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Performance and immunity of broiler due to addition of citric acid in low nutrient diet

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ABSTRACT

The study was conducted for 35 days to observe the performance and immune status of broiler providing low level of nutrients but using citric acid (CA) as feed additive. Day-old broiler chicks (216) were distributed into 4 dietary groups (6 replicates cages having 9 birds in each) as 1,control (22.1% CP and 2961 Kcal ME/kg); 2, low protein+0.5% CA; 3,low energy+0.5% CA; and 4,low protein and energy+0.5%CA. At the end of the trial, broiler chicks of the groups 1, 2, 3 and 4 gained their body weight 1608±23.03, 1635±26.41, 1638±30.21 and 1652±24.60 g respectively. Total feed intake was highest in low energy diet. Feed conversion ratio (FCR-kg feed intake/kg weight gain) varied among the groups, which was low in group 4. Due to addition of CA, the pH of diets reduced (5.91, 5.20, 5.33 and 5.43 in 1, 2, 3 and 4 respectively) but the changes disappeared in feaces expressing the pH level of 5.66, 5.62, 5.69 and 5.67 respectively. Cost of production per kg live weight of broiler was numerically lower in group 4, where both protein and energy was low and CA was added. In each case, 0.5% CA improved immunity to the broilers of the groups vaccinated against Newcastle disease. Therefore, supplementation of 0.5% CA in low nutrient diet (low protein and low energy) reflected similar weight gain of broiler with further benefits in the elevation of immunity. But further research is needed to find out the limit of lowering the protein and energy in diet by using certain level of CA.

Key words: Broiler, Citric acid, Energy, Immunity, Performance, Protein

Worldwide, antimicrobial feed additives are used in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, decreasing feed expenditure per gain and diminishing the risk of disease (Hays 1981, Gropp *et al*. 1992). But the unavoidable spread of bacterial resistance and cross-resistance to antibiotics used in veterinary and human therapy (Barton 1998, Khachatourians 1998) by the use of antibiotics is increasingly considered as a hazard, therefore the approval of antimicrobial growth promoters was phased out by many countries. Organic acids are important alternative to antibiotics exclusively used as growth promoter and for improvement of feed efficiency of farm animals (Esteive *et al.* 1997). European Union approved citric acid (CA), an organic acid, as feed preservative. It shows enough antimicrobial action in the gastrointestinal tract and ultimately improves the growth rate of poultry (Eidelsburger and Kirchgessner 1994). It also improves feed conversion efficiency as well as absorption of minerals (Moghadam *et al*. 2006, Nezhad *et al*. 2007, Chowdhury *et al.* 2009). It decreases pH of gastro-intestinal tract of broiler chicks (Jozefiak and Rutkowski 2005, Rahmani *et al.* 2005). It helps to alleviate the immune response of broiler (Abdel-Fattah *et al.* 2008, Rahmani *et al.* 2005). Considering these facts, CA is safe for human consumption and can be used as growth promoter in broiler production up to 0.5% level in their rations (Chowdhury *et al*. 2009). Now-a-days commercial diets are formulated providing higher amount of nutrients and additives that the growth arrived as per genetic potentiality, but increase the cost of feed. Further addition of CA would not show the potential effect on growth performance. So, the study was undertaken to know the growth and immune status of broiler using CA in low nutrient diet.

MATERIALS AND METHODS

Day-old (264; 42±1.1g/bird) broiler chicks (Hubbard Classic) were randomly divided into 4 groups having 6 replicate cages in each (9 birds per cage). The dietary groups were (i) control (22.1% CP and 2961 KcalME/kg), (ii) low protein + 0.5% CA, (iii) low energy + 0.5% CA, and (iv)

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Table 1. Formulation of feeds and their chemical composition (g/100g)

Formulation	Dietary groups[#]			
	1 (Control)	2 (LP) [a]	3 (LE) [a]	4 (LP and LE) [a]
Maize	40.15	47.65	43.55	49.00
Rice polish	15.00	13.00	15.00	14.50
Soybean meal (CP 44%)	30.80	25.00	28.90	26.42
Citric acid	0.00	0.50	0.50	0.50
Meat and bone meal (CP 45%)	7.00	7.00	8.00	5.00
Soybean oil	3.00	2.80	0.00	0.00
Oyster shell	0.10	0.10	0.10	0.30
DL-methionine	0.30	0.30	0.30	0.30
DCP	2.00	2.00	2.00	2.00
L-lysine	0.10	0.10	0.10	0.10
Vit-mineral premix[b]	1.00	1.00	1.00	1.00
Common salt	0.50	0.50	0.50	0.50
Choline chloride	0.05	0.05	0.05	0.05
Total	100	100	100	100
Composition (%)				
Dry matter	88.0	88.0	88.0	86.0
Crude protein	22.1	19.9	22.0	19.8
Crude fiber	4.35	3.75	3.95	4.20
Ether extract	2.85	2.40	2.35	2.55
Nitrogen free extract	47.07	51.43	48.40	51.15
Crude ash	11.63	10.52	11.30	10.30
ME (Kcal/kg DM)[c]	2961	2988	2791	2817

