EFFECT OF DIETARY CITRIC, ACETIC ACIDS OR THEIR MIXTURE ON BROILER CHICKS PERFORMANCE, CARCASS CHARACTERISTICS AND SOME INTESTINAL HISTOMORPHOLOGICAL PARAMETERS

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SUMMARY

A total number of 120, 1d old unsexed chicks were randomly distributed and divided equally into 4 dietary treatment groups with 3 replicates each. All broiler chickens were kept under similar managerial conditions. Basal starter and finisher diets were supplemented with two types of organic acids as follows: T1: basal diet (control, without supplementation), T2: basal diet + 2% citric acid (CA), T3: basal diet + 1% acetic acid (AC) and T4: basal diet + 1% citric acid + 0.5% acetic acid. Two corn-soybean based basal diets were formulated to be fed during starter (1 to 21 d, 22.13% CP and 3088 Kcal ME/ kg diet) and finisher (22 to 42 d, 19.82% CP and 3154 Kcal ME/ kg diet) periods. Growth performance parameters, feed conversion ratio, some carcass characteristics and some lymphoid organs were estimated. Some blood parameters (total protein, albumin, total cholesterol, creatinine, ALT and AST) and histomorphological samples and parameters were determined at 42 day. Also, European productive and economic efficiency were calculated. Results indicated that; chicks fed diets supplemented with a mixture of citric acid (1%) and acetic acid (0.5%) had significantly ($P \leq 0.05$) the highest values of body weight, body weight gain and the best feed conversion ratio, while recorded the lowest feed intake compared to the other treatments. Moreover, using mixture of supplementation significantly ($P \leq 0.05$) improved performance index (PI) in comparison with the control treatment. A significant ($P \leq 0.05$) beneficial effect of citric acid and acetic acid or their mixture as feed supplementation were found concerning dressing, giblets and some immune organs percentages at 42d of age compared to the control group. Mixture of citric acid and acetic acid supplementation significantly ($P \leq 0.05$) increased some serum biochemical constituents (total protein, albumin, globulin and liver enzyme; ALT), while, total lipids and cholesterol concentrations were significantly ($P \leq 0.05$) decreased. Histomorphological sections of the small intestine revealed villi height and villi widths were significantly ($P \leq 0.05$) increased with the supplementation of organic acid alone or in their mixture compared to the control group. Also, the mixture of 1% CA + 0.5% AC supplementation had beneficial effects on economical efficiency. In conclusion, there are some beneficial effects of using a mixture of citric acid and acetic acid (1% citric acid + 0.5% acetic acid) in the diets of chicks on productive performance, carcass traits, with no harm effect on health under experimental conditions.

Keywords*: Citric acid, acetic acid, carcass traits, serum biochemical consituents, histomorphological parameters and broiler chicks.*

INTRODUCTION

A modernistic challenge in the poultry production is to exploit the use of specific dietary supplements to boost the intrinsic potential of poultry for better growth. The nutritional and health status of birds are largely influenced by their gut health, which affects digestion, absorption, and metabolism of nutrients, as well as disease resistance and immunity (Yegani and Korver, 2008).

High levels of production and efficient feed conversion are critical to modern poultry industry, which could be achieved by using specific feed additives. Some of the commonly used feed additives are organic acids, probiotics, prebiotics, medicinal extract and exogenous enzymes. These feed additives were used as antimicrobial, antioxidants, emulsifiers, binders and pH control agents in the poultry diet (Rehman *et al*., 2016; Jasim and Fehan, 2017; Attia *et al*., 2018 and Adhikari *et al.*, 2020).

Organic acid treatments composed of individual acids or blends of several acids to perform antimicrobial activities similar to those of antibiotics (Wang *et al*., 2009). The European Union allowed the use of organic acids and their salts in poultry production because these are generally considered safe (Adil *et al.*, 2010). Organic acids are week acids, which modulate the intestinal pH. When these compounds are used correctly a long with good nutritional, management and biosecurity measures, they could be a powerful tool in maintating the health of the gastrointestinal tract (GIT) in poultry thus improving the performance (Sabour *et al*., 2019 and Adhikari *et al*., 2020). Organic acids had higher globulin concentration and better immune response and reduced serum cholesterol and total lipid (Youssef *et al*., 2017; Ali *et al*., 2019 and Mirderikvandi *et al*., 2019).

Citric acid (CA) is the most common organic acid used in poultry diets. It acts as a growth promoter through acidifying the gastrointestinal (GI) content. CA is an organic acid that has been recently employed to improve feed utilization and its influence seems to depend on the diet composition (Mohammadi and Khosravinia 2015 and AL-Harthi and Attia, 2016). In addition, citric acid was found to change the gut PH and increase the activity of some enzymes that need acidic conditions, such as pepsin and phytase, therefore enhancing the utilization of protein and some minerals (Wickramasinghe *et al*., 2014), increasing the digestibility of protein and fibre (Atapattu and Nelligaswatta, 2005), improving live weight gain, feed conversion effieciency and absorpition of minerals

(Sharifuzzaman *et al*., 2020). Citric acid also decreased pH of ceacal digesta (Jozefiak and Rutkowski, 2005), crop and gizzard (Andrys *et al*., 2003) and intestine (Dehghani and Jahanian, 2012 and Attia *et al*., 2018) in broiler chicks. It reduced microbial load and result in better immune response in broilers (Wickramasinghe *et al*., 2014).

Acetic acid are among greater attention for poultry industry (Kral *et al*., 2011 and Mohammadi *et al*., 2018). It is generally belived that use of acetic acid in broiler diets may inhibit pathogens like salmonella in both raw material and feed (Choct, 2001). The lower PH created can protect the animal from infection especially at their younger ages (Mohammadi *et al*., 2018). Acetic acid at a wide dose range from 0.5 to 5% has been implemented in diets for broiler chickens which mainly reduced growth of many pathogenic or non- pathogenic intestinal bacteria, therefore, reduced the risk of intestinal colonization and infectious processes, ultimately decrease inflammatory processes at intestinal mucosa, a phenomenon which in turn, increase villus highet and function of secretion, digestion and absorpition of nutrients by the mucosa (Pelicano *et al*., 2005). Acetic acid (AC) supplementation in diet improves growth performance (Seifi *et al*., 2015 and Abdel Razek *et al*., 2016), quicken intestinal tissue, resulting in changes in villus width, height and area of the duodenum, jejunum and ileum, which all are the promising factors for the better intestinal health and nutrient digestion and absorpition (Kum *et al*., 2010; Mohammadagheni *et al*., 2016 and Saleem *et al*., 2016). Citric acid (CA) and acetic acid (AC) have been used in diets due to their positive effect on bird's health and growth (Islam *et al*., 2008).

Therefor, the objective of the present study was to investigate the effects of dietary supplementation of some organic acids such as citric, acetic or the mixture citric and acetic at the levels of 2, 1, or 1 and 0,5%, as respectivily on growth performance, carcass characteristics, intestinal histomorphology, some serum blood parameters and economic efficiency in broiler chicks.

MATERIALS AND METHODS

The present study was conducted in a private farm in Menouf, Menoufia Governorate, Egypt, throughout the experimental period from May to June 2018 to study the effect of dietary supplementation of some organic acids (citric or acetic acids and their mixture) on growth performance, carcass characteristics, some histomorphological measurements, some serum biochemical parameters and economic efficiency of broiler chicks.

Experimental diets:

Two corn-soybean based basal diets were formulated to be fed during starter (1 to 21 d) and finisher (22 to 42d) periods. The broiler diets were formulated to be approximately isocacloric and isonitrogenous and meet or exceed the nutritional requirements according to National Research Council’s nutrient (NRC, 1994) and used to formulate the basal diet (Table 1). The basal corn – soybean meal starter diet contained approximately, 22.13% CP and 3088.38 Kcal ME/kg diet and 19.82% CP and 3154 Kcal/kg in finisher diet and both were offered in mash form. Proximate chemical analysis of the basal diets fed was performed according to AOAC (2011). Basal starter and finisher diets were supplemented with two types of organic acids,(OA)* as: citric acid, (CA) and acetic acid, (AC) as follows:

T_1: basal diet (control, without any supplementation).

T_2: basal diet + 2% citric acid.

T_3: basal diet + 1% acetic acid.

T_4: basal diet +1% citric acid + 0.5% acetic acid. Mixing of experimental diets was done weekly.

Chicks assay procedures:

On the day of hatch, one hundred and twenty, mixed sex Arbor Acres chicks were used in this experiment. Chicks were wing banded, weighed and randomly allotted to four treatment groups, 3 replicates of 10 chicks each nearly similar in initial body weight (45g). Groups were reared in pens with litter (wheat straw) from 1 day old up to 42 days of age.

