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Effect of Dietary Acidification on Some Blood Parameters and Weekly Performance of Broiler Chickens

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Abstract: A total number of 90 Ross 308 broiler chickens were randomly divided into three treatments with three replicates of 10 chicks each. The first treatment having 0% citric acid was served as control and fed corn-soybean meal base diet. While, the other two treatments were received the basal diet supplemented with citric acid at different levels of 3 and 6%, respectively. The experiment was lasted for 42 days. Some blood parameters (metabolites of serum, enzyme activities and plasma mineral concentrations), Gastrointestinal tract (GI-tract) morphology and weekly performance were measured. The results indicated that diets including citric acid had significant effect ($p<0.01$) on weekly feed intake, body weight gain and body weight but there was no significant improvement on feed conversion ratio during any experimental weeks. Also, citric acid had significant effect on carcass yield ($p<0.01$) and relative heart weight ($p<0.05$) but had no significant effect on relative weights of liver and abdominal fat. The effect of citric acid was significant on GI-tract morphology, cholesterol concentration, serum alkaline phosphatase and lactate dehydrogenase activities ($p<0.05$) but did not significantly affect urea, triglycerides, total protein concentrations and serum alanine aminotransferase and aspartate aminotransferase activities. Dietary effect of citric acid was significantly shown on plasma P and Fe concentrations ($p<0.05$) but not shown on plasma Ca, Mg and Zn concentrations. The results showed that addition of 3% citric acid significantly improved weekly growth performance but adding 6% citric acid had a negative effect on weekly performance factors of broiler chicks.

Key words: Citric acid, broiler, blood parameters, weekly performance, conversion, dietary effect

INTRODUCTION

Organic acids are mixed with the feed to create an acidified pH which provides a favorable environment in the digestive tract of broilers for the effective digestion of dietary nutrients such as proteins. They act as growth promoters and feed preservatives in poultry where they can also maintain feed hygiene. Also organic acids improve protein and energy digestibility by reducing the microbial competition nutrients of the host, endogenous nitrogen losses and ammonia production are other beneficial effects for broilers (Dibner and Buttin, 2002). Therefore, feeding organic acids by itself or with other feed additives have recently achieved more attention for animal future studies may be used to identify the most suitable organic acids for use alongside with different enzymes in poultry diets.

Researchers (Brzoska and Steck, 2007) have reported the beneficial effects of organic acids such as citric acid versus lactic acid as alternatives to some feed additives and antibiotics on performance, carcass quality and intestinal morphology of broilers. While feed intake and feed conversion ratios were not affected, the live body weight gain, carcass, thigh, breast, neck, liver and internal edible organs weights were improved with organic acid supplementation at 4 g kg^{-1} feed (Aksu *et al*., 2007). Positive effects of feeding organic acids to chickens were also reported by many researchers (Alcicek *et al*., 2004; Luckstadt *et al*., 2004). Luckstadt *et al*. (2004) supported the use of organic acids to preserve and protect feed from microbial and fungal destruction.

The later concluded that organic acids showed variable effects on the performance of broilers. The lower pH caused by the organic acids can protect the animal from infection especially at their younger ages. However, the effectiveness of organic acids in broiler diets may also depend on the composition and buffering capacity of the diet. The previous research have shown that the poultry digestive tract acidity is not desirable for complete hydrolyze (Brenes *et al*., 2003). Organic acids are mixed with the feed to create an acidified pH which provides a favorable environment in the digestive tract of broilers for

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the effective digestion of dietary nutrients. More recent research has indicated that Citric Acid (CA) is also very efficient in improving P utilization and other nutrients in chickens fed corn-soybean meal diets containing no supplemental phosphorus (Boling *et al*., 2000; Brenes *et al*., 2003; Snow *et al*., 2004; Rafacz-Livingston *et al*., 2005). In addition by modifying intestinal pH organic acids also improve the nutrients solubility, digestion and absorption of the feed ingredients.

Other studies reported that organic acids such as propionic, fumaric, sorbic and lactic acids and their salts have shown variable effects on egg production and egg quality parameters. This present study was carried out to investigate the effect of supplementing diet with CA on weekly performance (body weight, body weight gain and feed conversion ratio), metabolites of serum (urea, cholesterol, triglycerides and total protein), plasma minerals concentration (Ca, P, Mg, Fe and Zn), enzyme activities of serum (ALP, ALT, AST and LDH) and GI-tract morphology of broiler chicks.

