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# Effect of Feeding Citric Acid on Performance of Broiler Ducks Fed Different Protein Levels

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ABSTRACT

The present study was performed to investigate the effect of feeding citric acid with different protein levels on duck performance, carcass traits and blood parameters. A total number of 40 two weeks old Molar ducklings were randomly distributed into 4 equal groups, each of 10. The control diet was formulated to contain approximately crude protein (CP) (16%) and metabolizable energy (3000 kcal/kg diet) as recommended by NRC (1994). The first group was fed on control diet (16% CP) without any feed additives, while groups 2, 3 and 4 (T2, T3 and T4) were fed on basal diets containing 16, 14 and 12% CP respectively and supplemented with citric acid at 1.50% of the grower-finisher diet. The results showed that, birds fed on 16% protein diet supplemented with citric acid recorded significantly the best live body weight, body weight gain, feed intake and feed conversion compared with other treatments. There were no significant differences in hot carcass percentage, eviscerated percentage, dressing percentage and relative percentage of internal organs (gizzard, heart, liver and spleen) between different experimental groups. There were no significant differences in the level of serum total protein and triglycerides, while there were significant ($P<0.05$) differences in serum albumin, globulin, cholesterol and uric acid between control group and other treatments. There were no significant differences in the intestinal pH between different experimental groups. The relative economic feed efficiency was the highest in birds fed 16% protein diet supplemented with citric acid compared with other treated groups. It could be concluded that, dietary inclusion of 1.50 % citric acid in 16% protein diet improved body weight, weight gain, feed intake and feed conversion ratio and carcass traits.

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## Introduction

Beneficial effects of dietary additives such as probiotics, prebiotics and organic acids, on the energy and protein utilization of poultry have been reported (Samarasinghe *et al*., 2003; Angel *et al*., 2005; Pirgozliev *et al*., 2008; Yang *et al*., 2008). It has also been suggested that, feed additives may be more efficient when low nutrient diets are fed. Generally, low density diets are more profitable and resulted in less environmental pollution problems. In recent years, the high price of protein sources as well as environmental concerns related to high nitrogen excretion have resulted in increasing interest for using low protein diets in poultry production (Torres-Rodriguez *et al*., 2005).

Organic acids have been used for decades in feed preservation, either for protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed, e.g. silage. Organic acids are not antibiotics but, if used correctly along with nutritional, managerial and biosecurity measures, they can be a powerful tool in maintaining the health of the gastrointestinal tract of poultry, resulting in improving their perform-

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ances. Feeding of organic acids may suppress the growth of certain species of bacteria, particularly acid intolerant species such as *E. coli*, *Salmonella sp.* and *Campylobacter sp.* (Ricke, 2003; Dibner, 2004). Their principle rule is to lower and supplies the pH in the stomach and intestines so that the gut environment is too acidic for normal bacterial growth. Additionally, they improve protein digestion in young animal by stimulating pancreatic enzyme secretion (Mellor, 2000). Thus, dietary organic acids can suppress the growth of pathogenic bacteria, encourage the growth of beneficial microflora and ensure that, the enzymes function is at maximal capacity (Broek, 2000; Dibner and Winter, 2002; Ricke, 2003; Dibner, 2004).

Practically, organic acids work in poultry not only as a growth promoter but also as a meaningful tool of controlling all enteritis bacteria, both pathogenic and non-pathogenic (Naidu, 2000; Wolfenden *et al*., 2007). Moreover, feeding organic acids is believed to have several beneficial effects such as improving feed conversion ratio, growth performance, enhancing minerals absorption and speeding recovery from fatigue (Gornowicz and Dziadek, 2002) and also provided people with healthy and nutritious poultry products (Patten and Waldroup, 1988).

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 (Chowdhury *et al*., 2009).

## Materials and methods

*Birds, housing and feeding*

A total number of 40 two weeks old Molar ducklings were weighed (286±3.60) and randomly distributed into 4 equal groups, each of 10. Ducks were reared under similar environmental and managerial conditions during the period from 2-10 weeks of age.

The first group was fed a diet free from citric acid (CA) and considered as control. The other three groups were fed on diets with different protein levels (16, 14 and 12%) supplemented with citric acid at level of 1.50%.

