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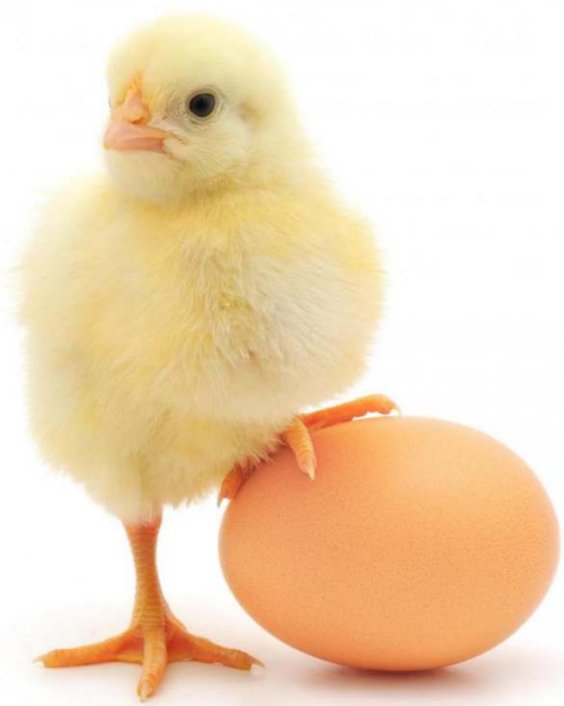

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Thyroid Activity, Some Blood Constituents, Organs Morphology and Performance of Broiler Chicks Fed Supplemental Organic Acids

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Abstract: A total number of 189 one d-old Hubbard broiler chicks were randomly divided into seven treatment groups of three replicates, 9 chicks each. The first group was served as control and fed the basal diets. While, the other six groups were received the basal diet supplemented with acetic acid (AC), citric acid (CA) or lactic acid (LA) at different levels of 1.5 and 3.0 % of diet, respectively. The experiment was lasted when chicks were 42 d old. Thyroid gland activity, some blood components, organ morphology, pH level of some gastrointestinal tract (GI-tract) segments and performance were measured. The results showed that dietary acidification elevated significantly concentration of T_3 as well as T_3:T_4 ratio, but T_4 level was not significantly affected. Moreover, the effect was clearly notable with CA and AC. Chicks fed acidified diets had better immune response as indicated by their higher serum globulin and relative lymphoid organs than the control. Similarly, higher calcium and phosphorus concentrations were noted. On the other hand, significant reduction in abdominal fat and serum level of cholesterol and total lipid was achieved due to dietary acidification. The liver functions did not adversely alter, in response to addition of organic acidifiers. Addition of any level and source of organic acids increased feed digestion and absorption as a result of increasing relative pancreas weight and small intestine density (indication of the intestinal villi dimension). The pH values in different GI-tract segments were insignificantly decreased with supplemental all types and doses of AC, CA and LA. Broiler chicks of dietary organic acids had superior improvement in live body weight (LBW), body weight gain (BWG) and feed conversion ratio (FCR) as compared to those of unsupplemented diet. No remarkable differences were noted between the addition of 1.5 and 3% of either AC, CA or LA in most studied traits.

Key words: Organic acids, broiler, thyroid hormones, serum, morphology, pH and performance

Introduction

The possibilities to achieve optimum broilers performance have led the producers to search for and use alternative promoters, in particular with the ban of using the antibiotic growth promoters. Thus, their use in feed rations of productive live stocks leads to the formation resistance against bacteria which are pathogenic to humans (Langhout, 2000). So that the researchers developed some physiological additives, such as acidifiers, to avoid scouring and improve performance. Physiological additives are those that help normal development of physiological functions of animals or that make up for their deficiencies (Huyghebaert, 2005).

Many attempts to replace antibiotic growth promoters with an acidification strategy have failed because it was based on deductions from swine nutrition and because the specific physiology of poultry was not respected. Gauthier (2002) reported that the efficacy of organic acids in animal's nutrition other than poultry has been proven time after time but in poultry this innovative approach is in its infancy. In a review of strategies to minimize problems after the removal of antibiotic growth promoters in poultry, Bedford (2000) did not even mention organic acids, in spite of the fact that it is probably the most efficacious one.