Throughout the experimental period (42d), chicks were given feed and water *ad libitum*. The management of broiler chickens was consistent with the guidelines (Arbor Acres Broiler Commercial Management Guide; http: //en. aviagen.com/assets/Tech_Center/AA_Broiler/AA-Broiler-Handbook2014i-EN.pdf). A 23 h of light and 1 h of darkness lighting schedule was maintained for the duration of the experiment. The initial temperature was 33 °C at the first day of age and decreased approximately 2 °C/ week until 24 °C, which was maintained at this temperature till the end of the experiment. Temperature, humidity, light and ventilation were the same for all treatments. Vaccination was performed according to breeder standards and was the same for all experimental treatments. All proper husbandry practices were followed.

The following parameters were measured:

Body weight (BW) an individual body weight was recorded while, ***body weight gain (BWG) and average daily gain (ADG)*** were calculated from the first day of the experiment and by weekly throughout the experimental periods.
Feed intake and feed conversion ratio*:*Total feed intake / dietary treatment group / day was recorded and expressed as feed (g) / bird / day. Feed conversion ratio was expressed as feed (g) / body weight gain (g).
Performance index (PI), mortality ratio (MO) and livability (Liv), %:
Performance index was calculated according to North (1984) as follows:

PI = Live body weight gain (Kg) ×100 / feed conversion ratio.

Mortality was recorded during the experimental periods, mortality % was calculated by subtracting the number of live birds at the end of the experiment from the total number of birds at the begining of the experiment. The product was multiplied by 100 to obtain the percentage mortality % as follow: MO,% = (No. of birds at the begining of a given period – No. of birds at the end of the same period) × 100 / No. of birds at the begning of a given period. Livability (Liv, %) = No. of birds at the end of the experiment period × 100 / No. of birds at the beginning of the experiment.

Slaughter and carcass information:

At the end of 42 days of age, three birds from each dietary treatment were weighed and slaughtered, after feed withdrawal for 12 hours to determine carcass traits: eviscerated carcass (without head, neck and legs) and total giblets (liver, gizzard and heart) weights were expressed relative to live body weight.

The bursa of fabricius, spleen and thymus (all lobes from left side of the neck) were cut and weighed to the nearest milligram. Data obtained was used for the calculation of dressing percentage as follows:

Dressing percentage (%) = carcass weight ×100 / live weight of bird.

Serum samples and biochemistry parameters:

Blood samples were taken at slaughter time from each bird, individual blood samples were collected. into tubes without heparin and serum was separated by centrifugation at 3500 rpm for 15 minutesand frozen at –20ºC until analysis Serum total protein (TP), albumin (A), total lipids (TL), cholesterol (Chol.), creatinine and glucose were determined using commercial kits. The globulin (G) value was obtained by subtracting the values of albumin from the corresponding values of total protein. Also, albumin/ globulin (A / G ratio) values were obtained by dividing the values of albumin on the values of globulins according to Coles, (1974). Also, liver function enzymes including aspartate amino transaminase (AST) and alanine amino transaminase (ALT) were calorimetrically estimated according to Reitman and Frankel (1956).

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*Organic acids, citric acid (CA) was supplied from Egyptian Company for Laboratory Services, Cairo, Egypt and Acetic acid, AC powder was obtained from AVITASA (ESP43100164) I C. Europa s/n pol Constanti (Tarragona) Spain and sale only from EVPCO group, Egypt veterinary pharmaceuticals company for animal and poultry feeding line only, Alexandria, Egypt.

Table (1): Composition and chemical analysis of the experimental diets fed during starting (1 –21) and finisher periods (22 – 42) days of age.

Ingredient	Starter diet	Finisher diet
Ground yellow corn (8.5%).	50.85	57.64
Soybean meal (44%).	40.47	33.92
Vegtable oil.	5.39	5.23
Limestone ground.	2.10	2.04
Di–calcium phosphate.	0.39	0.35
Vitamins and minerals mixture[1].	0.30	0.30
DL-Methonine[2].	0.20	0.22
Salt (sodium chloride).	0.30	0.30
Total	100	100
Calculated values[3]:		
Crude protein, %.	22.13	19.82
ME, kcal/ kg diet.	3088	3154
C/ P ratio.	140	159
Lysine, %.	1.29	1.11
Methionine, %.	0.57	0.54
Calcium, %.	1.02	0.97
Available phosphours, %.	0.44	0.42
Determined values:		
Dry matter, %.	89.40	88.49
Crude protien, %.	22.09	19.80
Ether extract , %.	4.56	6.01
Crude fiber, %.	3.64	3.58
Ash, %.	6.01	6.14

[1]*Vitamin and Mineral mixture at 0.30%of the diet supplies the following per kilogram of the diet: Vitamin A 12,000 IU, vitamin D_3 3,000 IU, vitamin E 40 mg, vitamin K_3 3 mg, vitamin B_1 2mg, vitamin B_2 6 mg, vitamin B_6 5 mg, vitamin B_{12} 0.02 mg, niacin 45 mg, biotin 0.075 mg, folic acid 2 mg, pantothenic acid 12 mg, manganese 100 mg, zinc 600 mg, iron 30 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1mg.*

[2]*DL – Methionine: 98% feed grade (98 % Methionine).*

[3]*Calculate according to NRC (1994).*

Histomorphological samples and parameter:

For the histological study of intestinal villi, at 42 days age, samples of duodenum, jejunum and ileum were obtained from the slaughtered birds. The specimens was 2 cm of the middle portions of the duodenum, jejunum and ileum were excised and fixed in 4% buffered formalin (Bancroft *et al.,* 1996). Segments referred to the midpoint of the duodenum (from gizzard to pancreo-biliary duct), jejunum (the midpoint between the entry of the common bile duct and the Meckel’s diverticulum) and ileum (from the Meckel’s diverticulum to ileocecal junction). Particular segments were gently flushed and rinsed with 0.9% physiological saline and then fixed in 4% neutral-buffered formalin solution for histological study.

Samples of the small intestine were transferred from formaldehyde after dehydration through passing tissue by sequences of alcohol solutions and then were cleared by xylene and embedded in paraffin. These fragments were opened longitudinally on styrofoam plates and washed with saline. The samples were fixed with Bouin’s solution for 24 h for histological analysis, according to Uni *et al.* (1999). The fragments cuts of 5 µm thick and stained with Hematoxylin and Eosin (H &E).

Morphmetric data from villus height and crypt depth were obtained from images captured by photomicroscope (Olympus).The tissue sections were taken for measuring the villus height, villus width and crypt depth. Morphometric measurements were performed on the selected 9 villi from each sample. The height of intestinal villi was measured from the tip to the base of villi at the opening crypt, and the villus width was measured at its midpoint (**Geyra *et al.,* 2001**). The intestinal crypt depth was measured from the base of the villi to submucosa, and the muscular thickness from the submucosa to the external layer of the intestine (Ebrahimi *et al.*, 2017).

Economical efficiency, relative economical efficiency and European productive efficiency, (%):

The economic efficiency was calculated from the input – output analysis (Heady and Jensen, 1954) assuming that other head costs were constant, as follows: [(price of kg weight gain-feed cost /kg gain)/ feed cost /kg gain × 100] under the experimental conditions and European productive efficiency was calculated as: EPE, % = (Mean body weight (Kg) × livability % ×100) / feed conversion ratio × marketing age, days), a cited by Soltan and Kusainova, (2012).

Statistical Analyses:

The experiment was conducted using a completely randomized design using SPSS (2011) program and the difference among treatment means were determined using Duncan's multiple range test (Duncan, 1955). Percentages were transformed to the corresponding arcsine values before performing statical analysis (Snedecor and Cochran, 1982) the following statical model was applied:

$$Yij = \mu + \alpha i + Eij.$$

Where:

Yij = Observed traits,

μ= Overall mean,

αi= Effect of treatment (I = 1, 2, 3 and 4) and

Eij= Experimental random error.