MATERIALS AND METHODS

Birds, feeding and management: A total of 90 feather-sexed Ross 308 day old broiler chicks were randomly assigned to 3 treatments of 10 chicks per each replicate of similar body weight among groups mean. The experiment was carried out using a Randomized Complete Block Design (RCBD). A control diet (without citric acid) was formulated on corn-soybean basis meal for grower (7-21 day) and finisher (22-42 day) periods according to NRC (1994) recommendations. Diets were provided in mash form. Broiler chicks were fed the following dietary treatments with equal energy and protein levels: basal diet+0% CA (control diet), basal diet+3% CA and basal diet+6% CA. Citric acid was supplied as monohydrate citric acid with 99.5% purity.

Ingredient composition and calculated nutrient composition are shown in Table 1. The temperature was regulated at 32±1°C in the 1st week and reduced by 3°C week to receive 23°C in the 4th week. Feed and water were provided *ad libitum* and a continuous lighting schedule were used all through out the experimental period.

Table 1: Ingredients and nutrient composition of basal diets grower and finisher period

Ingredient (%)	Grower (7-21 day)	Finisher (22-42 day)
Corn	57.00	58.60
Soybean meal	33.10	30.00
Fish meal	3.40	3.50
Soybean oil	2.00	3.50
Dicalsium phosphate	1.55	1.10
Oyster shell	1.03	1.18
DL-Methoinine	0.01	0.01
Common salt	0.26	0.26
Sand	0.65	0.85
Trace minerals mix[1]	0.50	0.50
Vitamins mix[2]	0.50	0.50
Total	100.00	100.00
Calculated composition ME (kca kg^{-1})	2910.00	3030.00
CP (%)	20.10	19.00
Ca (%)	0.95	0.90
Total P (%)	1.23	1.06
nPP (%)	0.45	0.36
Met (%)	0.50	0.38
Lys (%)	1.10	1.00
Met+cys (%)	0.83	0.71

[1]Mineral mix supplied the following per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg. [2]Vitamins mix supplied following per kg of diet: Vitamin A, 18000 IU; vitamin D_3, 4000 IU; vitamin E, 36 mg; vitamin K_3, 4 mg; vitamin B_{12}, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg

Collection of samples and measurements: Body weight gain and feed consumption were recorded on weekly intervals. Feed conversion ratio was adjusted concerning the feed consumption of the dead birds. At the end of the experimental period (42 days of age), two birds from each replicate were randomly taken and their carcass characteristics and morphology of digestive organs (proventriculus, gizzard, relative intestinal weight, relative jejunum and ileum length) were measured. Also, blood samples (approximatey 10 mL) were collected in heparinized vacuitainer tubes for measurement of plasma minerals concentrations (Ca, P, Mg, Fe and Zn), blood metabolites of serum (urea, cholesterol, triglycerides and total protein), serum enzyme Activity of Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH). Immediately after collection, tubes were placed in an ice bath and transferred to the laboratory.

Plasma was harvested subsequently by centrifuging the whole blood samples at 3000 rpm for 15 min. The heparinized plasma samples were stored at -20°C in Eppendorf tubes and analyzed subsequently. Blood plasma and serum were analyzed for some blood metabolites (urea, cholesterol, triglycerides and total protein), plasma minerals concentration (Ca, P, Mg, Fe and Zn) and enzyme activities of serum (ALP, ALT, AST and LDH) were analyzed using an automated chemistry analyzer of Zest Shimi Kit (Ziest Chem., Diagnostica, Cat No.10-508. 5256).

Statistical analyses: The data were subjected to an Analysis of Variance (ANOVA) through fitting General Linear Model (GLM) using SAS® (SAS Institute, 2006) software and the corresponding means were compared by Duncan's Multiple Range Test (DMRT). The statistical model was as follows:

$$Y_{ijk} = \mu + Ca_i + B_j + e_{ijk}$$

Where:

- Y_{ijk} = The individual observation
- μ = The experimental mean
- CA_i = The CA
- B_j = The block effect
- e_{ijk} = The error term with mean 0 and variance σ^2. Percentages of slaughter traits were divided by 100 and subjected to arc-sin transformation of the square root before analysis; however actual percentage means are presented