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 GI-tract of poultry, thus improving their zootechnical performances. If applied correctly, organic acids work in poultry, not only as a growth promoter but also as a meaningful tool of controlling all entrintic bacteria, both pathogenic and non-pathogenic (Naidu, 2000 and Wolfenden *et al*., 2007). Moreover, organic acids feeding is believed to have several beneficial effects such as improving feed conversion ratio, growth performance, enhancing mineral absorption and speeding recovery from fatigue (Vogt *et al*., 1981; 1982; Syed *et al*., 1994; Abdel-Azeem *et al*., 2000; Fushimi *et al*., 2001; Gornowicz and Dziadek, 2002; Abdo, 2004).

Contrary to antibiotics, organic acids have other properties like; lowering of the chyme pH consequently, enhancing of protein digestion (Gauthier, 2002). Afsharmanesh and Pourreza (2005) suggested that the reduction in gastric pH which occurs following organic acid feeding may increase pepsin activity (Kirchgessner and Roth, 1982). Moreover, peptides arising from pepsin proteolysis trigger the release of hormones, including gastrin and cholecystokinin, which regulate the digestion and absorption of protein (Hersey, 1987). Therefore, the acid anion has been shown to complex with Ca, P, Mg, and Zn, which results in an improved digestibility of these minerals (Kishi *et al.*, 1999).

In poultry production, limited studies have been conducted to explore the effects of supplemental organic acid on the physiological changes and growth performance of broiler chicks. Furthermore, the results in the literature are not consistent. Thus, the general objectives of this study were to assess the effect of using three types of organic acids on thyroid activity, some serum constituents, organs morphology and performance of broiler chicks.

Materials and Methods

A total number of 189 one d-old Hubbard broiler chicks were used in this study. The broiler chicks were nearly equal in the live body weight and divided randomly into seven treatment groups of 27 chicks each. Each experimental group was divided into three replicates (9 chicks/each). The first group was served as control and fed the basal diets. While, the other groups from 2 to 7 received the basal diet supplemented with acetic acid (AC), citric acid (CA) or lactic acid (LA) at different levels of 1.5 and 3.0 % of diet, respectively. This study was terminated when the birds were 42 days old. All chicks were vaccinated against Newcastle disease, at seven and twenty two days of age with Hitchener B1 and Lasota strain vaccine, respectively and Gumbora vaccine against Bursal disease at fourteen days of age.

The experimental period included two feeding phases (starter, from 1-21 days of age and finisher, from 21-42 days of age). Experimental diets were formulated to nearly meet the nutrient requirements of the broiler chicks (NRC, 1994). The composition and chemical analysis of the control basal diets are presented in Table 1. The chemical composition of the experimental diets was analyzed according to A.O.A.C. (1996).

Feed and water were supplied *ad-libitium* during the experimental periods. Chicks were grown in brooders with raised wire floors and exposed to 24 hours of constant light. All chicks were kept under the same managerial, hygienic and environmental conditions. Chicks were individually weighed at the beginning of the experiment, then at weekly intervals until the end of experiment. Live body weight (LBW), body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR, g feed/g gain) were recorded during these periods.

At the age of 42 d, ten birds (5 male and 5 female birds) from each experimental group were weighed and slaughtered by slitting the jugular vein, then scalded and defeathered. Carcasses were manually eviscerated and weighed. Liver, heart, gizzard, spleen, thymus (all lobes of both sides), bursa, small intestine and abdominal fat were removed and their relative percentages of live body weight was estimated. The intestinal length (cm) was also considered and the intestinal density (g weight/length, cm) was calculated. Values of pH in different parts of the gastrointestinal tract (GI -tract) were measured immediately by using a digital pH meter. To determine the pH, 10 g of gut content from gizzard, duodenum, jejunum and ileum were collected aseptically in 90 ml sterilized physiological saline (1: 10 dilution) and pH was determined (Al-Natour and Alshawabkeh, 2005).

Table 1: Composition of basal diets and Chemical analysis

Ingredients (%)	Starter (0- 3 weeks)	Finisher (3 -6 weeks)
Yellow corn	61.77	73.00
Soybean meal (44% CP)	24.50	13.60
Corn gluten meal	10.00	10.00
Bone meal	2.60	2.00
Limestone	0.30	0.60
Vit & Min Premix*	0.30	0.30
NaCl	0.25	0.25
Lysine	0.18	0.20
DL- Methionine	0.10	0.05
Total	100.00	100.00
Chemical analysis:-		
A-Detemined analysis:		
Crude protein %	22.06	18.15
Crude fiber %	3.53	3.13
Ether extract %	2.86	3.12
Total ash	6.77	6.58
B-Calculated analysis:		
ME (KCal/Kg diet)	2988	3120
Calcium %	1.02	0.90
Available phosphorous %	0.45	0.35
Lysine%	1.15	0.90
Methionine %	0.51	0.40
Cystine %	0.38	0.33
Meth. + Cys. %	0.89	0.73

