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Effect of Dietary Supplementation of Organic Acids on Performance, Nutrients Digestibility and Health of Broiler Chicks

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Abstract: This study was conducted to compare the effects of different types and levels of organic acids being Formic (0.25, 0.5 and 1.0% FA), Fumaric (0.5, 1.0 and 1.5% FUA), Acetic (0.25, 0.50 and 0.75% AC) and Citric Acids (1, 2 and 3% CA). A total number of 351 one d-old Arbor-Acres broiler chicks were randomly divided into 13 treatment groups, each in three replicates of 9 chicks per replicate. The first group was fed the basal diet without supplementation and served as control. While, the other groups were received the basal diet supplemented with the tested organic acids. The experiment was lasted when chicks were 42 d old. Performance, pH of some gastrointestinal tract (GI-tract) segments, organ morphology, cecal bacteria, blood metabolites and nutrients digestibility were measured. Results obtained could be summarized as follow: 1- Any of the tested organic acids increased significantly ($p<0.01$) body weight gain and European Production Efficiency Index compared with the control group. However, feed conversion ratio improved, but not significantly in case of formic and fumaric acids, while significantly improved with acetic and citric acids supplementation, compared to the control. No great differences in mortality rate were observed among all treatments as from 1 to 2 birds were died all over the experimental period due to natural causes and not due to nutritional treatments. 2- Dietary organic acids improved both ME and nutrients digestibility of the experimental diets compared to the control. 3- Dietary organic acids significantly reduced ($p<0.01$) the pH values in the different GI-tract segments of the chicks compared to chicks fed the control. 4- Feeding dietary FA and FUA led to sharp reduction in caeca content of lactobacilli bacteria, but increased number of coliforms bacteria and anaerobes was slightly increased. Feeding 0.75% AC and 2%CA increased Lactobacillus count and coliforms. Villus height was significantly higher ($p<0.01$) of all organic acid supplementation fed groups. 5- Blood serum content of calcium, phosphorus, total protein and globulin were increased significantly ($p<0.01$) by 0.5% FA, 0.5% FUA, 0.75% AC and 2%CA in the diet compared to the control. 6- Relative weights of lymphoid organs (spleen, bursa of fabrics and thymus gland) were significantly higher ($p<0.01$) by supplementing the tested organic acids compared to the control group. In conclusion, dietary 0.5% either of formic or fumaric as well as 0.75% acetic or 2% citric acids could be used safely to improve performance and health of broiler chickens.

Key words: Organic acids, broiler, performance, digestibility, serum, morphology, pH, bacteria

INTRODUCTION

Nowadays, the use of some antibiotics as growth promoter creates a huge problem for environmental conditions and healthy of consumers. Therefore, nutritionists trying to substitute those with different natural feed additive materials, such as Organic Acids (OAs) and probiotics. 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 bio-security measures, they can be a powerful tool in maintaining the health of the gastrointestinal tract of poultry, resulting in improving their performances. Feed of OAs may suppress the growth of certain species of bacteria, particularly acid intolerant species such as *E. coli*, *Salmonella* sp. and *Campylobacter* ssp. (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 OAs 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 (OAs) work in poultry not only as a growth promoter but also as a meaningful tool of controlling all

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enteritis bacteria, both pathogenic and non-pathogenic (Naidu, 2000; Wolfenden *et al*., 2007). Moreover, feeding OAs 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 objectives of this study aimed to investigate the optimal supplementation of organic acids being Formic Acid (FA), Fumaric Acid (FUA), Acetic Acid (AC) and Citric Acid (CA) on performance, nutrients digestibility, blood metabolites and health (small intestinal microbial flora, pH, tissue morphology) of broiler chickens.

MATERIALS AND METHODS

The experimental work of this study was carried out at Gezerit Elshier Poultry Research Station, AL- Kanater AL-Khairia. A total number of 351 day old male Arbor Acres broiler chicks were randomly taken and divided into 13 treatment groups, each in three replicates (9 birds / replicate). Feed and water were available all time. The study aimed to investigate the effect of different types and levels of organic acids (formic, fumaric, acetic and citric) on broiler performance, nutrients digestibility, some blood metabolites, organs morphology, pH level of some gastrointestinal tract segments, mortality and productive efficiency.

The experimental treatments included Formic acid (FA) which was supplemented at levels 0.25, 0.5 and 1.0%, fumaric acid (FUA) at 0.5, 0.1 and 1.5%, acetic acid (AC) at 0.25, 0.5 and 0.75% and citric acid(CA) at 1, 2 and 3% to the basal diet. In addition to the control group without organic acids supplementation. Basal diets were formulated (Table 1) to meet the nutrients requirements of Arbor Acres broiler at starter (1-14d), grower (15-28d) and finisher (29-42d) periods.