The ducklings in the four groups were fed *ad libitum* on the respective diets in pellet form and given free access to fresh and clean water (Table 1).

*Measurements*

Performance characteristics including body weight, body weight gain, feed intake and feed conversion ratio were calculated. The proximate analysis of the experimental feeds was performed using procedures detailed by the Association of Official Analytical Chemistry (AOAC, 1990).

*Carcass Traits*

At the end of the experiment, three birds from each group were randomly taken, individually weighed and slaughtered by severing the carotid artery and jugular veins. After four minutes of bleeding, each bird was dipped in a water bath for two minutes and feathers were removed by hand. After the removal of head, carcasses were manually eviscerated to determine some carcass traits including dressing % (eviscerated carcass without head, neck and legs) and giblets % (gizzard, liver, spleen and heart). The organ weight was expressed as relative weight proportionate to pre-slaughter live body weight.

*Serum samples and biochemistry*

At the end of the experiment, three randomly selected birds from each group were slaughtered after fasting overnight. Blood samples were collected from the selected birds of each treatment, allotted to clot at ambient temperature, centrifuged for 15 minutes at 3000 rpm and serum from each sample was extracted. The serum samples were kept at -20 °C until further analysis. Serum samples were assayed for estimation of total protein and its fractions (albumin and globulin), triglycerides, cholesterol and uric acid by spectrophotometer using commercial test kits (Spectrum, Cairo, Egypt).

*Measurement of pH in gastrointestinal content*

Values of pH in contents from different parts of gastrointestinal tract were measured immediately by using a digital pH meter. To determine the pH, 10 g of gut content from duodenum, jejunum and ileum were collected aseptically in 90 ml sterilized

Table 1. Composition and energy value of the experimental diets.

| Item | Treatments | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | T4 |
| Physical composition (%) | | | | |
| Yellow corn, ground | 51.30 | 51.20 | 58.21 | 62.65 |
| Soybean meal | 18.30 | 18.30 | 12.38 | 6.35 |
| Wheat bran | 20.00 | 20.00 | 20.00 | 22.00 |
| Sunflower oil | 7.15 | 7.15 | 5.94 | 5.51 |
| Citric acid | --- | 1.50 | 1.50 | 1.50 |
| Sodium phosphate dibasic | 1.30 | 1.30 | 1.35 | 1.35 |
| Limestone, ground | 1.35 | 1.35 | 1.40 | 1.40 |
| Common salt | 0.30 | 0.30 | 0.30 | 0.30 |
| Methionine | --- | --- | 0.02 | 0.04 |
| Premix* | 0.30 | 0.30 | 0.30 | 0.30 |
| Calculated chemical composition (%) | | | | |
| Dry matter | 87.35 | 87.27 | 86.88 | 86.71 |
| Crude protein | 16.01 | 16.00 | 14.00 | 12.00 |
| Ether extract | 10.21 | 10.21 | 9.16 | 8.87 |
| Crude fiber | 4.48 | 4.48 | 4.24 | 4.16 |
| Nitrogen-free extract | 53.40 | 53.34 | 56.39 | 58.70 |
| Ash | 3.25 | 3.24 | 3.09 | 2.98 |
| Calcium | 0.60 | 0.60 | 0.60 | 0.60 |
| Phosphorus, available | 0.30 | 0.30 | 0.30 | 0.30 |
| Lysine | 0.97 | 0.97 | 0.83 | 0.71 |
| Methionine | 0.31 | 0.31 | 0.30 | 0.30 |
| Calculated energy value | | | | |
| ME (kcal kg diet) | 3002 | 2998 | 2997 | 3000 |

*Each 3 kg contains : Vit. A, 1200000 IU ; Vit. D3, 300000 IU ; Vit. E, 700 mg ; Vit. k3, 500 mg ;Vit. B1, 500 mg ; Vit. B2, 200 mg ; Vit. B6, 600 mg ; Vit. B12, 3 mg ; Vit. C, 450 mg; Niacin, 3000 mg; Methionine, 3000 mg; Pantothenicacid, 670 mg ; Folicacid 300 mg; Biotin, 6 mg; Choline chloride, 10000 mg; Magnesiumsulphate, 3000 mg; Copper sulphate, 3000 mg; Ironsulphate, 10000 mg; Zinc sulphate, 1800 mg; Cobalt sulphate, 300 mg.

physiological saline (1:10 dilution) (Al-Natour and Alshawabkeh, 2005) and pH was determined.