*Each 3 kg of vit-mineral mixture contain vit A 10m IU, vit D_3 1m IU, vit E 10g, vit K_3 1g, vit B_1 1g, vit B_2 4.0g, vit B_6 1.5g, Nicotinic acid 20g, Pantothenic acid 10g,vit B_{12} 0.01g, Biotin 0.05g, Folic acid 30g, Choline chloride 50g, Iron 30g, Manganese 40g, Copper 3g, Iodine 0.45g, Zinc 45g and Selenium 0.1g.

Blood samples were collected from the ten slaughtered birds in nonheparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min. and serum obtained was stored at -20°C until analysis. Serum total protein, albumin, total lipids, total cholesterol, uric acid, creatinine, Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and minerals (calcium and phosphorous) were determined calorimetrically by using available commercial kits purchased from Diamond Diagnostics Company. The globulin values were calculated by subtracting the values of albumin from the corresponding values of total protein. Serum concentration of triiodothyronine (T_3) and thyroxine (T_4) were determined using commercial enzyme immunoassay test kit purchased from Taytec Incorporation (7278 Aldercrest Dr., Mississauga, ON, L5N 7N8, Canada).

The Economical efficiency (EE) was calculated

Table 2: Effect of supplemental organic acids on thyroid gland activity in broiler chicks

	Treatment							
Variable	Control	1.5% AC	3.0% AC	1.5% CA	3.0% CA	1.5% LA	3.0% LA	Sig.
T_3 (ng/ml)	0.573^{d}±0.025	0.75^{abc}±0.03	0.802^{ab}±0.038	0.84^{a}±.002	0.836^{a}±0.028	0.68^{c}±0.009	0.723^{bc}±0.06	***
T_4 (ng/ml)	6.824±0.11	6.327±0.30	7.02±0.05	6.56±0.27	6.54±0.33	6.52±0.07	6.56±0.78	NS
T_3:T_4	0.087^{c}±0.003	0.119^{ab}±0.002	0.112^{ab}±.0006	0.128^{a}±0.003	0.124^{a}±0.005	0.104^{bc}±0.01	0.11^{ab}±0.01	***

$^{a-d}$Means within the same row with different superscripts are significantly different. NS = Not significant, *** ($P \leq 0.001$). AC= acetic acid, CA = citric acid and LA = lactic acid.

Table 3: Effect of supplemental organic acids on some serum constituents in broiler chicks

	Treatment							
Variable	Control	1.5% AC	3.0% AC	1.5% CA	3.0% CA	1.5% LA	3.0% LA	Sig.
Total protein (g/dl)	3.89±0.41	4.03±0.51	4.23±0.08	4.13±0.14	4.15±0.23	3.94±0.24	4.01±0.28	NS
Albumin (A) (g/dl)	1.93±0.20	1.83±0.23	1.88±0.06	1.89±0.18	1.85±0.10	1.86±0.15	1.88±0.10	NS
Globulin (G) (g/dl)	1.96^{c}±0.25	2.20^{ab}±0.29	2.34^{a}±0.09	2.24^{ab}±0.26	2.30^{a}±0.18	2.08^{bc}±0.11	2.13^{b}±0.22	**
A/G ratio	0.98^{a}±0.07	0.83^{bc}±0.03	0.80^{c}±0.05	0.84^{bc}±0.02	0.80^{c}±0.06	0.89^{b}±0.06	0.88^{b}±0.05	*
Cholesterol (mg/dl)	393.33^{a}±11.70	287.08^{cd}±10.52	267.56^{d}±5.81	316.83^{bc}±8.96	279.40^{d}±5.38	328.33^{b}±23.10	317.83^{bc}±6.53	***
Total lipid (mg/dl)	880.37^{a}±43.07	620.55^{d}±52.83	604.61^{d}±12.24	805.21^{ab}±4.98	728.83^{bc}±29.55	818.68^{ab}±47.22	664.52^{cd}±21.60	***
Ca (mg/dl)	8.70^{b}±0.16	9.74^{a}±0.36	10.20^{a}±0.65	9.94^{a}±0.37	10.25^{a}±0.52	9.84^{a}±3.03	9.95^{a}±0.39	*
P (mg/dl)	5.94^{b}±0.18	7.17^{a}±0.37	7.27^{a}±0.40	7.19^{a}±0.35	7.93^{a}±0.41	6.88^{ab}±0.11	7.23^{a}±0.46	*
Uric acid (mg/dl)	4.26±0.04	3.97±0.06	3.88±0.01	3.75±0.01	3.87±0.02	4.05±0.00	4.16±0.02	NS
AST (U/l)	103.62±3.85	104.39±4.16	121.70±3.94	101.57±5.33	107.85±2.66	106.27±1.98	101.34±2.87	NS
ALT (U/l)	19.06±0.79	18.99±0.80	19.47±0.75	19.10±0.53	19.20±0.63	18.82±0.30	20.06±0.42	NS