Body weights (BW), feed consumption (FC) and mortality rate were recorded biweekly and average body weight gains (BWG), feed conversion ratio (FCR) and European Production Efficiency Index (EPEI) were calculated.

At 6 weeks of age, three birds from each treatment were randomly taken and housed in individual cages to determine the digestibility coefficients of nutrients for the experimental finisher diets. The analyses of feed and dried excreta were done according to A.O.A.C.(1990). Fecal nitrogen was determined according to Jakobson *et al.,* (1960).

At the end of the experiment, three birds / treatment were randomly taken and slaughtered to obtain the lymphoid organs. Blood samples were taken to determine serum content of total protein, Albumin, Calcium and Phosphor, using commercial kits. The pH in different parts of the gastrointestinal tract was determined according to Al-Natour and Alshawabkeh (2005), as well as the definition and count of the gastrointestinal tract microbial

Table 1: Composition and calculated analysis of basal diets

Ingredients %	Starter (1-2 wk)	Grower (3-4 wk)	Finisher (5-6 wk)
Yellow corn	59.38	65.15	71.80
Soybean meal 44%	24.25	19.00	13.00
Corn gluten meal 60%	10.00	10.00	10.00
Corn oil	1.80	1.70	1.10
Limestone	1.18	1.09	1.06
Di-Calcium phosphate	1.98	1.75	1.65
Vit and min. premix*	0.25	0.25	0.25
DL-Methionine	0.16	0.10	0.09
L-lysine HCL	0.50	0.46	0.55
Salt (NaCl)	0.50	0.50	0.50
Total	100.00	100.00	100.00
Calculated analysis**			
CP %	22.00	20.07	18.02
ME (kcal/kg)	3096.00	3159.00	3192.00
Calcium %	1.00	0.90	0.848
Available Phosphorus %	0.499	0.45	0.42
Lysine %	1.349	1.176	1.086
Methionine %	0.60	0.52	0.485
Methionine and cystine %	0.967	0.86	0.79
Sodium %	0.21	0.21	0.21

*Each 2.5 kg contains: Vit A 12.000.0000 IU, Vit D_3 2.000.00 IU, Vit E 10 gm, Vit K_3 2 gm, Vit B_1 1 gm, Vit B_2 5 gm, Vit B_6 1.5 gm, Vit B_{12} 10 mg, Nicotinic acid 30 gm, Pantothenic acid 10 gm, Folic acid 1 gm, Biotin 50 mg, Choline chloride(50) 250 gm, Iron 30 gm, Copper 10 gm, Zinc 50 gm, Manganese 60 gm, Iodine 1 gm, Selenium 0.1 gm, Cobalt 0.1 gm, Carrier ($CaCO_3$) to 25 kg.

**According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001)

content (Quinn *et al*., 1992). In addition villus height from part of the small intestine was measured according to the method outlined by Lillie (1965).

The obtained data were statistically analyzed using linear models procedure described in SAS users guide (SAS, 1990). Differences among means were tested using Duncans multiple range test (Duncanes, 1955).

RESULTS AND DISCUSSION

Performance and nutrients digestibility: The effects of dietary Formic Acid (FA) on growth performance and nutrients digestibility are listed in Table 2. The results indicated that at 42 days of age, the chicks fed 0.5% FA recorded the heaviest BWG (1689.74 g) followed by those fed 0.25% FA (1638.24 g), while, those fed control diet recorded significantly the lowest value (1457.54 g). In spit of the significant absence among all treatments in FCR during the overall period, but the group fed 0.5% FA recorded the best FCR. No great differences in mortality rate were observed among all treatments as from 1 to 2 chicks were died all over the experimental period due to natural causes and not due to the nutritional treatments.

Chicks fed 0.5% FA recorded significantly ($p<0.01$) the best EPEI value compared to the control group. This result may be due to the positive effect of FA on bacteria (*E. coli*, *Salmonella*) in both stomach and small intestine (Dhawale, 2005).

