05 ^{ab} ±0.08	2.24 ^b ±0.09	2.06 ^{ab} ±0.09	2.18 ^{ab} ±0.16	2.11 ^{ab} ±0.19

CA, Citric acid; LP, Low protein; LE, Low energy. Value indicate mean ± SD; ^{abc}Means with dissimilar superscripts are significantly different ($P < 0.05$).

Feed intake of bird under different groups during 1st, 2nd and 3rd weeks of trials differed significantly ($P < 0.05$) from each other but feed intake of group 7 was highest at 1st and 3rd weeks of trials shown in table 4. But cumulative feed intake of birds found similar in all the groups. At the end of the experiment feed conversion ratio (FCR= kg feed intake/ kg LWG) of the broiler were 2.01, 1.98, 2.17, 2.05, 2.24, 2.06, 2.18 and 2.11 in group 1, 2, 3, 4, 5,

6, 7 & 8 respectively (Table 5). Best FCR was observed in group 2 compared to control and others.

Highest tibia ash (%) was found in CA treated group compared to the control group (Table 6). Complex formulation of acidic anion with Ca, P, Mg, and Zn results in an improved digestibility of these minerals and ultimately increased tibia ash deposition.

Table 6: Tibia Ash% of birds in different dietary treatment groups

Group	1	2	3	4
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA
Ash (g)	1.02	1.04	0.94	0.99
Dry Matter (g)	2.31	2.10	2.01	2.10
Ash %	44.2	49.5	46.8	47.1

CA, Citric acid; LP, Low protein; LE, Low energy.

Economic analysis

Production cost was calculated by considering cost of bird, feed and CA (Table 7). The cost per kilogram feed was lowest in group 7 than others. Production cost per kilogram live weight of broiler was lowest (P<0.05) in group 8. Feed cost per kg bird was also highest in group 3 (BDT 96) and lowest in group 8 (86).

Discussion

Growth performance

The result in the present study revealed that, dietary CA can compensate the performance of broiler due to reducing nutrients level in diet. The probable reason may be the CA in diets could improve nutrient digestibility (Ziaei *et al.*, 2000) and prevents the growth of harmful microorganisms which give better performance and recover the deficiency of nutrients (Naidu, 2000 and Wolfenden *et al.*, 2007). Other researcher also found that, citric acid have positive effect (P<0.05) on live weight gain of broiler (Chowdhury *et al.*, 2009; Moghadam *et al.*, 2006 and Islam, 2007), which is also support by this study.

Relatively higher weight gain observed in group 2 than control (1047g) during 31 days of age. In case of weight gain CA fed groups showed better performance than non-CA group. The result is consistent with the finding of other researchers (Chowdhury *et al.*, 2009, Shenet *et al.*, 2005; Ivanov 2005 and Snow *et al.*, 2004) who reported that, inclusion of CA in broiler diet improved weight gain, but here compensated the lowering the nutrient content. So, the deficiency of protein and energy in diet was recovered by the better absorption of nutrients due to using 0.5% CA in

experimental diet then non-CA group. In general there was no significant difference for feed intake among the groups. The result is similar to the findings of Atapattu and Nelligaswatta, (2005) who found that in broiler chickens fed rice by-products based diet with CA (1 and 2%), though not significant, 2% dietary citric acid increased the feed intake which resulted in poor FCR.

Addition of CA in broiler by reducing protein and energy level improved feed conversion efficiency (FCE) than control (P>0.05). It was reported by Andryset *al.*, (2003); Shenet *al.*, (2005) and Chowdhury *et al.*, (2009) that the addition of CA increased FCR of broilers.

Tibia ash

There are several findings that the CA addition in diet increased bone mineral content, bone strength in different levels (Atapattu and Nelligaswatta, 2005; Islam *et al.* 2012; Haque *et al.* 2010). The increased mineral level in the bone is also related to the availability of the minerals in the blood, which is responsible for bone formation (Islam, 2012). In this study both bioavailability and its expression in blood as well as in bone is clear in this regards. So, replacement of commercial diet by rice bran is also found feasible not only for the performance but also for the mineral density in bone due to addition of CA in diet

This results of the bone ash of this present study agrees with the findings of Liem *et al.*,(2008), who reported significant effect of CA on bone ash percent of broilers. The results also coincide with the other researchers (Nezhadet *et al.*, 2007; Moghadam *et al.*, 2006).

Economic analysis

Reduction of protein and energy in diets result a progressive decrease in feed cost in all the dietary groups compared to that of control. Here we also can see that the CA group has more profit than non-CA group. The result indicates commercial importance of the use of CA as antimicrobial feed additives in broiler diet as it reduces production cost by ensuring better growth of birds. Under the above circumstances it may be concluded that diets containing low level of protein and energy up to 12% with 0.5% CA can compensate the performance of broiler and increase profits by reducing feed cost.

Table 7: Economic study (in BDT) of broiler production in different dietary treatments

Group	1	2	3	4	5	6	7	8
	Control	Control + 0.5 % CA	4% LP and LE	4% LP and LE + 0.5 % CA	8% LP and LE	8% LP and LE + 0.5 % CA	12% LP and LE	12% LP and LE + 0.5 % CA
Feed cost (per kg)	46.29	47.36	44.26	45.34	42.06	43.14	39.80	40.87
Feed cost/kg Weight gain	93.03	94.10	96.12	93.45	94.37	89.78	87.34	86.87
Cost (Feed+chick)/ bird	174.60	176.60	170.70	170.80	165.80	165.30	159.90	161.20
*Profit per bird	18.19	17.93	10.02	16.10	10.60	20.88	21.53	23.56

CA, Citric acid; LP, Low protein; LE, Low energy. *Market price 150.00BDT/kg BW.

Conclusion

From previous study it has shown that when citric acid added in broiler diet increased performance of broiler due to different positive effects of citric acid. From this study it may be concluded that reducing 8% protein and 8% Metabolizable energy would be possible without hampering any performance if diet contains 0.5% citric acid, but further reduction (12%) of energy and protein found cost effective as it reduces the cost of feed related to nutrient content.

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Conflict of interest

The authors would like to declare that there is no conflict of interest.

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