$^{a-d}$Means within the same row with different superscripts are significantly different. NS = Not significant, *($P \leq 0.05$), **($P \leq 0.01$), *** ($P \leq 0.001$). AC = acetic acid, CA= citric acid and LA= lactic acid.

according to the equation EE=((A-B)/B) x100, where A is the selling cost of the obtained gain and B is the feeding cost of this gain. Performance index was estimated according to North (1981): Performance index = (live body weight/feed conversion) x 100.

The obtained data were statistically analyzed using the general linear model procedure described in SAS User's Guide (SAS, 2001). Differences among means were tested using Duncan's multiple range test (Duncan, 1955). Percentages of slaughter traits were divided by 100 and subjected to arc-sin transformation of the square root before analysis; however actual percentage means are presented.

Results and Discussion

The fact that thyroid hormones had a major role in regulating oxidative metabolism of birds has been established (Sturkie, 1986). Triiodothyronine (T_3), the metabolically active thyroid hormone, plays an active role in energy metabolism and metabolic rate. Any pronounced alteration in thyroid function (hyperthyroidism or hypothyroidism) is reflected in an altered metabolic rate.

The results observed herein (Table 2) showed that, organic acidification of broiler chicks diets elevated ($P<0.003$) serum T_3 concentration. Indeed, the influence was more pronounced with the addition of either CA or AC at both levels (1.5 and 3%) followed by those received 3% LA comparable to the chicks of basal diet. Similar trend was nearly observed for T_3 : T_4 ratio, which implies the conversion rate of T_4 into T_3. However, serum T_4 level was not significantly affected.

These results pointed out superior metabolic and growth rate due to the addition of acidifiers into broiler chicks diet. The hyperthyroidism and peripheral conversion of T_4 to T_3 signified better immunocompetence and bursa growth, as well as lower fat deposition (Sturkie, 1986).

Data presented in Table 3 showed that, dietary supplementation of organic acids exhibited relatively noticeable increase, although insignificant in the serum concentration of total protein compared with non-supplemented control. This could be due to the achieved significant increase in the serum concentration of globulin level by the supplemented groups. Hence, serum albumin values showed no significant difference among all groups including the control. The present results are coincided with those obtained in growing rabbits (El-Kerdawy, 1996) and broiler chicks (Abdo, 2004) due to citric acid and acetic acid inclusion, respectively.

Broiler chicks whose diet was supplemented with either AC or CA at any level recorded significantly ($p \leq 0.01$) higher and lower values of serum globulin and A/G ratio, respectively as compared with those fed acidifiers free diet (control). Chicks received either 1.5 or 3% LA had intermediate values. These results indicated that supplemental organic acids may improve the immune response. Globulin level has been used as indicator of immune responses and source of antibody production. Griminger (1986) stated that high globulin level and low A/G ratio signify better disease resistance and immune response. This result is in harmony with those of Rahmani and Speer (2005) who found higher percentage of gamma globulin in broilers given organic acids than the control ones.

Table 4: Effect of supplemental organic acids on dressing and some organs as a percentage of body weight in broiler chicks