RESULTS AND DISCUSSION

Effect of dietary citric acid (CA), acetic acid (AC) or their mixture on broiler performance:

Body weight, weight gain and daily gain:

The effect of dietary citric acid (2% CA), acetic acid (1%AC) or their mixture (1% CA + 0.5% AC) supplementation on body weight (BW) , body weight gain (BWG) and average daily gain (ADG) of growing broiler chickens up to 42 days of age are shown in Table (2). The data showed a significant ($P \leq 0.05$) increased in body weight for birds fed dietaries 2% CA (T_2) and 1% acetic acid (T_3) or their mixture of organic acids (T_4, 1% CA + 0.5% AC) being 1232.23, 1220.06 and 1262.50 g, respectively compared to 1218.45g in the control group. As for BWG and ADG (Tables 2), chicks fed basal diets without supplementation had significantly lower ($P \leq 0.05$) body weight gain (751.74g) and ADG (53.79 g) during the period from 28 to 42 days of age. Broiler chicks fed 2% CA, 1% AC supplementation have the heighest BWG being (795.11 and 775.36g) and ADG values were 56.79 g and 55.50 g for T_2 and T_3, respectively during the period from 28 to 42 days of age, body weight gain and average daily gain were significantly increased ($P \leq 0.05$) with the mixture of citric acid and acetic acid supplementation being; 812.63 and 58.07g, respectively for T_4 compared to other treatment and control group.

In general, birds fed dietary the mixture of 1% CA + 0. 5%AC (T_4) or chicks fed dieary 1% AC (T_3) followed by those fed 2% CA supplementation (T_2) had the greatest BW, BWG and ADG, being (2075.13, 1995.42 and 2027.34g) for BW at 42 days of age; 2029.97, 1982.53 and 1948.15g in BWG and for ADG; were 50.02, 47.36 and 46.28g, respectively during the period frome 1 – 42 days of age compared to 1970.19 for BW at 42 days of age, 1924.98 and 46.13g for BWG and ADG, respectively for the control group during the period from 1 to 42 of age.

The improvement in BW, BWG and ADG of broilers fed diet contain of citric acid, acetic acid or thire mixture may be due to the following reasons a) acetic acid acts on pepsinogen and convert it to pepsin and increased the activity of pepsin and improves protein digestibility in broiler chicks (Ulaiwi *et al*., 2017), b) the antimicrobial property and low pH of organic acid inhibits the pathogenic intestinal bacteria and decreases the level of toxic bacterial products. So, energy and protein digestibility were improved (Adil *et al*., 2011 and Sultan *et al*., 2015), c) organic acids improve the protein digestibility by decreasing endogenous nitrogen losses and production of ammonia as well as other growth depressing metabolites (Dibner and Buttib 2002), d) intestinal cell proliferation and hence more available nutrients for absorption (Samanata *et al.*, 2010), and the acidic condition increases the nutrients availability for better performance (Boling *et al.*, 2001). Acetic acid has more effects on performance of broiler, addition of acetic acid to mash diet may lead to increase in palatability and improve mixing ration items

(Mohammadagheni *et al*., 2016). These results were a greement with the results reported by Khan and Iqbal (2016) and Youssef *et al.* (2017), who recorded that an improvement in BW and BWG for birds fed diets supplemented with organic acids meantime the growing period, which was due to the effect of keeping the beneficial bacteria population, improving nutrient digestion and may be impact the safety of microbial cell membrane or prohibit the nutrient transport and energy metabolism causing the bactericidal effect (Ricke, 2003). Many investigators reported that single or mixture of organic acid (CA and AC) can improve performance of chicks and had a significant effect of mean BW, WG and ADG on broiler chicks (Sabour *et al*. (2019); Adhikari *et al*., 2020; Sharifuzzaman *et al*., 2020 and Stamilla *et al*. (2020). On the other hand, Kopecky *et al*. (2012) and Flamand *et al.* (2014) showed that organic acid (citric and acetic acids) addition to the corn- soybean diet did not affect chick body weight, body weight gain and average daily gain.

Feed intake (FI), feed conversion ratio (FCR) and performance index (PI):

Data describing the effect of dietary organic acid as citric, acetic acids or their mixture on feed intake (FI, g / chick/ day) of broiler chickens during the period from 1 - 42 days of age are presented in Table 3. Broiler chickens fed citric and acetic acid supplemented to the basal diet significantly affect the feed intake of the treated- groups compared to the control group during 1 - 14 days. Also, basal diet contained citric acid and acetic acid (1% CA + 0.5% AC) significantly ($P \leq 0.05$) decreased feed intake of ArborAcres chicks during the experimental periods (14 – 28 days of age. Values of FI (g / chick / day) being; 90.17 g compared to other treatments T_2 (2% CA), T_3 (1% AC) and control group (0, without supplementation) being, 91.14, 93.45 and 95.71, respectively during 14- 28 d. Feed intake (FI) for treatments T_2, T_3 and T_4 were 128.01, 132.08 and 125.33 g), respectively compared to133.16g / chick/ day for control group at 28 – 42 days of age. In general, during the entire experimental period from 1- 42 days of age, chicks fed the basal diet with 1% CA + 0.5% AC (T_4) had significantly decreased feed intake 90.11g / bird / d in comparsion with the control group (95.67) and other experimental groups being, 91.45 and 93.96 g / b / d, respectivelyduring the period from 1 – 42 days of age.

The reduction in feed intake (g / chicks / d) was noticed during the starter and finisher period, this may be due to the decrease in palatability of acidified diets during experimental diets. They had a low tendency to free their H^+ ions and thus tended to have a strong taste associated with them which might have decrease the palatability feed and resulted in reduced feed intake Abdel Fattah *et al*. (2008); Adil *et al*. (2010) and Allahdoa *et al*. (2018). Also, organic acids increase the availability of nutrients from the feed which in turn decrease the feed consumption Pakhira and Samanta (2006) and sultan *et al.* (2015). These results is harmony with the results of Abdel Razek *et al.* (2016) who reported that chicks fed diets supplemented citric acid and acetic acid significantly consumed less feed ($P \leq 0.05$) compared to control group. It was observed that the lowest ($P \leq 0.05$) amount of feed intake was recorded for birds fed diets containing (1.61 and 1.70) of CA and (1.98 and 2.30) of AC. Additionally these results were in line with the finding of Ali *et al.* (2019); Mirdrikvandi *et al*. (2019); Omidi *et al.* (2020) and Stamilla *et al.* (2020) who reported decreased feed intake with higher level of organic acids such as citric and acetic acids application. On the other hand, the present results disagreed with those observed by Wickramasinghe *et al.* (2014), Seifi *et al*. (2015) and Araujo *et al*. (2018) who reported that adding different levels of citric acid and acetic acid to broilers did not have effect on feed intake. Results in Table 3 presents the effect of dietary different levels of citric acid, acetic acids or their mixture supplementation on feed conversion ratio (FCR) during the experimental periods from 1 to 42 days of age. Data revealed that FCR was significantly improved by the supplementation during the experimental period (1- 42 days of age).

Chicks consuming the basal control diet (T_1) had FCR 2.08 during 1 - 42 days of age, but feed conversion ratio was improved gradualy with the supplementation of citric acid (2%CA) and acetic acid (1% AC). The best value of FCR was 1.83 for chicks fed diet supplemented with themixture of citric and acetic acids (1% CA + 0.5% AC) at 1- 42 days of age.The significant improvement in FCR by the addition CA and AC may be due to: a) organic acids as the plays a very important role that acidification increasing gastric proteolysis and protein / amino acid digestibility by enhancing digestive system enzyme activities (Langhout, 2000).

The reason why protein is better used when CA is added to diets is due to the fact that pepsinogen is converted to pepsin, which increases pepsin activity and improves protein digestibility. Morever, peptides a rising from pepsin proteolysis trigger the release of hormones (including gastrin and cholecystokinin) which regulate the digesion and absorption of protein, b) organic acids due to the increasing efficiency of nutrients (Chowdhury *et al*. 2009), c) the numerical reduction in feed intake and concomitent increase in

BWG (Dehghani and Jahanian 2012) and d) organic acids due to the metabolic role of CA and AC in many metabolic path ways (Attia *et al.*, 2013).

Table (2): Effect of dietary citric acid, acitic acid or their mixtur supplementation on body weight, body weight gain and daily weight gain of broiler chicks during experimental periods (Means ± S.E.).

Dietary treatments[1]	Body weight		Body weight gain		Average daily gain	
	28 days of age	42 days of age	28-42 days of age	1-42 days of age	28-42 days of age	1-42 days of age
	………………………g……………………					
T_1	$1218.45^{c2,3}$ ± 20.23	1970.19^{d} ± 17.70	751.74^{d} ± 10.57	1924.98^{d} ± 11.95	53.79^{c} ± 0.83	46.13^{ab} ± 0.73
T_2	1232.23^{ab} ± 9.80	2027.34^{b} ± 12.25	795.11^{b} ± 9.94	1982.53^{b} ±10.25	56.79^{ab} ± 0.94	47.36^{b} ± 1.12
T_3	1220.06^{b} ± 16.58	1995.42^{c} ± 10.16	775.36^{c} ± 7.53	1948.15^{c} ± 13.15	55.50^{b} ± 1.14	46.28^{ab} ± 1.09
T_4	1262.50^{a} ± 9.49	2075.13^{a} ± 8.26	812.63^{a} ± 8.49	2029.97^{a} ± 10.26	58.07^{a} ± 1.19	50.02^{a} ± 1.02
Sig.	*	*	*	*	*	*

[1] T_1; control; basal diet without any supplementation, T_2; basal diet + 2% citric acid, T_3; basal diet + 1%aceticacid and T_4; basal diet + 1% citric acid + 0.5% acetic acid.