Table 2: Effect of feeding formic acid on performance and nutrients digestibility of broiler chicks at 42 days of age

					Digestibility %				
Level%	BWG (g)	FCR	MR %	EPEI %	CP	EE	CF	NFE	ME kcal/g
Control	1457.45^{b}	1.89	7.41	182.65^{b}	91.93^{b}	71.81	24.48	78.97	3.40
0.25	1638.24^{a}	1.89	3.70	212.03^{ab}	92.72^{a}	74.31	26.05	79.95	3.50
0.5	1689.74^{a}	1.80	0.00	225.16^{a}	93.16^{a}	77.39	28.27	80.89	3.53
1.00	1594.09^{ab}	1.87	0.00	202.93^{ab}	92.32^{b}	74.92	31.09	79.66	3.48

^{a,b}Means in the same column with different superscripts are significantly different (p<0.01). FCR = Feed Conversion Ratio (g feed/g gain), MR = Mortality Rate, EPEI = European Production Efficiency Index, BWG (g) = Body Weight Gain (g)

Table 3: Effect of feeding fumaric acid on performance and nutrients digestibility of broiler chicks at 42 days of age

					Digestibility %				
Level %	BWG (g)	FCR	MR %	EPEI %	CP	EE	CF	NFE	ME kcal/g
Control	1457.45^{b}	1.89	7.41	182.65^{b}	91.93^{b}	71.81^{b}	24.48	78.97^{b}	3.40^{c}
0.5	1672.15^{a}	1.82	0.00	233.49^{a}	93.21^{a}	86.46^{a}	20.46	83.63^{a}	3.84^{a}
1.0	1676.60^{a}	1.82	3.70	226.29^{ab}	93.52^{a}	85.86^{a}	23.95	82.77^{a}	3.78^{ab}
1.5	1696.10^{a}	1.82	0.00	224.10^{ab}	92.85^{a}	84.22^{a}	27.81	80.73^{ab}	3.74^{b}

^{a,b,c}Means in the same column with different superscripts are significantly different (p<0.01). FCR = Feed Conversion Ratio (g feed/g gain), MR = Mortality Rate, EPEI = European Production Efficiency Index, BWG (g) = Body Weight Gain (g)

Table 4: Effect of feeding acetic acid on performance and nutrients digestibility of broiler chicks at 42 days of age

					Digestibility %				
Level %	BWG (g)	FCR	MR %	EPEI %	CP	EE	CF	NFE	ME kcal/g
Control	1457.45^{b}	1.89	7.41	182.65	91.93	71.81^{b}	24.48	78.97^{b}	3.40^{b}
0.25	1632.08^{a}	1.78	0.00	224.64	92.39	81.27^{a}	23.12	82.18^{ab}	3.60^{a}
0.50	1600.98^{a}	1.83	0.00	216.55	92.52	81.77^{a}	26.97	83.96^{a}	3.66^{a}
0.75	1658.26^{a}	1.77	0.00	221.37	93.12	82.28^{a}	28.13	83.57^{a}	3.63^{a}

^{a,b}Means in the same column with different superscripts are significantly different (p<0.01). FCR = Feed Conversion Ratio (g feed/g gain), MR = Mortality Rate, EPEI = European Production Efficiency Index, BWG (g) = Body Weight Gain (g)

Several investigators reported that FA (0.1-1.0%) had positive effect on growth performance of broiler (Garcia *et al*., 2007; Bozkurt *et al*., 2009; Helen and Christian, 2010). While, Izat *et al*. (1990) and Hernadez *et al*. (2006) reported that FA (0.5-1%) did not affect BW, BWG, FC and FCR of broiler. Dietary FA improved both ME and nutrients digestibility compared to the control (Table 2). The best results obtained from 0.5% FA compared to other treatments. Similar trend was found by Hernadez *et al*. (2006); Garcia *et al*. (2007) and Helen and Christian (2010) on apparent ileal digestibility.

In regard to Fumaric Acid (FUA) shown in Table 3, there were significant (p<0.01) increase by adding FUA in BWG, while FCR improved insignificantly compared to the control group at 42 day of age. The mortality during the experimental period was absent on 0.5 and 1.5% FUA level. EPEI was significantly (p<0.01) higher at 0.5% FUA compared to the control group. Similar results were found by Rafacz *et al*. (2005a); Pirgozliev *et al*. (2008) and Banday *et al*. (2010) who reported that FUA (0.1-3%) improved BW, BWG, FC and FCR in broiler. The mortality rate during the experimental period was agrees with results obtained by Waldroup *et al*. (1995) and Islam *et al*. (2008) who used FUA at levels (0.125-1.25%) with broiler and found a positive response in growth performance from the addition FUA to diets. The digestibility of CP, EE, NFE and ME significantly improved by adding FUA compared to the control group, with 0.5% FUA being recorded the highest values (Table 3).