	Treatment							
Variable	Control	1.5% AC	3.0% AC	1.5% CA	3.0% CA	1.5% LA	3.0% LA	Sig.
Dressing	66.01±.038	67.32±0.44	67.30±0.70	66.67±0.58	67.79±0.44	68.66±0.43	67.51±0.52	NS
Liver	2.54±0.11	2.63±0.14	2.73±0.15	2.61±0.33	2.82±0.08	2.59±0.10	2.69±0.12	NS
Heart	0.52±0.01	0.54±0.01	0.52±0.02	0.57±0.06	0.57±0.03	0.53±0.03	0.50±0.01	NS
Pancreas	0.22^{d}±0.02	0.27^{bc}±0.02	0.31^{ab}±0.01	0.28^{bc}±0.01	0.32^{ab}±0.02	0.25^{cd}±0.02	0.34^{a}±0.02	**
Abdominal Fat pad	1.50^{a}±0.04	0.96^{bc}±0.06	1.09^{b}±0.02	0.94^{bc}±0.06	0.81^{c}±0.05	0.99^{bc}±0.08	0.86^{bc}±0.10	***
Intestinal morphology								
Relative SIW (g)	54.80^{c}±1.32	58.80^{ab}±1.71	60.00^{ab}±0.70	60.50^{ab}±0.86	62.20^{a}±1.15	58.00^{bc}±1.22	59.20^{ab}±1.35	*
SIL (cm)	190.60^{c}±1.80	192.80^{bc}±2.69	198.60^{ab}±1.02	194.00^{abc}±2.04	199.80^{a}±1.65	191.75^{bc}±3.03	192.80^{bc}±2.33	*
SID (wtg/Lcm)	0.287^{b}±0.004	0.305^{a}±0.005	0.302^{a}±0.002	0.312^{a}±0.003	0.311^{a}±0.004	0.302^{a}±0.004	0.307^{a}±0.006	*
Lymphoid organs								
Spleen	0.19±0.01	0.21±0.01	0.19±0.01	0.21±0.01	0.21±0.02	0.20±0.00	0.19±0.02	NS
Bursa	0.23^{c}±.02	0.27^{bc}±0.01	0.32^{a}±0.01	0.26^{bc}±0.01	0.30^{ab}±0.01	0.24^{c}±0.01	0.27^{ab}±0.02	***
Thymus	0.27^{c}±0.02	0.31^{bc}±0.03	0.41^{ab}±0.04	0.38^{ab}±0.03	0.44^{a}±0.04	0.40^{ab}±0.02	0.39^{ab}±0.05	*

a-dMeans within the same row with different superscripts are significantly different. NS = Not significant, *($P \leq 0.05$), ** ($P \leq 0.01$), *** (P=0.001). AC= acetic acid, CA= citric acid and LA= lactic acid. SIW= relative small intestine wt; SIL = small intestine length and SID = small intestine density.

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.

Results of serum cholesterol level showed that, chicks of the basal diet (control) were the highest (P<0.01) compared with the all supplemented groups. Additionally, the lowest levels were recorded in chicks received either AC (1.5 and 3%) or 3% CA followed by those of 1.5% CA and 3% LA. Similar trend was almost observed for serum concentration of total lipids. These findings are in agreement with El-Kerdawy (1996) and Abdo (2004) they reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. However, El-Afifi *et al.* (2001) found no significant effect on blood lipid profile in broiler chicks fed on citric acid.

The beneficial role of organic acids in reducing the blood lipid profile may be interpreted through their influence in decreasing the microbial intracellular pH. Thus, inhibits the action of important microbial enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of acid anions (Young and Foegeding, 1993). Also, the observed lower feed consumption (Table 6), during the early period of growth (starter), and consequently lower fat intake that resulted in fat depletion may also contribute in reducing blood lipid content. Pinchasov and Jensen (1989) and Zhang *et al.* (2005) observed an inhibition of feed intake in broiler breeder flocks due to chemical feed restriction induced by supplemental organic acids. Moreover, the observed hyperthyroidism associated with dietary organic acidification could also explain the observed reduction in serum lipid profile. Sturkie (1986) reported that hyperthyroidism (T_3 elevation) induced energy metabolism and decreased fat deposition. Hence, the concentration of avian blood lipids is influenced by the physical and nutritional status of the bird.

Chicks fed supplemental organic acids had significantly (p<0.05) higher blood Ca and P concentrations than those given unsupplemented diet. The increase of Ca and P levels in blood serum produced by addition of organic acids may be attributed to the lowering of GI-tact pH by using these acids, which increases the absorption of such minerals from the gut into the blood stream. Improving the utilization of calcium and phosphorus due to provision of organic acids was approved by Boling *et al.* (2001). Also, Abdo (2004) observed an increase in blood calcium of broiler chicks fed on dietary acidifier. In this respect, Li *et al.* (1998) and Edwards and Baker (1999) found that, the acidic anion has been shown to complex with Ca, P, Mg and Zn, which results in an improved digestibility of these minerals. Furthermore, Kishi *et al.* (1999) concluded that dietary acetic acid prevented osteoporosis, through reducing the bone turnover, as it enhanced intestinal Ca absorption by improving Ca solubility in ovariecomized rats.