[2] means ± S.E. of 3 replicates / treatment.

[3]a, b, c and ….etc. means within the same colum with each different superscript are significantly different ($P \leq 0.05$).

The findings are supported with that of (Mustafa *et al.* (2014); Sultan *et al.* 2015; Al- Harthi and Attia (2016) who suggested that the inclusion of organic acids (citric acid and acetic acid) in broiler chicks diet significantly enhanced FCR. Organic acids added to feeds should be protected to avoid their dissociation in the crop and in the intestine (high pH segments), where the bulk of the bacteria population located. The beneficial effect of growth promoter substances on performance is related to a more efficient use of nutrients, which in turn results in an improveing FCR. Similar obsrvations were noticed by Rahman *et al.* (2018); Sabour *et al.* (2019) and Omidi *et al.* (2020). However, Araujo *et al.* (2018) and Elmi et al. (2020) did not observe differences in FCR of broilers fed diets contained acetic acid and citric acid supplementation during 1- 35 d and 1- 42 days of age.

Results presented in Table (3) indicated that there were significant differences among treatments in performance index (PI) during the different experimental periods 14, 28 and 42 days of age. Performance index recorded 34.05, 29.48, 32.87 and 26.94 % for chicks fed experimental diets (T_4; 1% CA + 0.5% AC), T_3 (1% AC), T_2 (2% CA) and control diets (T_1), respectively at 14 days of age. The highest PI (72.97%) was recorded in chicks fed the mixture of organic acid (T_4) followed by 68.10, 66, 01% in groups fed 2% CA (T_2), and 1% AC (T_3),, respectively compared to 63.46% in the control group at 28 days of age. The best PI was obtained with chickens fed the mixture of 1% citric and 0.5% acetic acids (94.75%), while the lowest PI value was obtained in control group being 78.81%. The positive response on PI by organic acid in early life could be attributed to the decreasing pH and increasing the proteolytic enzyme activity and nutrient digestability along with bacteriostatic and bactericidal action to the pathogenic bacteria (Papatsiros *et al.* 2013).

Table (3): Effect of dietary citric acid, acitic acid or their mixture supplementaltion on average feed intake (FI), feed conversion ratio (FCR) and performance index (PI,%) of broilerchicks during experimental periods (Means ± S.E).

Parameters	Age (days)	Dietary treatments[1]				Sig.
		T_1	T_2	T_3	T_4	
Feed intake (FI, g / chick /day)	1 -14	57.87[a2,3]± 0.67	54.93[b]± 0.30	56.35[a]± 0.35	54.79[c]± 0.42	*
	14 -28	95.71[a] ±0.57	91.14[c]± 0.59	93.45[b] ± 0.79	90.17[d] ± 0.18	*
	28 - 42	133.16[a] ±0.49	128.01[b] ± 0.26	132.08[a] ± 0.22	125.33[c] ± 0.37	*
	1 - 42	95.67[a] ± 0.47	91.45[c] ± 0.43	93.96[b] ± 0.35	90.11[d] ± 0.56	*
Feed conversion ratio (FCR,g feed/ g gain)	1 -14	1.82[a] ± 0.08	1.60[c] ± 0.02	1.72[b] ± 0.01	1.57[d] ± 0.03	*
	14 -28	1.92[a] ± 0.01	1.81[c] ± 0.02	1.86[b] ± 0.02	1.73[d] ± 0.01	*
	28 - 42	2. 50[a] ± 0.03	2.25[c] ± 0.08	2.38[b] ± 0.08	2.19[d] ± 0.08	*
	1- 42	2.08[a] ± 0.18	1.89[c] ± 0.09	1.99[b] ± 0.02	1.83[d] ± 0.02	*
Performance index (PI,%)	14	26.94[d] ± 2.90	32.87[b] ± 2.20	29.48[c] ± 2.43	34.05[a2,3] ± 2.20	*
	28	63.46[d] ±1.43	68.10[b] ± 2.73	66.01[c] ± 1.73	72.97[a] ± 1.40	*
	42	78.81[d] ± 3.21	90.13[b] ± 2.26	88.84[c] ± 2.74	94.75[a] ± 2.33	*

[1] T_1; control; basal diet without any supplementation, T_2; basal diet + 2% citric acid, T_3; basal diet +1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic acid.
[2] means ± S. E. of 3 replicates / treatment.
[3]a, b, c and ….etc., means within the same row with each different superscript are significantly differen ($P \leq 0.05$).

Mortality, Livability and European productive efficiency:

Experimental results presented in Table 4 showed the effect of dietary citric and acetic acids or their mixture supplementation on mortality, livability and European productive efficiency at 42 days of age. Mortality % was obtained between 3 and 13%. The lower value of mortality percentage (3.33) was in T_4 and the higher mortality percentage was obtained in T_1: control (13.33). Moreover, livability % was significantly improved (96.67%) in chicks fed diet supplemented with the mixture of 1%CA+ 0.5% AC followed by chicks fed 1% AC and those fed 2% CA, being 93.33 and 90.00%, respectively compared to 86.67% in the control group. The same trend was noticed at European productive efficiency which recorded 218.61, 192.38, 186.31 and 162.62 for chicks fed T_4, T_2, T_3 and T_1, respectively at 42 days of age (Table 4). In summary, the better livability % and European productive efficiency (EPE) in organic acid (CA+ AC) addition in broiler diets may be due to the effect of organic acid in controlling pathogenic bacteria or maintaining the health of the GIT (Flamand *et al.* 2014).

Allahdoa *et al*. (2018); Adhikari *et al*. (2020) and Stamilla *et al*. (2020) observed that overall mortality was lower in organic acid supplementation compared to control groups. Generaly the positive impact of dietary organic acids as citric acid (CA), acetic acid (AC) or their mixture on growth performance may be attributed to: a) A reduction of the pH values in the feed and digestive tract, serving as a barrier against pathogenic micro - organisms which are sensitive to low pH values. b) The direct antimicrobial effect. Our results are disagree with the results of (Flamand *et al.*, 2014 and seifi *et al.*, 2015) who reported that there were no significant differences in mortality rate by adding levels of organic acids (0.5, 1, 1.5 and 2%) than control group during 22- 42 d. in broiler chickens.

Table (4): Effect of dietary citric acid, acitic acid or their mixture supplementation on mortality, livability and European productive efficiency of broiler chicks during experimental periods (Means ± S.E.).

Item	Dietary treatments[1]			
	T_1	T_2	T_3	T_4
Number of birds at the beginning of the experiment.	30	30	30	30
Number of birds at the end of the experiment.	26.00	27.00	28.00	29.00
Final body weight, (Kg).	1.97	2.02	1.99	2.08
Mortality percentage, (%).	13.33	10	6.67	3.33
Livability percentage, (%).	86.67	90.00	93.33	96.67
European productive efficiency,(EPE, %)[2] .	162.62	192.38	186.31	218.61

[1]*T_1; control; basal diet without any supplementation, T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic acid*

[2]*EPE, % = (Mean body weight, Kg. × livability % × 100) / feed conversion x marketing age, days), cited by Soltan and Kusainova, 2012.*

Effect of dietary citric acid and acetic acid supplementation on carcass characteristics and some immune responses parameters of broiler chicks:

Experimental results of the effect of adding CA, AC or their mixture supplementation to broiler chick diet on carcass characteristics and immune organs parameters at 42 days of age are shown in Tables 5 and 6. At 42 days of age addition of AC and CA mixture to broiler chicks significantly increased pre-slaughter and carcass weight (2243.76 and 1733.70g) for T_4 (1% CA + 0.5% AC) in comparsion with (2115.40 and 1593.40) T_2 (2% CA) and (1992.10 and 1517.70g) in T_3 (1% AC) compared to (1944.50 and 1428.00g) in T_1, control treatment, respectively. Weight and percentages of liver, gizzard were significantly affected by dietary levels of organic acids, 2% CA, 1% AC and their mixture at 42 days of age. The increased dressing yield on dietary organic acids might be due to increasing live weight (Mohammed 2016). The mixture addition of 1% citric acid with 0.5% acetic acid resulted in decreasing the level of abdominal fat percentage in carcass (1.27%) for group T_4 followed by (1.43, 1.49 and 1.64 %) for 1% AC, 2% CA and control groups, respectively at 42 days of age (Table 5).