Results presented in Table 4, summarize the effects of feeding the experimental diets having different levels of Acetic Acid (AC) on growth performance and nutrients digestibility of broiler. It was observed that all treatments significantly surpassed the control group for BWG and no significant differences between treatments were observed for FCR at 42 day of age, however, the 0.75% AC level achieved the best FCR. The mortality due to AC was almost absent among all treatments compared to control group. These results supported the finding of Abdel-Fattah *et al*. (2008) who reported that using 3%AC increased BWG and improved FCR. While, Pinchasov and Elmaliah (1994 and 1995) reported that growth performance of broiler did not affected by using 3% AC. The digestibility of EE, NFE as well as the ME for all treatments were significantly improved by acetic acid supplementation, while there were no significant differences for the digestibility of CP and CF compared to control group (Table 4). However, Furuse and Okumura (1989) found that protein, fat and energy retention were linearly lower as dietary acetic acid was increased from 12.7 up to 63.5 g/kg diet.

Growth performance of broiler fed Citric Acid (CA) are presented in Table 5. The results indicated that the BWG

Table 5: Effect of feeding citric on performance and nutrients digestibility of broiler chicks at 42 days of age

					Digestibility %				
Level %	BWG (g)	FCR	MR %	EPEI %	CP	EE	CF	NFE	ME kcal/g
Control	1457.45[b]	1.89	7.41	182.65[b]	91.93[b]	71.81[b]	24.48	78.97[c]	3.40[b]
1.0	1613.17[a]	1.88	3.70	213.28[ab]	93.16[a]	77.25[ab]	28.32	83.31[ab]	3.58[a]
2.0	1697.79[a]	1.81	0.00	241.44[a]	93.37[a]	84.75[a]	27.29	84.95[a]	3.73[a]
3.0	1712.04[a]	1.87	0.00	234.41[a]	93.07[a]	79.56[ab]	26.49	80.17[bc]	3.58[a]

[a,b,c]Means in the same column with different superscripts are significantly different ($p<0.01$). FCR = Feed Conversion Ratio (g feed/g gain), MR = Mortality Rate, EPEI = European Production Efficiency Index, BWG (g) = Body Weight Gain (g)

Table 6: Effect of formic acid on pH of GIT, cecal microbial count, villus height of duodenum, blood constituents and lymphoid organs of broiler chicks at 42 days of age

	FA (%)			
Item	Control	0.25	0.50	1.00
pH of GIT segments				
Crop	4.43[a]	4.26[b]	4.15[c]	4.14[c]
Gizzard	3.67[a]	3.39[b]	3.30[b]	3.31[b]
Duodenum	5.74[a]	5.60[ab]	5.48[b]	5.46[b]
Jejunum	5.80[a]	5.63[b]	5.37[c]	5.32[c]
Ileum	6.32[a]	6.05[b]	5.90[b]	5.84[b]
Ceca	6.31	6.28	6.24	6.22
Cecal microbial count (10 g^{10})*				
Lactobacillus	4.67[a]	3.69[d]	3.98[b]	3.78[c]
Coliforms	8.26[d]	8.79[b]	8.87[a]	8.76[c]
Anaerobes	8.93[c]	9.22[ab]	9.26[a]	9.11[b]
E. coli	2.52[ab]	2.60[a]	2.45[b]	2.53[ab]
Villus height (µm)				
Duodenum	45.55[b]	64.92[a]	66.30[a]	64.44[a]
Blood constituents**				
Calcium	10.59[b]	10.66[b]	12.14[a]	12.26[a]
Phosphorus	1.38[c]	2.29[b]	3.45[a]	2.18[b]
Total protein	2.80[c]	3.48[b]	4.42[a]	4.61[a]
Albumin	1.59[a]	1.02[b]	0.97[b]	0.98[b]
Globulin	1.20[c]	2.46[b]	3.45[a]	3.63[a]
Lymphoid organs (%)				
Spleen	0.12[b]	0.17[a]	0.17[a]	0.14[ab]
Bursa	0.09[b]	0.20[a]	0.20[a]	0.18[a]
Thymus	0.41[b]	0.42[b]	0.49[a]	0.51[a]

[a,b,c,d]Means in the same column with different superscripts are significantly different ($p<0.01$).
*Colony Forming Units (CFU/g sample).
**Ca, P (mg/dl), total protein, albumin and globulin (g/dl)

was significantly higher due to CA than control group at 42 day of age. Citric acid at 2% level achieved insignificantly an improvement in FCR and EPEI significantly than control group. These results are in harmony with the results of Boling *et al.* (2000); Snow *et al.* (2004); Rafacz *et al.* (2005a) and Ao *et al.* (2009) who reported that giving broiler CA (2-6%) improved BW, BWG, FE and FCR. On the contrary, Atapattu and Nelligaswatta (2005) and Rafacz *et al.* (2005b) reported that growth performance parameters were not significantly affected by dietary 2-4% CA. The mortality during the experimental period was absent on 2 and 3% CA level.