Data of uric acid, which is the major end product of protein metabolism in poultry (Sturkie, 1986) revealed that dietary addition of organic acid slightly reduced serum concentration of uric acid. This result could be referred to the better utilization of protein and amino acid digestibility. Zaghini *et al.* (1986) found that urea and creatinine levels in the blood of rabbits were unaffected significantly by feeding diets containing 1.5% citric or fumaric acid.

Several studies concluded that the organic dietary acidification increased the utilization and improved the digestibility coefficient of protein (El-Kerdawy, 1996; Abdel-Azeem *et al.*, 2000 and Abdo, 2004). Kommera *et al.* (2006) mentioned that the decreased gastric emptying rate is a possible mechanism to improve the protein digestion in the GI-tract.

From Table 3, it is clearly notable that insignificant differences were found among all experimental groups including the control one for both ALT and AST activity. This result pointed out that broiler chicks could tolerate the addition of organic acids up to 3% without any deleterious effects on kidney and liver factions. These results are in full agreement with those of El-Kerdawy

(1996). Abdel-Azeem *et al*. (2000) demonstrated that the level of AST was reduced in growing rabbits fed supplemental citric acid, although ALT was not significantly affected. On the other hand, Grassmann and Klasna (1986) reported that dietary addition of 3% citric acid significantly increased the activities of both AST and ALT enzymes.

As shown in Table 4, insignificant differences were noted among all treatments including the control in the relative dressing, liver and heart percentages. Whereas, the relative percentage of fat pad was significantly ($P<0.001$) reduced with the addition of organic acid particularly, 3% CA compared to the control group. These results confirmed those of Denli *et al*. (2003) who found that dietary organic acids had no effect on the carcass yield and liver weight of broiler chickens at 42d old. Also, Tawfeek *et al*. (1994) and El-Kerdawy (1996) reported similar findings in growing rabbits. On the other hand, Abdel-Azeem *et al*. (2000) declared that addition of citric acid to the diet was associated with higher and lower dressing and liver percentages, respectively.

The lack of significance in the relative liver weights between the acidified and control chicks may be ascribed to the more storage of glycogen and lower lipid repletion induced by dietary organic acid. This supposition may emphasize the hypothesis of Fushimi *et al*. (2001) who stated that dietary acidification might stimulate glycogenesis by increasing the influx of glucose 6-phosphate (G-6-P) into the glycogen synthesis pathway through the inhibition of glycolysis due to an increase in citrate concentration. Besides, the observed reduction of both serum lipid profile and abdominal fat pad (Table 3 and 4).

The present results showed significant elevation in the relative weights of pancreas with the addition 3% of LA, AC or CA when compared with control. The remaining groups recorded intermediate values. This result may be account for, the enlargement in the size and activity of pancreatic secretary cells consequently, enhancement of pancreatic enzymes secretion and activities with dietary acidification. In this respect, Gauthier (2002) illustrated that contrary to antibiotics, organic acids have the ability to stimulate the pancreatic secretions. This was also found by Jang *et al*. (2004) who reported that a blend of lactic acid with essential oils showed significant increases in digestive enzyme activities of the pancreas and intestinal mucosa, leading to an increase in growth.

Supplemental different sources and levels of acidifiers resulted in remarkable increase in the small intestine morphology (weight and length). Further, the effect was more prominent with the addition of CA at both doses (3 and 1.5%) and 3% of CA and LA (Table 4).

Concerning the impact of dietary acidification on small intestine densities (weight / length), which has been considered as an indication of the intestinal villi dimension of mucosa layer (Palo *et al*., 1995). The current findings revealed that chicks whose diets were provided by organic acid had longer and thicker villi than that of controls (Table 4). Consequently, better efficiency in feed digestion and absorption. These results agreed with those of Loddi *et al*. (2004) and Pelicano *et al* (2005). Also, Denli *et al*. (2003) who reported that supplementing the diet with the organic acids did not only result in the highest intestinal weight but also, intestinal length of broiler chicks at day 42.

The increase of villus height of the different segments of the small intestine may be attributed to the intestinal epithelium acting as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen (Paul *et al*., 2007).

It is well known that spleen, bursa and thymus are considered as a part of the immunity system (Sturkie, 1986) and this system is responsible for producing cells that protect the birds from the invaded microorganism.