Table (5): Effect of dietary citric acid, acitic acid or their mixture supplementation on carcass characteristics of broiler chicks at 42 days of age (Means ± S. E).

Item	Dietary treatment[1]				Sig.
	T_1	T_2	T_3	T_4	
Pre-slaughter weight, g	$1944.50^{c} \pm 19.99$	$2115.40^{b} \pm 16.05$	$1992.10^{c} \pm 4.35$	$2243.70^{a2,3} \pm 22.33$	*
Carcass weight, g.	$1428.00^{d} \pm 17.84$	$1593.40^{b} \pm 11.82$	$1517.70^{c} \pm 3.18$	$1733.70^{a} \pm 17.23$	*
Dressing percentage, %	$73.40^{d} \pm 1.67$	$75.30^{c} \pm 0.12$	$76.20^{b} \pm 0.12$	$77.30^{a} \pm 0.08$	*
Abdominal fat, g	$31.90^{a} \pm 0.43$	$31.50^{b} \pm 0.65$	$28.49^{c} \pm 0.45$	$28.49^{c} \pm 0.39$	*
Abdominal fat, %	$1.64^{a} \pm 0.04$	$1.49^{b} \pm 0.04$	$1.43^{c} \pm 0.04$	$1.27^{d} \pm 0.04$	*
Liver weight, g	$42.60^{c} \pm 0.47$	$55.50^{b} \pm 0.59$	$55.50^{b} \pm 0.42$	$64.70^{a} \pm 0.58$	*
Liver, %.	$3.00^{d} \pm 0.004$	$3.50^{c} \pm 0.01$	$3.70^{b} \pm 0.03$	$3.70^{a} \pm 0.004$	*
Gizzard weight, g	$48.70^{d} \pm 0.72$	$61.00^{c} \pm 0.35$	$65.00^{b} \pm 0.06$	$72.10^{a} \pm 0.81$	*
Gizzard%.	$3.40^{d} \pm 0.02$	$3.80^{c} \pm 0.01$	$4.30^{a} \pm 0.01$	$4.20^{b} \pm 0.01$	*
Heart weight, g	$8.40^{c} \pm 0.09$	$9.10^{b} \pm 0.21$	$7.00^{d} \pm 0.15$	$10.10^{a} \pm 0.06$	*
Heart, %.	0.60 ± 0.008	0.60 ± 0.01	0.50 ± 0.01	0.60 ± 0.01	NS
Giblets weight, g	$99.70^{c} \pm 1.23$	$125.5^{b} \pm 1.09$	$127.5^{b} \pm 0.34$	$146.8^{a} \pm 1.34$	*
Giblets %	$6.96^{c} \pm 0.02$	$7.88^{b} \pm 0.01$	$8.40^{a} \pm 0.03$	$8.47^{a} \pm 0.01$	*

[1] *T_1; control; basal diet without any supplementation, T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; asal diet + 1% citric acid + 0.5% acetic acid.*

[2] *means ± S. E. of 3 replicates / treatment.*

[3]*a, b, c and ….etc. means within the same row with each different superscript are significantly different ($P \leq 0.05$).*

The present results indicated that organic acids used for 42d maintained sufficient undissociated acid molecules, which appeared to produce bacteriostatic or bactericidal effect leading to better carcass yield and lower abdominal fat than diets without organic acids. The reduction in abdominal fat might be supplemented dietary acidification in broiler diets and has a role in lipid metabolism (Leeson *et al*., 2005). Also,The results are conincides with Youssef *et al*. (2017) who indicated that chicks fed diet supplemented with CA or AC had significantly increased carcass yields. Also, Sharifuzzaman *et al*. (2020) reported that the highest dressing percentage (69%) was noticed where 0.75% citric acid was added to diet. While, Lakshmi and Sunder (2013) who observed that abdominal fat percent was lower in broiler fed 1 and 2% citric acid up to 42 days of age. On contrary, Rehman *et al*. (2016), Saleem *et al*. (2016), Ali *et al*. (2019) and Sabour *et al*. (2019) observed that there was no significant differences in dressing percentage by having diets supplemented with citric and / or acetic acid.

Data presented in Table (6) showed the effect of dietary 2% CA, 1% AC or their mixture on some immune organs of relative weights and percentage (thyme, spleen and bursa of fabricus) at the end of experiment (42 days of age broiler chicks). Data exhibited the relative weights and percent of thymus and bursa increased by addition of single citric or acetic acids or their mixture. The highest values were reported for (1% CA + 0.5% AC, T4), being (3.99g % and 0.23 %) of thymus % and (3.34g and 0.19 %) for bursa %, respectively at 42 d in comparsion with other treatments.While, percentages of spleen did not significantly differ between all treatments. The improvement in immunity might be due to the inhibitory effects of these OA on gut pathogens. In addition, OA supplementation caused hyper - thyroidism and peripheral conversion of $T_4 – T_3$ which indicate that these birds had better immune competence and bursa growth (Abdel Fattah *et al*., 2008). The major constituents of the avian immune system are the lymphoid organs as bursa, spleen and thymus had mild to moderate hyperplasia of their lymphoid follicles which indicate that the immune response was increased (Attia *et al.* 2018).

The relative increase in weight organs of bursa, spleen and two primary lymphoid organs were conciderd as an indication of immunological advances. The establishment of immune response associated with dietary acidification could be on account for their inhibitory effects against the pathogenic microorganisms throughout the GI tract providing scope for improved nutrient absorption. The higher immunity due to organic acids can also be attributed to higher nutrient efficiency, which might have triggened immunogenic cells to record higher immune response (Ram Rao *et al*., 2004). The results of the peresnt experiment reported herein were also in agreement with those of Mohammadi and Khosravinia (2015) and Attia *et al*. (2018) who noted that the use of organic acid leads to an increase in the number of contributing cells in immune and then increase the bursa of fabricius weight. However, the results of Saleem *et al*. (2016) and Allahdoa *et al*. (2018) disagree with of the peresnt study

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Table (6): Effect of dietary citric acid, acitic acid or their mixture supplementation on immune organs of broiler chicks at 42 days of age (Means ± S. E).

Item	Dietary treatment[1]				Sig.
	T_1	T_2	T_3	T_4	
Thymus weight, g.	$2.17^d \pm 0.06$	$3.03^c \pm 0.15$	$2.92^b \pm 0.12$	$3.99^{a2,3} \pm 0.17$	*
Thymus, %.	$0.15^c \pm 0.005$	$0.19^b \pm 0.02$	$0.19^b \pm 0.01$	$0.23^a \pm 0.01$	*
Spleen weight, g.	$1.15^d \pm 0.13$	$1.59^b \pm 0.06$	$1.36^c \pm 0.12$	$2.08^a \pm 0.16$	*
Spleen, %.	0.08 ± 0.01	0. 10 ± 0.004	0.09 ± 0.01	0.12 ± 0.01	NS
Bursa, g.	$2.04^d \pm 0.15$	$2.75^c \pm 0.15$	$2.92^b \pm 0.12$	$3.34^a \pm 0.15$	*
Bursa, %.	$0.14^c \pm 0.01$	$0.17^b \pm 0.01$	$0.19^a \pm 0.01$	$0.19^a \pm 0.01$	*

[1] *T_1; control; basal diet without any supplementation, T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic aci*

[2] *means ± S. E. of 3 replicates / treatment.*

[3] *a, b, c and ….etc. means within the same row with each different superscript are significantly different (P ≤ 0.05).*
NS: Non Significant.

Effect of dietary citric acid and acetic acid supplementation on some serum biochemical parameters of broiler chicks:

Data concerning the effect of citric and acetic acids or their mixture as a diet supplemetation on blood serum constitunets at 42 days are shown in Table 7. The mixture of citric acid and acetic acid (1%CA + 0.5% AC; T_4) significantly ($P \leq 0.05$) improved total protein, albumin, globulin and A/ G ratio compared to the control group. Globulin is a source of antibody production, so its level in the serum is a good indicator of immune responses and conseqantly better disease resistance (Griminger and Scances 1986). Also, Ghazalah *et al*. (2011) noticed a higher level of globulin in serum blood concentaration which used as an indicator for measuring immunity response by addition of organic acids, suggesting that the improvement in bird immunity could be related to the inhibitory effects of organic acids on gut system pathogenes and enhancing the density of the lymphocytes in the lymphoid organs, enhancing the non specific immunity (Haque *et al*., 2010). In this study, the concentration of serum blood creatinine was not significantly ($P \leq 0.05$) differe between all dietary supplemented treatments compared to T_1, (un-supplemented) during 42 days of age. Also, the concentration of blood glucose was significantly ($P \leq 0.05$) differences by organic acid (CA and AC or their mixture) during 42 days of age. The peresnt results showed that broiler chicks fed diets contained CA and AC or their mixture had significantly lower serum total lipids and cholesterol. Meanwhile, broiler chicks fed diet containing a mixture of CA + AC showed the lowest total lipidsm(TL) and total cholesterol (TCH); being 430.27 and 133.11 mg / dl for $T_{4;}$ (1% CA+ 0.5%AC), respectively, when compared to the control treatment (550.39 and 159.13 mg / dl) for TL and TCH, respectively.