Total feed cost, total production cost, price of body weight, net revenue and economic feed efficiency were calculated.

*Statistical analysis*

All data were analyzed using one way analysis of variances (ANOVA) followed by LSD test using SPSS 11.0 statistical software (SPSS, Inc, Chicago, IL,2001), www.spss.com.

## Results

The results of body weight and gain of ducks (Table 2 and 3) indicated that, the live body weight of ducks fed 16% protein diet supplemented with CA was significantly (P< 0.05) higher than those fed control diet at the fourth week of the trial and continued until the end of experiment.

There was no significant (P>0.05) difference in live body weight between ducks fed 14% protein diet supplemented with CA and the control at the whole period of the experiment.

The obtained results also indicated that the live body weight of ducks fed12% protein diet supplemented with CA was significantly (P<0.05) lower than those fed control one.

The results also cleared that, the birds fed 16 and 14% protein diet supplemented with CA have higher total gain (3244 and 2868g, respectively), while birds fed 12% protein diet supplemented with CA gave the lower one (2339g) compared with the control (2784g).

Comparing the results among citric acid treatment groups cleared that, the birds fed 16% protein diet supplemented with CA have significantly (P<0.05) higher body weight than those fed 14 and 12% protein diet supplemented with CA from 4-8 weeks of the experiment.

The results in Table 4 showed that, the total feed

Table 2. Body weight development (g/bird) of ducklings during the experiment

| Exp. period (week) | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| 0* | 294±17.59$^{a}$ | 288±9.65$^{a}$ | 284±15.84$^{a}$ | 286±9.70$^{a}$ |
| 1 | 589±38.79$^{a}$ | 600±20.47$^{a}$ | 605±27.37$^{a}$ | 558±16.73$^{a}$ |
| 2 | 984±48.54$^{a}$ | 988±33.01$^{a}$ | 1005±42.27$^{a}$ | 913±35.94$^{a}$ |
| 3 | 1341±53.25$^{a}$ | 1474±48.87$^{a}$ | 1391±59.65$^{a}$ | 1368±77.17$^{a}$ |
| 4 | 1751±49.39$^{b}$ | 2022±52.20$^{a}$ | 1794±78.99$^{b}$ | 1671±74.40$^{b}$ |
| 5 | 2106±91.52$^{b}$ | 2523±65.43$^{a}$ | 2170±95.69$^{b}$ | 2048±77.80$^{b}$ |
| 6 | 2593±89.30$^{b}$ | 2959±58.68$^{a}$ | 2670±87.78$^{b}$ | 2284±81.99$^{c}$ |
| 7 | 2878±51.49$^{b}$ | 3300±56.83$^{a}$ | 2923±84.95$^{b}$ | 2499±76.35$^{c}$ |
| 8 | 3078±81.65$^{b}$ | 3533±51.05$^{a}$ | 3152±96.48$^{b}$ | 2685±92.45$^{c}$ |

* * 0= 2 weeks of age
Means within the same row with different superscripts are significantly different (P < 0.05).
T1: the control diet 16% protein ; T2 : 16% protein ; T3: 14% protein ; T4 : 12% protein.