From Table 4 it is clearly observed that supplemental organic acids significantly increased the relative weight of both primary lymphoid organs (bursa and thymus) comparable to the basal diet control. However, this effect was not attained for the relative weight of spleen among all groups. These results might imply that broiler chicks fed on acidifiers had better immune response and disease resistance. In this respect, Katanbaf *et al*. (1989) reported that the increase in the relative organs weight is considered as an indication of the immunological advances.

The pH level 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 the pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens (Boling *et al*., 2001 and Rahmani and Speer, 2005).

The effect of dietary acidification on pH values of different GI-tract segments are presented in Table 5. The results indicated that organic acid supplementations reduced gizzard, duodenum, jejunum and ileum pH values compared with control group. However, the differences were not significant. These results are in harmony with the results of Denli *et al*. (2003) who reported that giving broiler organic acid mixture showed insignificant reduction in the intestinal pH. Similarly, Paul *et al*. (2007) who used different organic acid salts and found no significant difference in the pH values of different segments of the GI-tract. Moreover, Hernandez *et al*. (2006) reported no effect on intestinal pH with the use of a product containing combination of propionic acid and formic acid. These authors referred this insignificant effect to the strong buffering action of the GI-tract in poultry.

The effects of dietary organic acid supplementations on growth performance of broiler chicks are listed in Table

Table 5: Effect of supplemental organic acids on pH values of some gastrointestinal tract segments in broiler chicks

	Treatment							
Variable	Control	1.5% AC	3.0% AC	1.5% CA	3.0% CA	1.5% LA	3.0% LA	Sig.
Gizzard	3.21±0.02	3.08±0.0.02	3.16±0.04	3.09±0.06	3.00±0.05	3.12±0.01	2.83±0.07	NS
Deudenum	5.86±0.06	5.80±0.07	5.81±0.03	5.78±0.04	5.73±0.01	5.83±0.06	5.71±0.02	NS
Jejnum	6.64±0.04	6.60±0.07	6.54±0.06	6.49±0.03	6.43±0.06	6.61±0.04	6.58±0.08	NS
Ileum	7.24±0.05	7.23±0.04	7.19±0.02	7.21±0.05	7.15±0.06	7.23±0.06	7.22±0.04	NS

Means within the same row with different superscripts are significantly different. NS = Not significant, AC = acetic acid, CA = citric acid and LA = lactic acid.

Table 6: Effect of supplemental organic acids on growth performance of broiler chicks

	Treatment							
Age	Control	1.5% AC	3.0% AC	1.5% CA	3.0% CA	1.5% LA	3.0% LA	Sig.
				----- Live body weight (gm/ bird) -----				
3 weeks	608.3±2.1	616.7±4.8	594.2±18.2	614.6±24.1	623.6±29.7	613.7±17.9	600.0±12.5	NS
6 weeks	1593.1^{b}±26.2	1739.6^{a}±7.8	1718.3^{a}±3.8	1720.9^{a}±16.5	1717.0^{a}±16.5	1693.4^{a}±25.1	1744.9^{a}±16.4	**
				----- Body weight gain (gm/bird) -----				
0-3 weeks	570.8±8.6	578.4±32.0	556.2±13.4	576.1±27.2	585.2±31.8	575.7±10.4	562.3±4.8	NS
3-6 weeks	984.9^{c}±27.7	1122.9^{ab}±55.9	1124.1^{ab}±19.6	1106.3^{ab}±27.1	1093.4^{ab}±45.6	1079.7^{b}±31.6	1144.9^{a}±23.3	**
0-6 weeks	1555.7^{b}±36.2	1701.3^{a}±24.1	1680.3^{a}±18.8	1682.4^{a}±19.0	1678.6^{a}±20.7	1655.4^{a}±29.7	1707.2^{a}±25.9	**
				----- Feed consumption (gm/bird) -----				
0-3 weeks	886.1^{a}±2.5	755.8^{b}±25.0	717.7^{c}±20.1	768.3^{c}±19.2	784.2^{b}±21.0	765.5^{b}±27.5	746.6^{b}±33.1	**
3-6 weeks	2469.3^{b}±33.5	2529.0^{a}±50.8	2513.0^{ab}±32.4	2465.9^{b}±72.6	2480.0^{ab}±34.4	2426.5^{b}±28.9	2576.0^{a}±33.1	**
0-6 weeks	3355.4^{a}±32.6	3284.8^{a}±76.2	3230.7^{b}±29.2	3234.2^{b}±91.3	3264.2^{ab}±14.0	3192.0^{b}±7.0	3322.6^{a}±32.7	**
				----- Feed conversion ratio (gm feed/gm gain) -----				
0-3 weeks	1.55^{b}±0.05	1.31^{a}±0.12	1.29^{a}±0.10	1.33^{a}±0.05	1.34^{a}±0.08	1.33^{a}±0.08	1.33^{a}±0.09	*
3-6 weeks	2.51^{b}±0.05	2.25^{a}±0.13	2.24^{a}±0.04	2.23^{a}±0.12	2.27^{a}±0.06	2.25^{a}±0.06	2.25^{a}±0.05	**
0-6 weeks	2.16^{b}±0.05	1. 93^{a}±0.07	1.92^{a}±0.03	1.92^{a}±0.07	1.94^{a}±0.02	1.93^{a}±0.05	1.95^{a}±0.05	**
				----- Performance index (PI) -----				
0-6 weeks	73.75^{b}±2.6	90.13^{a}±3.7	89.49^{a}±1.9	89.63^{a}±2.3	88.51^{a}±1.7	87.74^{a}±2.3	89.48^{a}±3.2	**
				----- Economic efficiency (Eef) -----				
0-6 weeks	83.5^{b}±3.5	94.9^{a}±7.2	93.4^{a}±3.1	98.4^{a}±6.7	97.6^{a}±2.0	95.3^{a}±4.1	94.3^{a}±5.3	**
				----- Relative economic efficiency -----				
	100.00	113.65	112.35	117.84	116.89	114.13	112.93	