The decrease in blood lipid profile may be due to the beneficial role of organic acids 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, lead into an intracellular accumulation of acid anions (Young and Foegeding, 1993). Organic acid treatments insignificantly influenced each of ALT and AST compared to the control treatment at 42 days of age, chicks fed diet supplemented with 2% CA (T_2), 1% AC (T_3) and 1% CA + 0.5%AC (T_4) had the lowest value of ALT (14.78, 14.81 and 14.35), respectively in comparsion with control treatment (15.14), while AST enzyme was insignificantly influenced by dietary treatments ($P > 0.05$) at 42 days of age (Table 7). This result is harmony with those of Capcarova *et al*. (2014); Rahman *et al*. (2018) and Ali *et al*. (2019) who report that total protein, globulin and A / G ratio were ($P \leq 0.05$) higher in birds fed diets supplemented with single citric and acetic acids or their mixture supplementation in broiler diets.

Where, Abdel Razek *et al*. (2016) and Mirderikvandi *et al*. (2019) reported that total cholesterol was significantly ($P < 0.05$) decreased in citric acid supplementation of broiler chickens at 5 weeks of age. Other studies noticed negative effects on total protein, globulin, glucose, albumin and A / G ratio in corn-based diets supplemented with organic acids of Mohammadi and Khosravinia (2015) and Mirderikvandi *et al*. (2019). Generally, serum biochemical constituents reflect the health, nutrition, climate and management conditions which the animals are submitted (Minafara *et al*., 2010). The levels of biochemical parameters in the blood can be used as an indicator of the productive performance of the birds and of metabolic disease (Rotava *et al*., 2008). Measurements of AST and ALT activities are indicatives of liver damage in broiler chicks and its therefore available tool for determination of a safe inclusion rate for feed additives.

Table (7): Effect of dietary citric acid, acitic acid or their mixture supplementation on some blood serum contituents of broiler chicks at 42 days of age (Means ± S.E.).

Item	Dietary treatment [1]				Sig.
	T1	T_2	T_3	T_4	
Total protein, g / dI.	$4.38^{d}\pm 0.006$	$4.43^{c}\pm 0.011$	$4.51^{b}\pm 0.006$	$4.56^{a2,3}\pm 0.006$	*
Albumen (A), g / dI.	$2.40^{d}\pm 0.006$	$2.47^{c}\pm 0.006$	$2.52^{b}\pm 0.012$	$2.58^{a}\pm 0.012$	*
Globulin (G), g / dI.	$1.94^{c}\pm 0.006$	$1.96^{b}\pm 0.006$	$1.99^{a}\pm 0.006$	$1.98^{a}\pm 0.006$	*
A / G ratio	$1.24^{d}\pm 0.005$	$1.26^{c}\pm 0.001$	$1.27^{b}\pm 0.003$	$1.30^{a}\pm 0.002$	*
Creatinine, mg / dI.	1.43 ± 0.006	1.42 ± 0.006	1.40 ± 0.001	1.42 ± 0.001	NS
Total lipids, mg / dI.	$550.39^{a} \pm 0.57$	$448.13^{b}\pm 0.02$	$435.88^{c}\pm 0.58$	$430.27^{d}\pm 0.58$	*
Total cholesterol, mg / dI.	$159.13^{a}\pm 0.58$	$149.00^{b}\pm 0.57$	$137.18^{c} \pm 0.33$	$133.11^{d}\pm 0.01$	*
Glucose, mg / dI.	$234.07^{a} \pm 0.02$	$231.66^{b}\pm 0.57$	$232.91^{c}\pm 0.58$	$227.11^{d}\pm 0.59$	*
ALT, U / L.	$15.14^{a} \pm 0.059$	$14.78^{c}\pm 0.058$	$14.81^{b}\pm 0.059$	$14.35^{d}\pm 0.058$	*
AST, U / L.	42.11 ± 0.59	42.03 ± 0.57	42.14 ± 0.57	42.03 ± 0.57	NS

[1] T_1; control; basal diet without any supplementation, T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic aci

[2] means ± S. E. of 3 replicates / treatment.

[3]a, b, c and ….etc. means within the same row with each different superscript are significantly different ($P \leq 0.05$).

NS: Non Significant.

Effect of dietary citric acid, acitic acid or their mixture supplementation on intestinal morphological

parameters of broiler chicks:

The average villus height, width , crypt depth, muscular thickness and villi height: crypt depth of duodenum, jejunum and illum of broiler chickens measurments at 42 days of age are presented in Tables (8, 9 and 10) and illustrated in Figures (1, 2 and 3). Data showed significant increase ($P \leq 0.05$) in villi hieght of duodenum, jejnum and illum with the addition of citric and acetic acids mixture (1% CA + 0.5% AC, T_4) ; being 2173.99, 1453.94 and 970.07 µm as comparion with the other treatments (1741.36, 1339.26 and 1028.18 µm, T_2), (1783.06, 1101.42 and 784.07 µm, T_3) and in the control (T_1) group were 1485.17, 1079.76 and 718.09 µm, respectively.The increase in villus height of the different segments of the small intestine epithelium play key roles in the digestion, absorption and assimilation of nutrients (**Wang and Peng, 2008**). Organic acidifiers especially acetic acid 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 muscosa, which increase villius height, function of secretion, digestion and absorption of nutrients can be appropriately performed by the muscosa (**Loddi *et al*., 2004).** Also**, Pelicano *et al*. (2011)** reported that increased villius heights in duodenum and jejunum with most of the organic acids reduces the growth of many pathogenic or non pathogenic intestinal bacteria, decreasing the intestinal colonization and infectious processes, ultimately decreasing the inflammatory reactions at the intestinal muscosa, which increases the villius height, the functions of secretion, digestion and absorption of nutrients by the muscosa.

Table (8): Effect of dietary citric acid, acitic acid or their mixture supplementation on the intestinal morphology (duodenum) of broiler chicks at 42 days of age (Means ± S.E.).

Histological parameter	Dietary treatment [1]				Sig.
	T_1	T_2	T_3	T_4	
Villi height, µm.	$1485.17^{c} \pm 13.58$	$1741.36^{b} \pm 55.37$	$1783.06^{b} \pm 41.49$	$2173.99^{a,2,3} \pm 17.84$	*
Villi width, µm.	$156.32^{d} \pm 10.55$	$241.09^{b} \pm 9.31$	$198.85^{c} \pm 5.68$	$313.10^{a} \pm 15.83$	*
Crypt depth, µm.	$140.85^{c} \pm 4.55$	$266.37^{a} \pm 21.42$	$195.87^{b} \pm 5.07$	$261.94^{a} \pm 13.44$	*
Muscular thickness, µm	$220.44^{d} \pm 3.06$	$327.94^{a} \pm 13.85$	$264.37^{c} \pm 4.12$	$293.84^{a} \pm 12.68$	*
Villi height / Crypt depth	$9.69^{a} \pm 0.58$	$7.29^{c} \pm 0.44$	$9.03^{b} \pm 0.44$	$7.04^{c} \pm 0.39$	*

[1] T_1; control; basal diet without any supplementation ,T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic acid.
[2] means ± S. E. of 3 replicates / treatment.
[3]a, b, c and ….etc: means within the same row with each different superscript are significantly different ($P \leq 0.05$).

Villi intestine width values being; 313.10, 217.74 and 213.87 µm for T_4 of duodenum, jejnum and ileum compared to other treatments which were (241.09, 207.94 and 177.52 µm, T_2) and (198.85, 183.67 and 169.44 µm, T_3) and for control group were (156.32, 143,02 and 132.15 µm), respectively. Also, crypt depth and muscular thickness of duodenum, jejnum and ileum of broiler chicks measured at 42 days of age and presented in Tables 8, 9 and 10 and illusterated in Figures (1, 2 and 3). Muscularis thickness were increased in all segments of small intestine by the addition of citric acid, acetic acid or their mixture compared to the control group. The villus crypt considered as the villus factory, deeper crypts indicate fast tissue turn over to permit renewal of the villus as needed in response to normal slougring or inflammation from pathogens or their toxins and high demands for tissue (Yason *et al*., 1987).