Table 3. Weight gain (g/bird) of ducklings during the experiment.

| Exp. Period (week | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| 1 | 295±23.20$^{a}$ | 312±11.34$^{a}$ | 321±14.33$^{a}$ | 273±7.53$^{a}$ |
| 2 | 395±15.20$^{a}$ | 388±15.96$^{a}$ | 400±17.56$^{a}$ | 355±22.94$^{a}$ |
| 3 | 357±14.77$^{c}$ | 486±26.28$^{a}$ | 386±23.40$^{bc}$ | 455±41.99$^{ab}$ |
| 4 | 411±11.14$^{b}$ | 548±37.38$^{a}$ | 403±32.27$^{b}$ | 303±15.94$^{c}$ |
| 5 | 354±49.55$^{b}$ | 501±16.93$^{a}$ | 376±37.90$^{b}$ | 377±22.85$^{b}$ |
| 6 | 487±24.59$^{a}$ | 436±16.88$^{a}$ | 500±57.41$^{a}$ | 236±13.55$^{b}$ |
| 7 | 285±50.40$^{ab}$ | 341±20.81$^{a}$ | 252±24.43$^{ab}$ | 215±13.56$^{b}$ |
| 8 | 200±40.74$^{a}$ | 232±22.09$^{a}$ | 229±25.10$^{a}$ | 186±41.52$^{a}$ |
| Total | 2783.60 | 3244.40 | 2867.60 | 2399.20 |

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 4. Feed intake (g/bird) of ducklings during the experiment

| Exp. period (week) | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| 1 | 654.46 | 729.61 | 896.91 | 471.64 |
| 2 | 999.35 | 1043.18 | 1161.16 | 985.98 |
| 3 | 1159.60 | 1399.10 | 1202.76 | 1437.45 |
| 4 | 1388.50 | 1787.13 | 1377.92 | 997.86 |
| 5 | 1389.25 | 1648.29 | 1309.18 | 1589.67 |
| 6 | 2108.71 | 1500.53 | 1836.47 | 1231.92 |
| 7 | 1417.44 | 1439.86 | 1213.56 | 1355.39 |
| 8 | 1105.78 | 1187.56 | 1263.53 | 1197.27 |
| Total | 10223.10 | 10735.28 | 10234.48 | 9537.18 |

intake of ducklings fed 16% protein diet (T2) is slightly higher than the control by 512g/bird, while the feed intake of birds fed 14% protein diet (T3) was decreased by 11g/bird. The birds fed 12% protein diet (T4) highly decreased in feed intake which reached to 686 g/bird.

The results in Table 5 cleared that, inclusion of 1.5% citric acid improved Feed conversion ratio (FCR) compared with the control one who has the same level of protein by the ratio 0.36, while duckling fed 14% protein diet supplemented with CA has nearly the same feed conversion ratio. Conversely, the birds fed 12% protein diet supplemented with CA lowered the feed conversion ratio by 0.31.

Table 5. Feed conversion ratio of ducklings during the experiment

| Exp. period (week) | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| 1 | 2.22 | 2.34 | 2.71 | 2.72 |
| 2 | 2.53 | 2.69 | 2.90 | 2.78 |
| 3 | 3.25 | 2.88 | 3.12 | 3.16 |
| 4 | 3.38 | 3.26 | 3.42 | 3.29 |
| 5 | 3.92 | 3.29 | 3.48 | 4.22 |
| 6 | 4.33 | 3.44 | 3.67 | 5.22 |
| 7 | 4.97 | 4.22 | 4.81 | 6.31 |
| 8 | 5.54 | 5.11 | 5.52 | 6.43 |
| Average | 3.67 | 3.31 | 3.57 | 3.98 |

The obtained data in Table 6 revealed that, no significant differences in preslaughter weight, hot carcass percentage, eviscerated carcass percentage, dressing percentage and relative percentage of internal organs (heart, liver, spleen and gizzard) between different experimental groups. Birds fed on 16% protein diet supplemented with CA (T2) recorded significantly (P<0.05) higher weights of hot carcass, eviscerated carcass and dressed carcass than other treatments. However, birds fed on 12% protein diet supplemented with CA recorded significantly (P<0.05) lower gizzard weight than the other treatments.

The obtained results in Table 7 cleared that, a significant (P<0.05) increase in globulin and a significant (P<0.05) decrease in albumin, albumin/globulin ratio, cholesterol and uric acid and no significant difference in triglycerides between duckling fed 16,14 and 12% protein diets supplemented by citric acid and the control one. There were no significant differences in serum total protein for duckling fed 14 and 12% protein diet supplemented by citric acid and the control one. Birds fed on 16% protein diets supplemented by citric acid had significantly (P<0.05) higher serum total protein than the control one.