Fortunately, 2% of CA significantly surpassed the control group in nutrients digestibility and ME. In general, adding 2% CA to the diet was the best level for all traits studied. These findings are in agreement with Atapattu and Nelligaswatta (2005) and Ao *et al.* (2009) who found that CA (1 and 2%) increased the digestibility of CP, NDF and CF of broilers.

Generally the positive impact of dietary acidifiers on growth performance may be attributed to: (1) a reduction of the pH values in the feed and digestive tract, serving as a barrier against pathogenic microorganisms which are sensitive to low pH values; (2) the direct antimicrobial effect; (3) the reduction in buffering capacity in conjunction with improving digestibility coefficients of CP, EE, CF and ME.

Effect of organic acids on pH of GIT, cecal microbial count, villus height of duodenum, blood constituents and lymphoid organs of broiler chicks at 42 days of age: The effect of dietary Formic Acid (FA) on pH values of different Gastrointestinal Tract (GIT) segments are presented in Table 6. The results indicated that FA supplementations significantly reduced ($p<0.01$) crop, gizzard, duodenum, jejunum and ileum pH values compared to control group. These results were in harmony with the result of Al-Tarazi and Alshawabkeh (2003) and Alshawabkeh and Kanan (2005) who reported that adding 0.5-1.5% FA to broiler diet reduced significantly the intestinal pH. However, Hernadez *et al.* (2006) and Al-Natour and Alshawabkeh (2005) found insignificant reduction in the intestinal pH for broiler when used 0.5-1.5% FA.

Caeca contents of the birds fed control diet had significantly higher ($p<0.01$) counts of lactobacilli followed by 0.5% FA. While, 0.5% FA had higher caeca counts of coliforms and anaerobes bacteria and lower *E. coli* content (Table 6). In this connection, Alshawabkeh and Kanan (2005) and Al-Natour and Alshawabkeh (2005) found that addition of FA levels from 0.5-1.5% reduced significantly the viability of the *Salmonella gallinarum*.

Villus height was significantly ($p<0.01$) increased by FA used compared with the control group. Garcia *et al.* (2007) noted higher villi height in duodenum and jejunum with 5,000 or 10,000 ppm FA added to broiler diets.

Data presented in Table 6 indicated that dietary supplementation of FA showed higher blood Calcium (Ca) and Phosphorus (P) concentrations than control

Table 7: Effect of fumaric acid on pH of GIT, cecal microbial count, villus height of duodenum, blood constituents and lymphoid organs of broiler chicks at 42 days of age

	FUA (%)			
Item	Control	0.5	1.0	1.5
pH of GIT segments				
Crop	4.43^{a}	4.24^{b}	4.24^{b}	4.29^{b}
Gizzard	3.67^{a}	3.56^{ab}	3.36^{b}	3.47^{ab}
Duodenum	5.74^{a}	5.61^{ab}	5.65^{a}	5.32^{b}
Jejunum	5.80^{a}	5.42^{b}	5.69^{a}	5.31^{b}
Ileum	6.32^{a}	6.07^{b}	6.07^{b}	6.00^{b}
Ceca	6.31	6.04	6.05	6.12
Cecal microbial count (10 g^{10})*				
Lactobacillus	4.67^{a}	4.22^{b}	4.02^{c}	3.94^{d}
Coliforms	8.26^{b}	8.75^{a}	8.78^{a}	8.75^{a}
Anaerobes	8.93^{d}	9.09^{c}	9.34^{a}	9.21^{b}
E. coli	2.52^{a}	2.33^{b}	2.43^{ab}	2.53^{a}
Villus height (μm)				
Duodenum	45.55^{b}	67.50^{a}	67.23^{a}	64.57^{a}
Blood constituents**				
Calcium	10.59^{c}	12.55^{a}	11.22^{bc}	12.12^{ab}
Phosphorus	1.38^{b}	2.78^{a}	1.98^{ab}	1.89^{ab}
Total protein	2.80^{c}	4.50^{a}	4.44^{a}	3.83^{b}
Albumin	1.59^{a}	0.88^{b}	0.92^{b}	0.97^{b}
Globulin	1.20^{c}	3.62^{a}	3.53^{a}	2.86^{b}
Lymphoid organs (%)				
Spleen	0.12^{b}	0.19^{a}	0.13^{ab}	0.18^{ab}
Bursa	0.09^{c}	0.20^{a}	0.14^{b}	0.20^{a}
Thymus	0.41^{b}	0.51^{a}	0.51^{a}	0.49^{a}