RESULTS AND DISCUSSION

The results showed that adding 6% CA to diet caused significantly decrease ($p<0.0001$) in body weight, body weight gain and feed intake in all experimental weeks, nonetheless, addition of 3% CA to basal diet had a tendency to improve weekly body weight, body weight gain and feed consumption, also, the CA had no effect on feed conversion ratio (except for 1st week) Table 2. For carcass yield, two levels of citric acids (3 and 6%) were found to be significantly different from each other ($p<0.0018$). However, the CA caused a significant decrease in heart weight ($p<0.0347$) but had no significant effect on liver and abdominal fat pad weights. The results, also indicated that addition of 6% CA caused a significant increase in relative weight of proventriculus ($p<0.0444$), gizzard ($p<0.0020$) and intestines ($p<0.0173$), relative length of jejunum ($p<0.0002$) and ileum ($p<0.0003$) Table 3.

The effect of CA on some blood parameters are shows in Table 4. The data indicated that CA caused a significant decrease in cholesterol ($p<0.0171$) but had no significant effect on urea, triglycerides and total protein of serum concentrations. Also, CA significantly decreased enzyme activity of Alkaline Phosphatase (ALP) ($p<0.0276$) also it increased enzyme activity of lactate dehydrogenase shown by increase in enzyme activity of LDH ($p<0.0001$) but had no significant effects on activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). According to the obtained results, CA caused a decrease in plasma P ($p<0.0004$) and Fe ($p<0.0248$) concentrations, whereas, it had no significant effect on plasma Ca, Zn and Mg concentrations (Table 4).

The present study showed that the effect of CA was effective on performance factors (body weight, body weight gain, feed consumption) of broiler male chickens.

In this respect, the positive utilization effect of CA in diets was reported by researchers. Snow *et al.* (2004), Rafacz-Livingston *et al.* (2005) and Afsharmanesh and Pourreza (2005) who stated that addition of CA to diets caused significant increase in feed intake, body weight and body weight gain. Boling *et al.* (2000) have also reported similar results. They speculated that this might be because of the dilution of energy in the diet due to the inclusion of CA. The addition of the highest level of CA (60 g kg^{-1}) to diets depressed body weight, body weight gain and feed intake. These results are similar to those published by Brenes *et al.* (2003).

Boling-Frankenbach *et al.* (2001) that also, found a negative effect on performance of chickens by adding 60 g kg^{-1} CA to diets containing adequate available phosphorus (4.5 g kg^{-1}). Boling *et al.* (2000) and Boling-Frankenbach *et al.* (2001) indicated that citric acids

Table 2: Effect of citric acid on weekly growth performance of broilers

Time of experiment (day)	Diet: 0% CA (Control)	3% CA	6% CA	SEM	Probabilities
Body weight (g)					
14	278.73[a]	288.27[a]	233.87[b]	7.20	0.0001
21	502.83[b]	560.43[a]	423.33[c]	14.30	0.0001
28	801.93[b]	884.77[a]	699.00[c]	23.50	0.0001
35	1193.30[b]	1278.23[a]	995.27[c]	33.92	0.0001
42	1599.99[b]	1683.43[a]	1334.92[c]	53.11	0.0002
Body weight gain (g)					
7-14	143.57[a]	154.27[a]	97.20[b]	5.68	0.0001
15-21	224.10[b]	272.17[a]	189.47[c]	9.17	0.0001
22-28	299.10[ab]	324.33[a]	275.67[b]	12.25	0.0229
29-35	388.03[a]	393.47[a]	296.27[b]	14.67	0.0001
36-42	419.17[a]	403.97[a]	324.46[b]	16.47	0.0040
Feed consumption (g)					
7-14	256.70[a]	277.57[a]	207.00[b]	4.93	0.0013
15-21	419.80[b]	478.43[a]	368.87[c]	11.08	0.0057
22-28	638.60[a]	673.07[a]	533.03[b]	19.06	0.0144
29-35	830.50[a]	823.57[a]	604.10[b]	12.74	0.0004
36-42	921.40[a]	925.53[a]	674.10[b]	22.96	0.0024
Feed conversion ratio (g g^{-1})					
7-14	1.79[b]	1.80[b]	2.13[a]	0.03	0.0018
15-21	1.87	1.76	1.96	0.06	0.1786
22-28	2.14	2.07	1.96	0.09	0.4657
29-35	2.12	2.09	2.05	0.08	0.8148
36-42	2.25	2.30	2.02	0.12	0.3085