The effect of dietary acidification on pH values of different GI-tract segments are presented in Table 8. The results indicated that, citric acid supplementation reduced duodenum, jejunum and ileum pH values compared with control group. However, the differences were not significant.

Table 6. Carcass trait parameters of ducklings in the experiment

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| Pre-slaughter weight (g) | $3133\pm88.19^{ab}$ | $3550\pm76.38^{a}$ | $3046\pm66.57^{b}$ | $2743\pm254^{b}$ |
| Hot carcass weight (g) | $2578\pm90.42^{b}$ | $3125\pm141^{a}$ | $2495\pm57.65^{b}$ | $2317\pm233^{b}$ |
| Hot carcass (%) | $82.25\pm0.64^{a}$ | $87.95\pm2.05^{a}$ | $81.92\pm0.19^{a}$ | $84.33\pm0.88^{a}$ |
| Eviscerated carcass wt (g) | $2248\pm65.94^{b}$ | $2703\pm214^{a}$ | $2153\pm52.62^{b}$ | $2026\pm190^{b}$ |
| Eviscerated carcass (%) | $71.74\pm0.53^{a}$ | $75.95\pm4.37^{a}$ | $70.69\pm0.30^{a}$ | $73.84\pm0.72^{a}$ |
| Dressing weight (g) | $2409\pm76.18^{b}$ | $2874\pm219^{a}$ | $2316\pm58.24^{b}$ | $2153\pm206^{b}$ |
| Dressing (%) | $76.88\pm0.61^{a}$ | $80.77\pm4.42^{a}$ | $76.04\pm0.33^{a}$ | $78.43\pm0.83^{a}$ |
| Liver (%) | $1.59\pm0.05^{a}$ | $1.64\pm0.02^{a}$ | $1.54\pm0.00^{a}$ | $1.61\pm0.04^{a}$ |
| Heart (%) | $0.83\pm0.07^{a}$ | $0.78\pm0.03^{a}$ | $0.81\pm0.04^{a}$ | $0.74\pm0.01^{a}$ |
| Gizzard (%) | $2.72\pm0.08^{ab}$ | $2.40\pm0.03^{b}$ | $3.00\pm0.04^{a}$ | $2.25\pm0.17^{b}$ |
| Spleen (%) | $0.08\pm0.01^{a}$ | $0.07\pm0.01^{a}$ | $0.05\pm0.00^{a}$ | $0.06\pm0.01^{a}$ |

Means within the same row with different superscripts are significantly different (P < 0.05).

Total feed cost, total production cost, price of body weight, net revenue and economic feed efficiency were calculated and presented in Table 9. From the table it was observed that, revenue and economic feed efficiency were increased with 16 and14% protein diets supplemented with citric acid and were decreased with 12% protein diet supplemented with citric acid compared with control one.

The results cleared that, the birds fed 16% protein diet supplemented with citric acid gave the best economic feed efficiency (49.89%), while birds fed 12% protein diet supplemented with citric acid gave the worst one (30.42%). The birds fed 14% protein diet gave the intermediate values (42.52%)

Table 7. Blood parameters of ducklings during the experiment.

| Item | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| Total protein g/dl | 3.38±0.24$^{b}$ | 4.20±0.28$^{a}$ | 3.43±0.02$^{b}$ | 3.80±0.18$^{ab}$ |
| Albumin g/dl | 1.85±0.06$^{a}$ | 1.39±0.04$^{bc}$ | 1.32±0.06$^{c}$ | 1.56±0.05$^{b}$ |
| Globulin g/dl | 1.53±0.19$^{c}$ | 2.81±0.25$^{a}$ | 2.11±0.04$^{b}$ | 2.24±0.14$^{b}$ |
| Alb/Glob ratio | 1.24±0.12$^{a}$ | 0.50±0.03$^{c}$ | 0.63±0.04$^{bc}$ | 0.70±0.03$^{b}$ |
| Triglycerides mg/dl | 26.27±5.77$^{a}$ | 31.34±9.54$^{a}$ | 30.60±0.12$^{a}$ | 33.88±1.72$^{a}$ |
| Cholesterol mg/dl | 323.98±1.14$^{a}$ | 173.31±12.28$^{c}$ | 188.24±0.14$^{c}$ | 287.78±3.14$^{b}$ |
| Uric acid mg/dl | 3.06±0.29$^{a}$ | 1.98±0.02$^{b}$ | 1.81±0.06$^{b}$ | 1.62±0.22$^{b}$ |