^{a,b,c,d}Means in the same column with different superscripts are significantly different (p<0.01).
*Colony Forming Units (CFU/g sample).
**Ca, P (mg/dl), total protein, albumin and globulin (g/dl)

Table 8: Effect of acetic acid on pH of GIT, cecal microbial count, villus height of duodenum, blood constituents and lymphoid organs of broiler chicks at 42 days of age

	AC (%)			
Item	Control	0.25	0.50	0.75
pH of GIT segments				
Crop	4.43^{a}	4.27^{b}	4.24^{b}	4.28^{b}
Gizzard	3.67^{a}	3.31^{b}	3.25^{b}	3.26^{b}
Duodenum	5.74^{a}	5.60^{a}	5.56^{a}	5.50^{a}
Jejunum	5.80^{a}	5.84^{a}	5.62^{b}	5.64^{b}
Ileum	6.32^{a}	6.04^{b}	6.05^{b}	6.09^{b}
Ceca	6.31	6.18	6.20	6.21
Cecal microbial count (10 g^{10})*				
Lactobacillus	4.67^{c}	4.65^{d}	4.78^{b}	4.86^{a}
Coliforms	8.26^{d}	8.74^{b}	8.61^{c}	8.76^{a}
Anaerobes	8.93^{c}	9.25^{ab}	9.29^{a}	9.18^{b}
E. coli	2.52	2.53	2.50	2.53
Villus height (μm)				
Duodenum	45.55^{b}	66.40^{a}	65.83^{a}	65.62^{a}
Blood constituents**				
Calcium	10.59^{c}	12.00^{b}	11.45^{bc}	13.26^{a}
Phosphorus	1.38^{c}	2.08^{b}	2.78^{a}	2.25^{b}
Total protein	2.80^{b}	4.00^{a}	3.78^{a}	3.83^{a}
Albumin	1.59^{a}	1.08^{b}	0.94^{b}	0.88^{b}
Globulin	1.20^{b}	2.92^{a}	2.85^{a}	2.95^{a}
Lymphoid organs (%)				
Spleen	0.12^{b}	0.16^{a}	0.17^{a}	0.19^{a}
Bursa	0.09^{b}	0.19^{a}	0.19^{a}	0.20^{a}
Thymus	0.41^{b}	0.43^{b}	0.49^{a}	0.49^{a}

^{a,b,c,d}Means in the same column with different superscripts are significantly different (p<0.01).
*Colony Forming Units (CFU/g sample).
**Ca, P (mg/dl), total protein, albumin and globulin (g/dl)

group. The increase of Ca and P levels in blood produced by addition of FA may be attributed to the lowering of GI-tract pH by using these acids, consequently, increases the absorption of such minerals from the gut into blood stream. Total protein and globulin content of blood serum were significantly higher compared to control group by adding FA to the diets. While, serum albumin showed significantly decrease in all treatments studied compared to the control group. These results indicated that FA may improve the immune response. Globulin level has been used as an indicator of immune responses as source of antibody production. However, Hernadez *et al.* (2006) showed that using formic acid at 0.5, 1.0% in broiler diets had no effect in blood metabolites compared to control.

The relative weight of primary lymphoid organs (spleen, bursa and thymus) were significantly (p<0.01) improved by supplemental FA especially for 0.5% compared to the basal diet (Table 6). These results indicated that broiler chicks fed acidifiers in their diets 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.

Data in Table 7 show the effect of the different levels of Fumaric Acid (FUA)on pH of the GIT segments at 42 days of age. The reduction of pH values occurred by using FUA were significantly only for crop and ileum, while gizzard, duodenum, jejunum and caeca pH values were insignificantly reduced.

Lactobacillus counts (Table 7) were higher in the control group followed by 0.5% FUA, while, Coliforms and anaerobes were significantly lower with the control group compared to FUA. The population of *E. coli* was significantly lower with feeding 0.5% compared to control and 1.5% FUA. In this connection, Pirgozliev *et al.* (2008)found that birds offered diets supplemented with FuA at 0.5, 1.0 or 1.5% had lowered numbers of Lactic acid bacteria and Coliforms in the ileum and caeca, compared to control birds.

The duodenum and villi height were significantly increased by using all levels of FUA compared to the control group. In this respect, Banday *et al.* (2010) found that addition of FUA (2 or 3%) increased the villus height in the small intestines.