Increases in the VH and VH to CD ratio directly correlated with increased epithelial cell turnover and longer villi are associated with activated cell mitosis (Awad *et al*., 2009). These results agreed with those of Rehman *et al*.(2016); Allahdoa *et al*. (2018); Sabour *et al*. (2019) and Stamilla *et al*.(2020) who showed that supplementaion of dietary organic acid had significantly effects in small intestine histology. On the other hands, Mohammadagheni *et al*. (2016) and Attia *et al*. (2018) found no significant effects of dietary organic acids on muscular thickness of small intestine of broiler chicks.

Table (9): Effect of dietary citric acid, acitic acid or their mixture supplementation on the intestinal Morphology (Jejunum) of broiler chicks at 42 days of age (Means ± S.E.).

Histologicalparameter	Dietary treatment [1]				Sig.
	T_1	T_2	T_3	T_4	
Villi height, µm.	1079.76^{c}± 15.57	1339.26^{b}± 8.93	1101.42^{c}± 20.71	1453.94^{a2,3}± 35.25	*
Villi width, µm.	143.02^{c}±6.47	207.94^{a}± 9.67	183.67^{b}±11.52	217.74^{a}± 5.16	*
Crypt depth, µm.	175.69^{b} ± 4.75	193.36^{b}±7.67	188.15^{b}±1.24	240.26^{a}± 11.46	*
Muscular thickness, µm	255.34^{b}± 10.65	237.65^{b}± 11.91	282.48^{a} ± 4.31	238.08^{b}±3.25	*
Villi height / Crypt depth	7.66^{a} ± 0.41	6.51^{b}± 0.32	6.11^{b}± 0.38	6.71^{ab}± 0.26	*

[1] T_1; control; basal diet without any supplementation ,T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic acid.
[2] means ± S. E. of 3 replicates / treatment.
[3]a, b, c and ….etc: means within the same row with each different superscript are significantly different (P ≤ 0.05).

Table (10): Effect of dietary citric acid, acitic acid or their mixture supplementation on the intestinal morphology (Ileum) of broiler chicks at 42 days of age (Means ± S.E.).

Histologicalparameter	Dietary treatment [1]				Sig.
	T_1	T_2	T_3	T_4	
Villi height, µm.	718.09^{b}±5.56	1028.18^{a}±50.40	784.07^{b}±22.80	970.07^{a2,3}± 11.46	*
Villi width, µm.	132.15^{c}±4.86	177.52^{ab} ±12.81	169.44^{b}±12.26	213.87^{a}±15.56	*
Crypt depth, µm.	126.43^{c}±9.17	170.56^{b}±8.50	142.29^{bc}±9.70	208.66^{a}±12.54	*
Muscular thickness, µm.	214.25^{c}±2.73	261.10^{b}± 11.31	249.57^{b}±1.56	287.03^{a}±8.45	*
Villi height / Crypt depth	5.47^{a} ± 0.21	5.95^{a}± 0.50	4.79^{a}± 0.46	4.70^{a}± 0.36	*

[1] T_1; control; basal diet without any supplementation ,T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T_4; basal diet + 1% citric acid + 0.5% acetic acid.
[2] means ± S. E. of 3 replicates / treatment.
[3]a, b, c and ….etc: means within the same row with each different superscript are significantly different (P ≤ 0.05)

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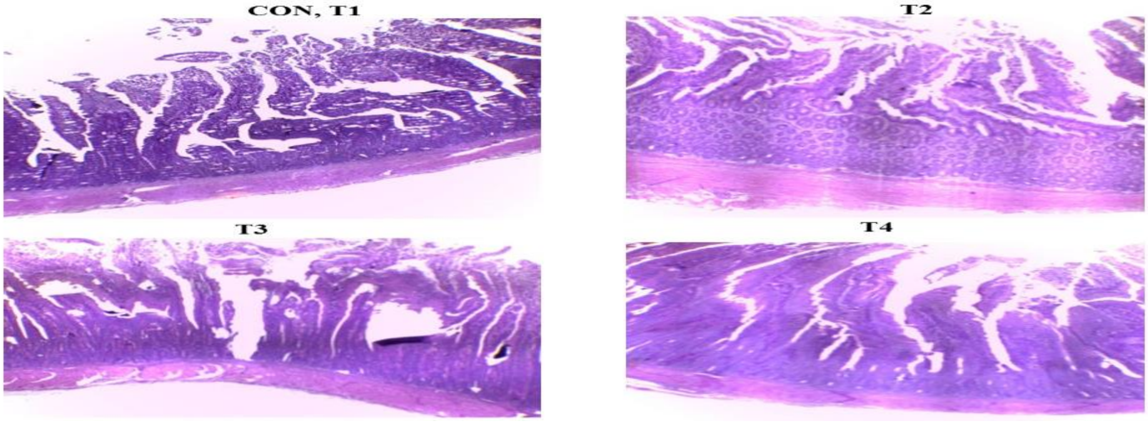

Figure (1): Photomicrographs of intestinal villi from the duodenum of broiler chicks on 42 days of age in T_1, control, basal Diet without supplementation; T_2; basal diet +2% citric acid; T_3 ; basal diet+ 1% acetic acid and T_4; basal diet+1% cetric + 0.5% acetic acids.

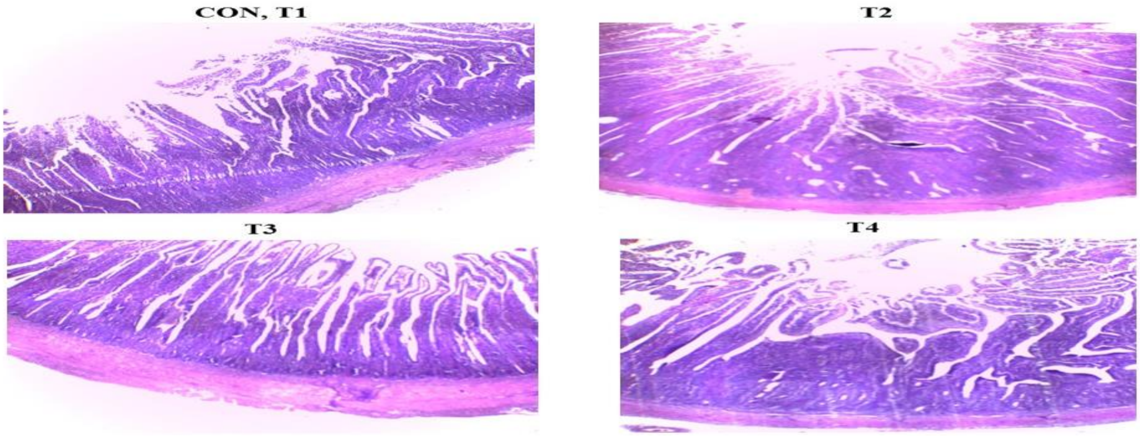

Figure (2): Photomicrographs of intestinal villi from the jujunum of broiler chicks on 42 days of age in T_1, control, basal dietwithout supplementation; T_2; basal diet +2% citric acid; T_3 ; basal diet+ 1% acetic acid and T_4; basal diet+ 1% cetric+ 0.5% acetic acids.

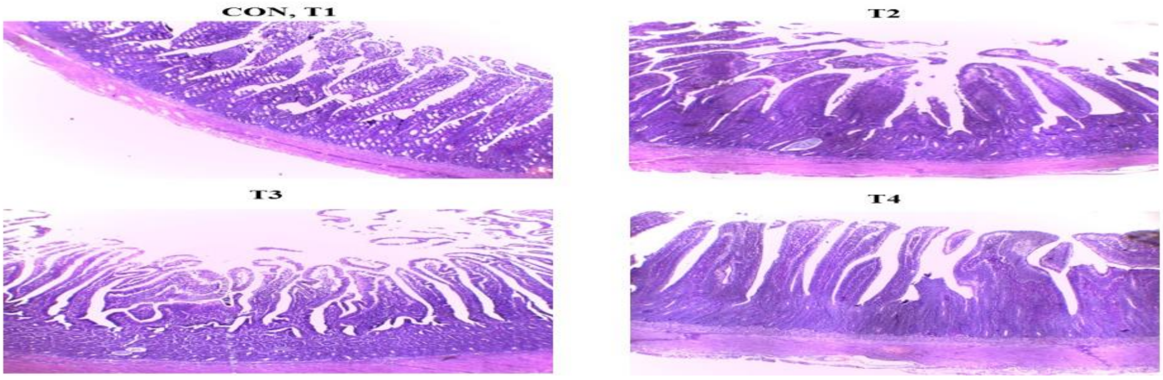

Figure (3): Photomicrographs of intestinal villi from the ileum of broiler chicks on 42 days of age in T_1, control, basal diet without supplementation; T_2; basal diet +2% citric acid; T_3 ; basal diet+ 1% acetic acid and T_4; basal diet+ 1% cetric + 0.5% acetic acids.

