ORIGINAL ARTICLE

Effect of citric acid supplemented diets on aflatoxin degradation, growth performance and serum parameters in broiler chickens[#]

Efecto de dietas suplementadas con ácido cítrico en la degradación de aflatoxinas, el crecimiento y los parámetros sanguíneos de pollos de engorda

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RESUMEN

Este estudio fue realizado para investigar los efectos del ácido cítrico (CA) sobre la degradación de las aflatoxinas, el crecimiento y algunos componentes del suero sanguíneo de pollos de engorda. 300 pollos de un día de edad (Ross) fueron divididos aleatoriamente en cinco grupos con tres repeticiones de 20 pollos cada uno. Cuatro grupos recibieron la dieta suplementada con CA (6,25, 12,5, 25 y 50 g/kg), mientras que el otro sirvió como grupo control. La dieta fue preparada con base en las recomendaciones de la NRC, y el experimento fue terminado cuando las aves alcanzaron 28 días de edad. Los resultados mostraron que las aflatoxinas en la dieta a una concentración de 39 ng/g fueron degradadas (92%) por el procedimiento de acidificación. En general, el peso vivo (LBW) fue ligeramente mayor en los animales alimentados con la adición de CA; la dieta con la concentración más alta (50 g CA/kg) resultó en un aumento significativo en el LBW y una mejora en el índice de conversión. Sin embargo, a medida que se incrementó la concentración de CA, valores altos en la actividad de la enzima aspartato aminotransferasa fueron registrados. Por el contrario, el hematocrito, las proteínas totales y la albúmina no fueron afectados por cualquier nivel de inclusión de CA en la dieta. De estos resultados, se concluye que el CA puede ser usado como un aditivo para degradar a las aflatoxinas en la dieta, así como para promover el crecimiento en pollos de engorda jóvenes.

Key words: broilers, aflatoxins, citric acid, serum parameters.

Palabras clave: pollos de engorda, aflatoxinas, ácido cítrico, parámetros sanguíneos.

INTRODUCTION

The poultry industry is continuously searching for additives to improve feed efficiency and animal health; among these compounds, organic acids are promising alternatives. Dietary organic acids and their salts inhibit microorganism growth in feed, and maintain the microbial balance in the gastrointestinal tract (GIT) (Naidu 2000). Several authors have reported positive effects of certain organic acids in poultry diets (Runho *et al* 1997, Jin *et al* 1998), since acidifiers might improve poultry performance by reducing colonization of pathogenic microorganisms and toxic bacterial metabolites such as ammonia and amines (Chaveerach *et al* 2004). Moreover, Méndez-Albores *et al* (2007) have observed that citric acid degrades aflatoxins in the ration.

Aflatoxins (AF) are a group of acutely toxic metabolites produced by toxigenic strains of *Aspergillus flavus* Link, *Aspergillus parasiticus* Speare, and *Aspergillus nomius* Kurtzman *et al* (Feibelman *et al* 1998, Weidenbörner 2007). These toxins have closely similar structures and

form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Four principal AF are produced by those fungi: AFB_1, AFB_2, AFG_1, and AFG_2. AF causes a wide range of clinical and subclinical problems in poultry related to many different toxic effects; among them, reduced performance, hepatic intoxication, adverse effects on carcass as well as on egg shell quality, immunosuppression, and carcinogenicity have been reported by different authors (Charmley *et al* 1995, Hussein and Brasel 2001, Kermanshahi *et al* 2007).

At present, unfortunately, aflatoxins are considered unavoidable contaminants of feed and foods. The Food and Agriculture Organization (FAO) estimates that at least 25% of world cereal production is contaminated with mycotoxins (Dowling 1997). For this reason, developments of detoxification procedures are needed. Such detoxification procedures should not only reduce the concentration of toxins to "safe" levels (below regulatory limits), but also to prevent production of new toxic products derived from the aflatoxin degradation, and of course non-reduction of the nutritional value of the treated commodities. A number of methods have been investigated in connection with their effectiveness to inactivate aflatoxins in contaminated feedstuffs; the aims of these methods are either to remove or to destroy the toxin, and can be classified into physical, biological and chemical methods.