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 8. Intestinal pH value of ducklings during the experiment.

| Item | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| Dudenum | 6.43±0.09$^{a}$ | 6.37±0.07$^{a}$ | 6.37±0.03$^{a}$ | 6.33±0.07$^{a}$ |
| Jeujenum | 6.53±0.09$^{a}$ | 6.43±0.03$^{a}$ | 6.40±0.06$^{a}$ | 6.37±0.03$^{a}$ |
| Ileum | 6.70±0.06$^{a}$ | 6.60±0.00$^{a}$ | 6.53±0.03$^{a}$ | 6.63±0.03$^{a}$ |

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 9. Economical evaluation of the different experimental diets.

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Control group | Citric acid groups | | |
| | T1 | T2 | T3 | T4 |
| Average feed intake (kg/bird) | 10.22 | 10.74 | 10.24 | 9.54 |
| Price/kg feed (L.E) | 3.18 | 3.44 | 3.25 | 3.09 |
| Total feed cost (L.E) | 32.51 | 36.93 | 33.29 | 29.47 |
| Total production cost (L.E) | 54.51 | 58.93 | 55.29 | 51.47 |
| Body weight (kg/bird) | 3.077 | 3.533 | 3.152 | 2.69 |
| Price/kg body weight (L.E) | 25 | 25 | 25 | 25 |
| Total revenue (L.E) | 76.93 | 88.33 | 78.80 | 67.13 |
| Net revenue (L.E) | 22.42 | 29.40 | 23.51 | 15.66 |
| Economic feed efficiency (%) | 41.12 | 49.89 | 42.52 | 30.42 |
| Relative economic feed efficiency | 100 | 121.3 | 103.4 | 73.97 |

## Discussion

The body weight of ducks supplemented with citric acid was significantly (P<0.05) higher than ducks in control group. The obtained results are in harmony with the results of Abdel-Fattah *et al*. (2008); Islam *et al*. (2008); Chowdhury *et al*. (2009); Ghazalah *et al*. (2011); Islam *et al*. (2012) who reported that, giving broiler CA (0.25 – 3%) improved body weight and body weight gain. On the contrary, Öztürk *et al*. (2004); Atapattu and Nelligaswatta (2005), Ao *et al*. (2009); Talebi *et al*. (2010); Kopecký *et al*. (2012) revealed that, no effect on body weight of broilers by addition of acidifiers to diets. The improved body weight gain is probably due to the beneficial effect of organic acids on the gut flora. The organic acids may affect the integrity of microbial cell membrane or cell macromolecules or interfere with the nutrient transport and energy metabolism causing the bactericidal effect (Ricke, 2003). Use of organic acid mixture decreases the total bacterial and gram negative bacterial counts significantly in the broiler chicken (Gunal *et al*., 2006). Besides, organic acids supplementation has pH reducing property, although non significant, in various gastrointestinal segments of the broiler chicken (Abdel-Fattah *et al*., 2008). The reduced pH is conducive for the growth of favourable bacteria simultaneously hampering the growth of pathogenic bacteria which grow at a relatively higher pH. However, it is worth mentioning that the effects of organic acids down the digestive tract diminish because of the reduction in concentration of acids as a result of absorption and metabolism (Bolton and Dewar, 1964). Thus, it can be hypothesized that the effect of organic acids in the distal segments of gastro-intestinal tract could be due to the reduced entry of pathogenic bacteria from the upper parts of gastrointestinal tract as a compensatory mechanism but no valid literature regarding such mechanism was found. The beneficial microbiological and pH-decreasing abilities of organic acids might have had resulted in the inhibition of intestinal bacteria leading to the reduced metabolic needs, thereby increasing the availability of nutrients to the host. This also had decreased the level of toxic bacterial metabolites as a result of lessened bacterial fermentation, causing an improvement in the protein and energy digestibility, thus ameliorating the weight gain and performance of experimental birds. Furthermore, the organic acids improve the villus height in the small intestines and also have a direct stimulatory effect on the gastro-intestinal cell proliferation as reported by Tappenden and McBurney (1998) that short chain fatty acids increase plasma glucagon-like peptide-2 (GLP-2) and ileal proglucagon mRNA, glucose transporter (GLUT2) expression, and protein expression, which are all signals which can potentially mediate gut epithelial cell proliferation. These histological changes in small intestines probably had increased the intestinal surface area, facilitating the nutrient absorption to a greater extent and, thus boosted the growth promoting effect of organic acid supplementation.