Means in rows with no common superscript differ significantly ($p<0.05$), CA = Citric Acid, SEM = Standard Error of Mean

Table 3: Effect of citric acid on carcass traits and GI-tract morphology of broilers

Variables	Diet: 0% CA (Control)	3% CA	6% CA	SEM	Probabilities
Carcass traits					
Carcass yield (%)	71.0[ab]	71.8[a]	70.1[b]	0.28	0.0018
Liver (g kg^{-1})	19.9	20.0	19.7	0.86	0.9770
Heart (g kg^{-1})	6.4[a]	5.4[b]	5.7[ab]	0.24	0.0347
Abdominal fat (g kg^{-1})	12.3	12.4	14.6	1.05	0.2288
GI-tract morphology					
Proventriculus (g kg^{-1})	4.5[b]	4.7[ab]	5.0[a]	0.14	0.0444
Gizzard (g kg^{-1})	15.7[b]	18.8[a]	19.1[a]	0.62	0.0020
Intestine weight (g kg^{-1})	45.1[ab]	40.6[b]	46.9[a]	1.41	0.0173
Jejunum length (cm kg^{-1})	50.1[b]	51.2[b]	57.5[a]	1.03	0.0002
Ileum length (cm kg^{-1})	45.0[b]	45.5[b]	51.4[a]	0.96	0.0003

Means in rows with no common superscript differ significantly ($p<0.05$), CA = Citric Acid, SEM = Standard Error of Mean

Table 4: Effect of citric acid on some blood parameters of broilers

	Diet				
Variables	0% CA (Control)	3% CA	6% CA	SEM	Probabilities
Metabolites					
Urea (mg dL^{-1})	2.2	2.4	2.9	0.34	0.3344
Cholesterol (mg dL^{-1})	136.4^{ab}	137.8^{a}	129.7^{b}	1.89	0.0171
Triglycerides (mg dL^{-1})	43.4	43.7	43.3	1.51	0.9807
Total protein (g dL^{-1})	2.1	2.2	2.1	0.07	0.7111
Enzyme activity					
ALP (U L^{-1})	2740.1^{a}	2600.5^{ab}	2519.4^{b}	52.4	0.0276
AST (U L^{-1})	247.0	252.8	248.9	7.7	0.8615
ALT (U L^{-1})	1.7	1.6	1.6	0.05	0.1263
LDH (U L^{-1})	1876.5^{b}	1831.7^{b}	2184.4^{a}	41.13	0.0001
Plasma minerals					
Ca (mg dL^{-1})	10.1	9.9	10.0	0.13	0.6288
P (mg dL^{-1})	6.0^{a}	6.2^{a}	5.6^{b}	0.08	0.0004
Mg (mg dL^{-1})	2.1	2.0	1.9	0.06	0.0561
Fe (µg dL^{-1})	1251.4^{ab}	1278.5^{a}	1146.9^{b}	32.05	0.0248
Zn (µg dL^{-1})	249.5	288.4	272.3	18.77	0.3630

Means in rows with no common superscript differ significantly ($p<0.05$), CA = Citric Acid, SEM = Standard Error of Mean

(20-60 g kg^{-1}) had a positive effect on performance only in low-AP diets (1.0-2.5 g kg^{-1}) and with a Ca:AP ratio similar to or >4:1. The reason for a negative response or absence of response to CA is unknown. Thus the results of current study suggesting that 6% CA may be to high for diets containing adequate AP.

The the study also, indicated the CA at 6% in the diet showed a tendency to improve Feed Conversion Ratio (FCR). These results are in agreement with other studies (Boling-Frankenbach *et al*., 2001; Snow *et al*., 2004; Ebrahimnezhad *et al*., 2008; Liem *et al*., 2008). The reason that different CA levels on FCR in the findings was not significant probably was due to taking into account both body weight and feed intake simultaneously, thus they together could not show an effect on FCR as a combination.

The current study showed that the addition of CA was effective on carcass yield. This result is in agreement with investigations by Abdel-Fattah *et al*. (2008) and Ebrahimnezhad *et al*. (2008). The relative percentage weight of heart was significantly reduced with the addition of 3% CA and the relative percentage weight of abdominal fat pad was not significantly increased by addition of CA compared to the control treatment.