Also, broiler fed dietary FUA recorded higher Ca and P concentrations of blood serum than control group (Table 7). The increase of Ca and P levels in blood serum produced by addition of FUA may be attributed to the lowering of GIT pH by using, consequently, increases

Table 9: Effect of citric acid on pH of GIT, cecal microbial count, villus height of duodenum, blood constituents and lymphoid organs of broiler chicks at 42 days of age

	CA (%)			
Item	Control	1.0	2.0	3.0
pH of GIT segments				
Crop	4.43^{a}	4.22^{b}	4.20^{b}	4.11^{c}
Gizzard	3.67^{a}	3.38^{b}	3.31^{b}	3.35^{b}
Duodenum	5.74^{a}	5.47^{b}	5.35^{b}	5.45^{b}
Jejunum	5.80^{a}	5.55^{b}	5.54^{b}	5.67^{ab}
Ileum	6.32^{a}	6.05^{b}	6.02^{b}	6.09^{b}
Ceca	6.31	6.09	6.14	6.12
Cecal microbial count (10 g^{10})*				
Lactobacillus	4.67^{c}	4.96^{b}	5.15^{a}	4.98^{b}
Coliforms	8.26^{d}	8.65^{b}	8.74^{a}	8.44^{c}
Anaerobes	8.93^{c}	9.18^{a}	9.12^{b}	8.20^{d}
E. coli	2.52^{a}	2.40^{b}	2.43^{ab}	2.37^{b}
Villus height (µm)				
Duodenum	45.55^{b}	58.63^{a}	60.00^{a}	52.55^{ab}
Blood constituents**				
Calcium	10.59^{b}	10.43^{b}	13.38^{a}	11.86^{ab}
Phosphorus	1.38^{b}	2.14^{a}	2.11^{a}	1.89^{a}
Total protein	2.80^{b}	2.91^{b}	3.32^{a}	2.97^{b}
Albumin	1.59^{a}	1.12^{b}	0.91^{c}	1.05^{bc}
Globulin	1.20^{b}	2.00^{ab}	2.19^{a}	1.92^{b}
Lymphoid organs (%)				
Spleen	0.12^{b}	0.16^{ab}	0.19^{a}	0.15^{ab}
Bursa	0.09^{c}	0.17^{b}	0.19^{a}	0.18^{ab}
Thymus	0.41^{b}	0.48^{a}	0.52^{a}	0.48^{a}

^{a,b,c,d}Means in the same row with different superscripts are significantly different (p<0.01).
*Colony Forming Units (CFU/g sample).
**Ca, P (mg/dl), total protein, albumin and globulin (g/dl)

the absorption of such minerals from the gut into blood stream. Improving the utilization of Ca and P due to provision of FUA had been approved by Banday *et al.* (2010) who reported that serum Ca and P concentrations were increased by using 2 or 3% FUA in broiler diets. However, Liem *et al.* (2008) noted that plasma Ca level was not affected when broilers fed the basal diet supplemented with 2.9% FUA, while plasma P had been increased when compared with the control. Total protein and globulin were significantly increased, but, albumin significantly decreased in all treatments studied compared to the control group. These results indicated that FUA may improve the immune response, as globulin level has been used as an indicator of immune responses as source of antibody production.

The relative weight of spleen, bursa and thymus were significantly (p<0.01) improved by supplemental FUA especially at 0.5% FUA compared to the basal diet (Table 7).

The pH in the different GI-tract segments of the chicks fed Acetic Acid (AC) was significantly (p<0.01) lower than in the chicks fed control diet except caeca pH value which was insignificantly (Table 8) lower. In this respect, Abdel-Fattah *et al.* (2008) found that AC at levels of 1.5 and 3.0% of diet, reduced gizzard, duodenum, jejunum and ileum pH values compared to control, however, the differences did not reach significance.

The lactobacillus and coliforms counts of the caeca were significantly higher in the 0.75% AC group compared to other treatments. Anaerobes count were significantly higher in all treatments compared to control group. The difference in *E. coli* was insignificant for all studied levels of AC (Table 8). The duodenal villus height was highest (p<0.01) in AC compared to the control.

Blood serum constituents (calcium, phosphorus, total protein and globulin) were significantly increased by using AC compared to control group (Table 8). The best result obtained from using 0.75% AC for all blood serum constituents compared to control group. Improving the utilization of Ca and P due to provision of AC was approved by Abdel-Fattah *et al.* (2008) who observed an increase in blood calcium of broiler fed 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 absorbability of these minerals.