The decrease in feed intake of birds fed 14 or 12% protein diet supplemented with citric acid may be attributed to the low level of protein and not to the citric acid. Nezhad *et al*. (2007) found that, the addition of citric acid did not affect feed intake in broilers supplemented with citric acid and similar results were found by Chowdhury *et al*. (2009). However, this observation was not found by Moghadam *et al*. (2006); Islam *et al*. (2008) who reported that, the effects of citric acid on feed intake of broilers were significant.

The lower values of FCR of birds fed 14 or 12% protein diet supplemented with citric acid may be attributed to the low level of protein and not to the citric acid. These results are in concordance with the reports of earlier researchers (Abdel-Fattah *et al*., 2008; Chowdhury *et al*., 2009) who found that, dietary inclusion of citric acid significantly improve feed conversion ratio in broiler chickens compared with control group. At the opposite direction, the present results disagreed with that reported by previous studies (Atapattu and Nelligaswatta, 2005; Islam *et al*., 2008; Ghazalah *et al*., 2011) who reported that, dietary inclusion of organic acids had no significant effect on feed conversion ratio in broiler chickens.

The present data agreed with that reported by Öztürk *et al*. (2004); Adil *et al*. (2010), who stated that, addition of organic acids had no significant effect on the carcass characteristics (dressing percentage, liver and spleen weights) of broiler chickens. Moreover, Wang *et al*. (2010) reported that, eviscerated carcass percentage was not influenced by dietary CP concentration. However, Abdel-Hakim *et al*. (2009) declared that addition of citric acid to the diet was associated with higher liver percentage (P<0.05).

The obtained results indicated that, supplemental organic acids may improve immune response. Globulin level has been use as indicator of immune responses and source of antibody production. This established enhancement of immune response associated with dietary acidification could be account for their inhibitory effects against the pathogenic microorganisms throughout the GI-tract. This results in harmony with Rahmani and speer (2005) who found higher percentage of gamma globulin in broilers given organic acids than the control one. These findings are in agreement with Abdo (2004); Abdel-Fattah *et al*. (2008) who observed that, blood total lipids and cholesterol decreased significantly by dietary acidifiers. However, Adil *et al*. (2010) found no significant effect on serum cholesterol in broiler chicks fed on organic acids.

The pH values in specific areas of the GI-tract is a factor which establishes a specific microbial population, and also affects the digestibility and absorptive value of most nutrients. Most of pathogens grow in a pH close to 7 or slightly higher. In contrast beneficial microorganisms live in acidic pH (5.8-6.2) and compete with pathogens (Boling *et al*., 2001; Rahmani and Speer, 2005).

Results of the present study are in harmony with the results of Denli *et al*. (2003); Öztürk *et al*. (2004) who reported that, giving broiler diets contain organic acid mixture showed insignificant reduction in the intestinal pH. Similarly, Atapattu and Nelligaswatta (2005); Abdel-Fattah *et al*. (2008) noticed that CA at level of 1-3% of diet doesn't alter the pH of the GI-tract after gizzard. These authors referred this insignificant effect to the strong buffering action of the GI-tract in poultry. However, Ghazalah *et al*. (2011) found that CA at levels of 1, 2 and 3.0% of diet significantly ($P<0.05$) reduced duodenum, jejunum and ileum pH values compared to control group.

## Conclusion

It could be concluded that citric acid supplementation to 16% protein diet had positive effect on growth performance and carcass traits parameters of molar ducks.

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