[#]1,Control (22.1% CP and 2988 kcal ME/kg); 2, Low protein; 3,Low energy; 4,Low protein and low energy [a]addition of 0.5% citric acid (5 g/kg feed)

[b]Vitamin mineral premix: Vitamin A, 4 800 000 IU/kg; D_3, 1 000 000 IU/kg; E, 8,000 mg/kg, K_3 1600 mg/kg, B_1 600 mg/kg, B_2 2000 mg/kg, B_3 1600 mg/kg, B_6 1600 mg/kg, B_{12} 4 mg/kg, 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.

low protein and low energy + 0.5% CA (Table 1). Diets were formulated using maize, rice polish, soybean meal, meat and bone meal, soybean oil, oyster shell, DL-Methionine, di-calcium phosphate, L-lysine, vitamin-mineral premix, common salt and choline chloride (following nutrient recommendation guide for broiler). Feeds and water were supplied to all birds on *ad lib* basis. Birds were reared carefully considering animal welfare guideline (Harlan 1986).

Birds from three replicate cages from each group were vaccinated through ocular method against Newcastle disease at the age of day 7 and booster dose at day18. It was mentioned that maternal antibody in chicks derived from vaccinated breed is retained up to 12 days (Chowdhury *et al.* 1982) and 15 days (Islam *et al.* 2003). Saeed *et al.* (1988) also reported that, maternal derived antibody decline to zero 15–20 days and totally zero at day 25.

The sample of feed and feaces were taken for determination of pH. Blood sample from day-old-chick was collected directly from the heart. At day 17 and 28 of age, blood was also collected from the wing vein. The serum from the blood was centrifuged at 1,500 rpm for 15 min to obtain clear serum. After inactivation of the serum at 56°C for 30 min in water-bath it was preserved at –20°C until used (Samad 2001).

Blood from non-vaccinated chicks was collected from wing vein using 4% tri-sodium citrate (1:10) and subjected to washing with phosphate buffer saline (PBS) by centrifugation at 1 000 rpm for 10 min. Packed final RBC was found after 3 consecutive washing; 0.25 ml of the pelleted RBC was mixed to 49.75 ml of PBS to prepare 50 ml of 0.5% RBC suspension. Heamagglutination inhibition (HI) test was performed to determine the antibody titre of the sera collected from different groups of the chicks. The test was conducted by using constant virus (4 HA unit) and decreasing serum antibody level.

Feed samples were analyzed for proximate components according to AOAC (1990). Data were analyzed by using statistical SPSS 11.0 program for one way analysis of variance (ANOVA). Duncan's multiple range test was done to know the differences among the groups at 5% level of significance (Duncan 1955, Steel and Torrie 1980).

RESULTS AND DISCUSSION

Initial weight was similar in all the groups (42±1.1g/chick) but during the age of day 14, 21 and 28 the live weight of bird was different ($P<0.05$) among the groups (Table 2). Finally at the age of 35, the birds of the group 4 attained highest weight, which was 2.74% higher than control and almost similar to the birds of group 2 and 3, although was different significantly ($P>0.05$). This study revealed that, although reducing either protein or energy or both in diet but addition of 0.5% CA showed similar performance as control. The probable reason may be the CA in diets could improve nutrient digestibility (Ziaei *et al.* 2000 and Atapattu and Nelligaswatta 2005) and prevents the growth of harmful microorganisms (Naidu 2000; Wolfenden *et al.* 2007) which helps to maintain the performance even lowering the protein and energy level. Findings of other researcher also agreed and indicated that the CA has positive effect on live weight of broiler (Chowdhury *et al.* 2009, Deepa *et al.* 2011).

Feed intake seems significantly higher in low energy diet ($P<0.05$). Ultimately low in both protein and energy diet reflected better feed conversion efficiency at the end of the trial ($P<0.05$). In the present study, feed intake of broilers of different experimental groups was significantly increased ($P<0.05$). This might be due to low energy content in diet, because feed intake was more to recover the energy requirements (Plavnik *et al.* 1997, Nahashon *et al.* 2005). Better feed conversion ratio was observed in the birds of group 4 compared to other groups ($P>0.05$), which would be

Table 2. Live weight (g), live weight gain (g), feed intake (g) and feed conversion ratio of broilers in different dietary groups

Parameters	Dietary groups#				
	1(Control)	2(LP) [a]	3(LE) [a]	4(LP and LE) [a]	Sig.
Day-old-weight on d 14	42±1.1325[a]±4.80	42±1.1329[ab]±6.31	42±1.1335[b]±6.90	42±1.1345[c]±11.30	NS*
Weight on d 21	666[a]±7.97	676[ab]±11.01	685[b]±8.70	690[b]±13.84	*
Weight on d 28	1131[b±]14.98	1144[ab]±21.86	1128[b]±11.56	1159[a]±26.05	*
Weight on d 35	1608±23.03	1635±26.41	1638±30.21	1652±24.60	NS
Total BWG	1561±23.19	1587±26.08	1591±29.78	1604±24.61	NS
Feed intake	3532[b]±53.74	3548[b]±16.37	3615[a]±43.89	3532[b]±47.85	*
FCR(kgFI/kgLWG)	2.26[a]±0.03	2.24[ab]±0.03	2.27[a]±0.06	2.20[b]±0.04	NS

[abc]·means with dissimilar superscripts are significantly different (P<0.05)*; NS: not significant (P>0.05).