Effect of dietary citric acid and acetic acid or their mixture supplementation on economic efficiency of broiler chickens:

Data partaining to dietary citric, acetic acids or their mixture supplementation on the relative economical efficiency (REE) are presented in Table (11). In comparsion with the control treatment (100%), the supplementation of citric and acetic mixture improved REE by 20. 72% for T_4 which was supplemented with (1% CA + 0.5% AC). This may be due to better feed conversion obtained in chicks received the experimental diets. The low values of economical efficiency were obtained for chicks fed diet supplemented with 1% AC (T_3) and 2% CA (T_2), respectively compared to T_4 (1% CA + 0.5% AC) but higher than the control group. This may be due to the increase in the price of acetic acid in the markets. Abdel Fattah *et al*. (2008) observed that the economic and relative economic efficiency were increased by using citric and acetic acids at (1.5 and 3 %) levels. Ghazalah *et al.* (2011) found that 0.25% AC and 3% CA supplementation gave the best value of European economic efficiency compared to the control group at 42 days of age. On the other hand, Mirderikvandi *et al*. (2019) showed that acidified diet (20 mg acetic acid / kg diet) significantly reduced economic efficiency of birds by about 16.77%. Also, Omidi *et al*. (2020) indicated that acetic acid supplementation to diet decreased economic efficiency by 40 units compared to control birds during 14 to 42 days of age.

Table (11): Effect of dietary citric acid, acitic acid or their mixture supplementation on economic effeciency of broiler chicks during experimental period.

	Dietary treatment [1]			
Item	T_1	T_2	T_3	T_4
Initial body weight, g.	45.21	45.17	45.24	45.17
Final body weight, kg.	1.97	2.02	1.99	2.08
Body weight gain, kg.	1.92	1.97	1.94	2.03
Total revenue[2], L.E.	61.44	63.04	62.08	64.96
Feed intake, kg.	4.02	3.84	3.95	3.78
Price of one kg feed, L.E.	7.25	7.30	7.36	7.34
Feed cost, L.E.	29.15	28.03	29.07	27.75
Net revenue[3], L.E.	32.29	35.01	33.01	37.21
Economical efficiency[4].	1.11	1.25	1.14	1.34
Relative economic efficiency, %.	100	112.61	102.70	120.72

[1] T_1; control;basal diet without any supplementation ,T_2; basal diet + 2% citric acid, T_3; basal diet + 1% acetic acid and T4; basal diet + 1% citric acid + 0.5% acetic acid.
[2] Total revenue = live body weight gain × marketing price (32 L.E. according to pricices in June, 2018).
[3] Net revenue = Total revenue – Feed cost.
[4] Economical efficiency = Net revenue / Feed cost.

CONCLUSION

In general, based on the obtained experimental results reported herein and from the nutritional and economical point of view, there are some beneficial effects of using mixture of citric acid and acetic acid (1% citric acid + 0.5% acetic acid) to broiler (Arbor Acres) diets showed the best productive performance, carcass characteristics, some serum componterty, some histological parameters and better economic efficiency without any adverse effects on health under our local environmental conditions

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تأثير إضافة حامضى الستريك ، الخليك أو مخلوطهما فى علائق كتاكيت التسمين على أداء النمو ، صفات الذبيحة وبعض القياسات الهستولوجية للأمعاء

عاطف محمد حسن أبو عاشور ، منال كمال عبدا لعليم أبو النجا ، إيمان عاشور محمد حسين و زينب مصطفى عبد الحميد البنا

قسم انتاج الدواجن والأسماك- كلية الزراعة- جامعة المنوفية- مصر.

استخدم فى هذه الدراسة عدد 120 كتكوت تسمين أربورإيكرز Arbor Acres عمر يوم غير مجنس - قسمت عشوائياً إلى 4 معاملات غذائية تجريبية – كل منها قسمت إلى 3 مكررات. تم رعاية الطيور تحت نفس الظروف، أضيفت الأحماض العضوية (حامض الستريك ، حامض الخليك) إلى عليقة البادئ والناهى كما يلى: المعاملة الأولى: العليقة الأساسية بدون أى إضافة (مقارنة)، المعاملة الثانية: العليقة الأساسية + 2% من حامض الستريك، المعاملة الثالثة: العليقة الأساسية + 1% من حامض الخليك - المعاملة الرابعة: العليقة الأساسية + (1% من حامض الستريك + 0.5 % من حمض الخليك). غذيت جميع كتاكيت التجربة من عمر يوم حتى 21 يوم على عليقة بادئ تحتوى على 22.13% بروتين خام و3088 كيلو كالورى طاقة ممثلة/ كجم عليقة، ومن عمر 22 حتى 42 يوم على عليقة ناهى تحتوى على 19.82% بروتين خام و3154 كيلو كالورى طاقة ممثلة/ كجم عليقة. تم تقدير أداء الطيور، معدل تحويل الغذاء، صفات الذبيحة وبعض أعضاء جهاز المناعة ، بعض مكونات سيرم الدم (البروتين الكلى، الالبيومين، الكوليستيرول الكلى، الكرياتينين، و كذلك إنزيمات وظائف الكبد ALT and AST) وذلك عند عمر 42 يوم، وتم تقدير بعض القياسات الهستولوجية للأمعاء عند عمر42 يوم، كما تم حساب الكفاءة الإنتاجية الأوربية والكفاءة الإقتصادية.

وفيما يلي أهم النتائج المتحصل عليها: ارتفع معنوياً متوسط وزن الجسم ومعدل الزيادة فى وزن الجسم فى كتاكيت المعاملة الرابعة (T_4) المغذاة على العلائق المضاف إليها مخلوط الأحماض العضوية (1% حامض الستريك + 0.5 % حامض الخليك). وسجلت أفضل معدل تحويل غذائى وأقل معدل استهلاك علف مقارنة بباقى المعاملات. ارتفع دليل الكفاءة الإنتاجية فى الكتاكيت المغذاة على علائق مضاف اليها مخلوط الستريك والخليك بمستوي (1% حامض الستريك + 0.5 % حامض الخليك ، المعاملة الرابعة) مقارنة بالكتاكيت المغذاة على العليقة الأساسية (المقارنة) . أدت إضافة حامض الستريك أوالخليك أومخلوطهما انخفاضاً معنوياً عند مستوى (0.05) فى معدلات النفوق خلال فترة التجربة (من عمر 1 – 42 يوم) مقارنة بالمعاملة المقارنة. أدت إضافة الأحماض العضوية إلى زيادة معنوية فى كلاً من نسبة التصافى ونسبة الأحشاء الداخلية المأكولة و بعض أعضاء جهاز المناعة مقارنة بالمعاملة المقارنة عند عمر 42 يوم. أدت إضافة مخلوط الأحماض العضوية (1% حامض الستريك + 0.5% حامض الخليك) الى تحسن معنوى لبعض مكونات سيرم الدم (البروتين الكلى، الألبيومين، الجلوبيولين وإنزيم الكبد ALT) - بينما انخفض معنوياً مستوى الدهون الكلية والكوليسترول فى الدم. أوضحت القياسات الهستولوجية فى الأمعاء زيادة إرتفاع وعرض الخملات والنسبة بينهما مقارنة بالمعاملة المقارنة نتيجة إضافة الأحماض العضوية سواء بصورة منفردة أو مخلوطة. لوحظ أن خملات الإثنى عشر فى طيور المعاملة الرابعة المغذاة على العلائق المضاف إليها مخلوط الأحماض العضوية (1% حامض الستريك + 0.5% حامض الخليك) كانت أطول وأعرض وأكثر عمقاً وسمكاً مقارنة بالمعاملة الثالثة والثانية والأولى 133.30، 100.29، 85.97 و 46.38% على التوالى. بصفة عامة - وبناءً على النتائج المتحصل عليها من التجربة ودراسة الكفاءة الإقتصادية يتضح أن إضافة مخلوط الأحماض العضوية (1% حامض الستريك + 0.5% حامض الخليك) إلى علائق كتاكيت التسمين (Arbor Acres) أدى الى تحسن فى الأداء الانتاجى، صفات الذبيحة، بعض مكونات سيرم الدم، القياسات الهستولوجية والكفاءة الاقتصادية بدون أى تأثيرات سلبية على الصحة العامة تحت ظروف التجربة.