$^{a-c}$Means within the same row with different superscripts are significantly different. NS = Not significant, * (P ≤ 0.05), ** (P = 0.01), AC = acetic acid, CA = citric acid and LA = lactic acid.

6. The results indicated that, LBW, BWG and FCR were significantly improved with supplemental any type and level of tested organic acid compared with un-supplemented group. In spit of, the absence of significance among all treatments in LBW and BWG during the starter period (0-3 wks of age), highly significant effect was established during the grower stage (3-6 wks of age) due to dietary organic acidification. Additionally, FCR did not significantly differ in between the acidifier groups. The improvement in LBW, BWG and FCR are closely associated with the observed enhancement in the thyroid gland activity (Table 2). Also, the attained reduction, however not significant, in pH values of different GI-tract segments (Table 5).

Thus, decreasing of pH in GI-tract have a beneficial effect in the inhibition of intestinal bacteria competing with the host for available nutrients and a reduction of possibly toxic bacterial metabolites, e.g. ammonia and amines, thus improving weight gain of the host animals. Furthermore, the growth inhibition of potential pathogen bacteria, e.g. *E. coli* and *Salmonella*, in the feed and in the GI-tract is of benefit with respect to animal health (Iba and Berchieri, 1995; Berchieri and Barrow, 1996 and Thompson and Hinton, 1997). Kirchgessner and Roth (1988) found that dietary acidification increases gastric proteolysis and protein and amino acid digestibility. They also added that, organic acids serve as substrates in intermediary metabolism.

Feed consumption (FC) was slightly decreased by addition of organic acid compared with control group. The reduction in FC was noticed during the starter period while the differences were disappeared during the grower one. This may be due to shock decrease in palatability of acidified diets during the first stage. However, during the second stage the birds could accustom the dietary acidification. Organic acids are known as weak acids, they have a low tendency to free their H^+ ions and so tend to have a strong taste associated with them.

This suggestion was partially agreed with the earlier findings of Cave (1984) who reported that addition high levels of organic acid would strongly decrease palatability which reduce feed intake.

The results indicated also that there were clearly improvements in performance index, economical efficiency and relative economic efficiency in acidifier groups compared with control ones. Birds fed diets containing CA at both levels (1.5 and 3%) had the best values of either economic or relative economic efficiency compared with the other sources and levels of organic acids used in the present experiment.

In conclusion, this study pointed out the importance of using organic acid as physiological additives to promote the growth performance of broiler chicks through their physiological action in inducing the growth and activities of some endogenous mechanisms responsible for better performance. As well, under the condition of this experiment, no further benefits were achieved as a result of increasing the dietary organic acid level. Further studies are needed to throw more light on the developmental effects of those organic acids on the bird's physiological functions, with the consideration of using different combinations.

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