Table 3. pH of diet and feaces of birds of different dietary groups

Parameters	Dietary groups#			
	1 (Control)	2 (LP) [a]	3 (LE) [a]	4 (LP and LE) [a]
pH in diet	5.91	5.20	5.33	5.43
pH of feaces	5.66	5.62	5.69	5.67

Table 4. Result of HI test of different group of chicken vaccinated with NDV vaccine: (Average antibody titre)

	Dietary groups#			
	1 (Control)	2 (LP) [a]	3 (LE) [a]	4 (LP and LE) [a]
0 day*				
Non-vaccinated	80.0	80.0	80.0	80.0
Vaccinated	80.0	80.0	80.0	80.0
17 days**				
Non-vaccinated	4.00	6.00	4.00	8.00
Vaccinated	22.40	42.67	96.00	42.00
28 days***				
Non-vaccinated	<4.00	<4.00	<4.00	<4.00
Vaccinated	22.00	44.00	148.00	96.20

*Maternal antibody titre (MAT); **average antibody titre after first vaccination; ***average antibody titre after second vaccination.

the reflection of result of weight gain and feed intake supported by other researchers (Eidelsburger and Kirchgessner 1994, HuiFang *et al.* 2005, Chowdhury *et al.* 2009).

Due to addition of CA, the pH of diets reduced but the changes disappeared in feces expressing the similar pH level shown in Table 3. Nourmohammadi *et al*. (2011) found that the addition of CA in broiler diet decrease the pH of feed which increased towards lower gut of the bird, these findings are similar to the present result. Different biochemical reactions and buffering capacity changed the pH of feed ingested throughout the tract. Overall mortality was 0.9% which is lower than accepted limit of commercial broiler. Only 2 birds died belonged to the groups of 2 and 3 during age of day 35.

Highest antibody titre (GMT value was 80 HI) was in the serum of day-old birds (Table 4) which was maternally derived antibody (MDA). The MDA titre was disappeared in the non-vaccinated group of birds on day 17. In vaccinated groups, serum titre against ND was highest in group with diet containing LE and CA (96.00 HI) followed by 42.67 and 42.00 in group 2 and 4, respectively, but in control group it was only 22.40. At the age of 28, the HI titre was highest (148) in group fed diet contained LE with CA followed by 96, 44 and 22 in group 4, 2 and 1 respectively.

Schmidt and Schmidt (1955) reported the minimum protection level of serum HI antibody titre i.e 32. They also mentioned that the HI titre of serum above 32 resisted the chickens against challenge with virulent NDV. So, the diet containing CA has potentiality to resist the virulent challenge by helping the increasing level of antibody titre in the broiler. The CA enhance antibody titre of vaccinated birds, because CA increase digestibility of crude protein (Atapattu and Nelligaswatta 2005) which increase the amino acid level and protein (globulin) in blood stream are important to produce antibody upon introducing antigen/vaccine.

Reduction of protein and energy in diets result a

Table 5. Economic study (in BDT) of broiler production in different dietary treatments

	Dietary groups#			
	1 (Control)	2 (LP) [a]	3 (LE) [a]	4 (LP and LE) [a]
Cost/kg feed[@]	27.35	27.24	26.10	25.00
Cost(feed/broiler)	96.59	96.65	98.21	88.28
Cost (feed+chick)/ broiler	121.59	121.65	123.21	113.28
Cost (feed+chick)/kg live weight	77.88	76.65	77.47	70.62
*Sale revenue/bird	128.64	130.80	131.04	132.16
Profit/bird	7.05	9.15	7.83	18.88

[@], price of feed during November 2009.*Market price of broiler Tk.80 per kg.

progressive decrease in feed cost compared to control (Table 5). The cost of chick and feed (BDT/kg broiler) was lowest in 4 (70.62) which were responsible for highest profit of that group. As other cost remain similar so profit per bird calculated as deducing the cost of feed and chick.

It is clear that the diet which contains low level of protein and energy the performance might be maintained by the addition of 0.5% CA. Moreover, the CA enhances to increases the antibody level against ND which is a clear indication of improving immunity of birds. Further research is needed to find out the limits of lowering the protein and energy in diet by using certain level of CA.

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