Dietary AC exhibited relatively noticeable increase (p<0.01) in serum concentration of total protein and globulin compared to the control, indicating that the immune response improved by addition of AC (Table 8). Supplemental AC increased significantly (p<0.01) the relative weight of both spleen, bursa and thymus especially at 0.75% comparable to the basal diet (control). This might indicate that broiler chicks fed acidifiers supplemented diets had better immune response and disease resistance. The present results are coincided with those obtained Abdel-Fattah *et al.* (2008) who reported that supplemental AC increased significantly (p<0.01) the relative weight of spleen, bursa and thymus.

The effect of Citric Acid (CA) on pH in the different GI-tract segments of the chicks was significantly (p<0.01) lower than in the chicks fed control diet except caeca pH value which was insignificantly (Table 9) reduced. In this respect, Waldroup *et al.* (1995) noticed that the pH value was altered when citric acid (0.25-2.00%) was continually fed to broilers. EL-Afifi *et al.* (2001) shown that ileal pH values were slightly decreased as dietary CA increased gradually (0.0, 0.2, 0.4, 0.6 and 0.8%). Also, Abdel-Fattah *et al.* (2008) found that CA at levels of 1.5 and 3.0% of diet reduced gizzard, duodenum, jejunum and ileum pH values compared to control group. However, the differences were not significantly. Moreover, citric acid significantly decreased the pH of the crop content (6.2 vs. 5.4), when used CA at 0 or 1.5%, in place of equivalent amounts of corn in the basal diet (Ao *et al.*, 2009). However, Atapattu and Nelligaswatta (2005) noticed that CA at 0, 1 and 2 % doesn't alter the pH of the GIT after gizzard, while decreased pH in the crop.

The total viable number of lactobacillus and coliforms in caeca contents were significantly (p<0.01) higher at 2.0% CA compared to other treatments while 1.0% CA showed higher number of anaerobes. *E. coli* was highest (p<0.01) with control group than all treatments studied (Table 9). In the same connection, EL- Afifi *et al.* (2001) found that feeding broiler on CA at 0.2, 0.4, 0.6 and 0.8% led to sharp reduction in ileal content of coliforms bacteria and bacterial total count, but the number of lactobacilli bacteria was slightly increased. However, Biggs and Parsons (2008) found that 4% CA decreased (p<0.05) the concentration of bifidobacteria when compared to the basal diet. The populations of *E. coli* and *C. perfringens* were unaffected by any of dietary CA treatments.

The duodenum villus height was significantly increased with the different levels of CA compared to the control. In this respect, Abdel-Fattah *et al.* (2008) indicated that using 1.5 and 3.0% CA of diet showed an increase in the small intestine morphology (weight and length).

As shown in Table 9, blood constituents (calcium, phosphorus, total protein and globulin) were significantly increased by using 2% CA compared to the other treatments.

Improving the utilization of Ca and P due to provision CA was approved by Abdel-Fattah *et al.* (2008) who observed an increase in blood calcium of broiler fed dietary CA. While, Boling *et al.* (2001) found that CA had no effect on Ca availability or the Ca requirement. Also, Liem *et al.* (2008) noted that CA and malic acid additions did not increase plasma P levels.

Adding CA increased significantly (p<0.01) total protein and globulin content of blood serum compared to the control. Also, supplemental CA increased significantly (p<0.01) the relative weight of both spleen, bursa and thymus especially at 2% level comparable to the control diet (Table 9). These results might indicate that broiler chicks fed the acidifiers supplemented diets had better immune response and disease resistance. The present results are coincided with those obtained with Abdel-Fattah *et al.* (2008) who reported that supplemental CA increased significantly (p<0.01) the relative weight of spleen, bursa and thymus. However, there was no effect on bursa or spleen weight when fed broilers on CA at 0.2, 0.4, 0.6 and 0.8% (EL-Afifi *et al.*, 2001).

In general, the increase of villus height of the different segments of the small intestine of chicks having FA, FUA, AC or CA 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 (Podolsky, 1993). So, organic acidifiers reduce the growth of many pathogenic or non-pathogenic intestinal bacteria, therefore, reduce intestinal colonization and reduce infectious processes, ultimately decrease inflammatory processes at the intestinal mucosa, which increase villus height and function of secretion, digestion and absorption of nutrients than can be appropriately performed by the mucosa (Loddi *et al.*, 2004; Pelicano *et al.*, 2005).

Therefore, from data obtained under such experimental conditions, it can be concluded that 0.5% of either formic or fumaric, 0.75% acetic or 2% citric acids could be used safely in broiler diets to improve their performance, digestion and health.

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