These results confirmed those of Ebrahimnezhad *et al*. (2008) who found that dietary CA had affected the relative weight of liver and heart of broiler chickens at 42 day old. On the other hand, Abdel-Azeem *et al*. (2000) declared that addition of CA to the diet was associated with higher and lower dressing and liver percentages, respectively. The lack of having significance in the relative liver weights between the acidified and control chicks may be ascribed to more storage of glycogen and lower lipid repletion that were induced by dietary organic acid. This theory may emphasize the hypothesis of Fushimi *et al*. (2001) who stated that dietary acidification might stimulate glycogenesis by increasing the influx of glucose 6-phosphate (G-6-P) into the glycogen synthesis pathway through the inhibition of glycolysis as a result of increasing in citrate concentration.

Supplementation of different sources and levels of acidifiers resulted in remarkable increase in the small intestine morphology (weight and length). These values are in agree with the findings of Abdel-Fattah *et al*. (2008) and Kaya and Tuncer (2009). Further, the effect was more prominent with the addition of CA at 6%. Concerning the impact of dietary acidification on small intestine densities (weight/length) has been considered as indication of the intestinal villi dimension of mucosa layer (Palo *et al*., 1995).

Abdel-Fattah *et al*. (2008) observed a tendency to increase in total protein concentration and lipid metabolites reduction (cholesterol and triglycerides) by addition of different levels of citric acids (0, 1.5 and 3%). The decrease in serum TP could be due to a decrease in synthesis of protein caused by liver disorders, small intestinal malabsorption or increased loss of proteinuria due to renal diseases or malnutrition (Zantop, 1997). Kaya and Tuncer (2009) after organic acids addition to broiler diets found a decrease in total protein and triglycerides and an increase in cholesterol concentration. The present study does not agree with these observations. Decrease in serum ALP activity is associated with CA that might be reflected by increase observed from Zn retention.

Because Zn has a specific role in the reactivation of chicken intestinal ALP after acid exposure. Total serum ALP measures composition of several Zn metalloenzymes isoenzymes by cells in a number of organs, such as; liver, bone, muscle, small intestine and kidney (Moss, 1982). In this respect, Zantop (1997) indicated that increases of serum ALP is most related to liver diseases, even though the level of ALP activity in this organ is low.

Viveros *et al*. (2002) and Brenes *et al*. (2003) reported that decreasing aP level of diet increased ALP activity, CA through the above mentioned mechanism above facilitate liberation of phytate P and so increase plasma P concentration (as observed in our study) that resulted in decreased in ALP activity. There are five LDH isoenzymes in birds; each occurring in several tissues, including skeletal muscle, cardiac muscle, liver, kidney, bone and red blood cells that are found to decrease LDH activity which could be related to liver diseases because this enzyme decreases quickly as the disease progresses (Zantop, 1997).

In this respect, Abdel-Fattah *et al.* (2008) found that adding different levels of CA (1.5 and 3%) had changed blood Ca and P concentrations and caused an increase on minerals concentrations. The increase of Ca and P levels in blood serum produced by addition of organic acids may be attributed to the lowering of GI-tact pH by using these acids to increase the absorption of such minerals from the gut into the blood stream. Improving the utilization of calcium and phosphorus was due to provision of organic acids that was shown by Boling-Frankenbach *et al.* (2001). Also, Zeinb (2004) observed an increase in blood calcium of broiler chicks fed on dietary acidifier.

In contrast, Ebrahimnezhad *et al.* (2008) observed increase in plasma Ca and P concentration by addition of different levels of CA (2.5 and 5%). This researchers surveyed the effect of CA on phytate phosphorus utilization in laying hen and found that addition of CA in two levels (2 and 4%) that did not have any effect on Ca concentration.

CONCLUSION

Based on the results of the study, addition of 3% CA to the diet improved performance factors and minerals availability of intestinal absorption, nonetheless, adding 6% CA to control diet had a negative effect on growth performance. As well, under the condition of this experiment, no further benefits were achieved as a result of increasing the dietary CA levels. Clearly, the role of CA in poultry diets has been emphasised by many researchers. However, there is still a need to conduct more research in order to establish the suitability of adding CA to broiler diets to increase satisfactory effects and enhance feed utilization and broiler production.

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