Citric acid (CA) is one of the most widely-used food additives, which is commonly used as a preservative, acidulant, pH control agent, flavor enhancer, and antioxidant in many foods. Global production (mainly through microbiological fermentation) is estimated to be approaching 4^5 tons per year (Kristiensen *et al* 1999). The US Food and Drug Administration classify CA as a GRAS (Generally Recognised as Safe) substance.[1] However, some toxic effects of CA have been also studied (Aktaç *et al* 2003[a], 2003[b], Yılmaz *et al* 2008, Abd-AlGadir *et al* 2009).

Our recent studies indicated that aqueous CA had a detoxification effect when used to treat aflatoxin-contaminated feeds (Méndez-Albores *et al* 2009). Considering that the acidic treatment protects ducklings from chronic aflatoxin toxicity and greatly reduces the mutagenic and carcinogenic activity of aflatoxins in the Ames test (Méndez-Albores *et al* 2005), the purpose of the present study was to determine the effect of dietary CA on the stability of B-aflatoxins in feed, as well as on growth performance and biochemical parameters in the blood of young broiler chickens.

MATERIAL AND METHODS

CHEMICALS

Anhydrous citric acid (99.9% purity) was obtained from Mallinckrodt Baker (JT Baker, Xalostoc, Mexico). The chemical properties of citric acid are as follows: chemical formula $C_6H_8O_7$, and molecule weight 192.13 g/mol. All other chemicals used were analytical reagent grade.

DIETS

A control sorghum-soybean meal based diet was prepared based on National Research Council (NRC 1994) recommendations. The composition and chemical analysis is presented in table 1. For preparing other treatments, the control diet was supplemented with aqueous CA at levels of 6.25, 12.5, 25 and 50 g/kg, respectively. No antibiotic or anticoccidial drug was used in those diets. The moisture content (M.C.) of the diets was adjusted to 30% by adding 265 ml/kg of aqueous CA solutions. Samples were mixed at low speed for 15 min in a mixer (model C-100, Hobart Corp., Troy, OH). After mixing, samples were transferred to plastic bags and stored at 4ºC for 72 h, in order to achieve M.C. equilibration. The acid-treated feed was dried in a vacuum oven at 40ºC for 48h, and thoroughly mixed. The final average M.C. of the diets was 12%, determined by drying replicate portions of 5–10 g each of feed at 103 ºC for 72 h, with percentages calculated on a wet-weight basis. The control diet (non CA added) was treated similarly to the CA treated diets regarding M.C. adjustment, mixing, equilibration and

drying. The chicks were fed with five diets: (1) CA-supplemented feed with 6.25 g/kg; (2) CA-supplemented feed with 12.5 g/kg; (3) CA-supplemented feed with 25 g/kg; (4) CA-supplemented feed with 50 g/kg citric acid; and (5) the control diet, consisting of CA-free feed. The pH of the diets was determined according to the 02-52 AACC method (AACC 2000). All diets were tested for total aflatoxins before and after CA addition.

AFLATOXIN ANALYSIS

The total aflatoxin content in the feed was determined according to the 991.31 AOAC (1995) method using monoclonal antibody columns for aflatoxins B1 and B2 (VICAM Science Technology, Watertown, MA, USA). The detection limit for aflatoxins with the immunoaffinity column (IAC) via fluorescence measurement is approximately 0.5 ng/g (Hansen 1990). Aflatoxin identification was performed by a modification of the HPLC-AFLATEST procedure. A Waters HPLC equipment with two pumps (Model 510. Waters Associates, Milford, MA), and a Waters nova-pak C18 reverse phase column (5 μm, 3.9mm 150mm) was used.

Table 1. Ingredients and nutrient composition of the control experimental diet.

Ingredientes y composición de nutrientes de la dieta control experimental.

Ingredient	%	Calculated analysis[4]	%
Sorghum	50.33	ME (Kcal/Kg)[5]	3010
Soybean meal (48)	41.52	Crude protein	24
Sunflower oil	3.46	Calcium	1
Orthofosfate	1.86	Phosphorus, total	0.5
Calcium carbonate	1.56	Methionine + Cystine	1.6
Salt (NaCl)	0.41	Lysine	1.5
Alimet 88[1]	0.35	Threonine	0.97
L-Lisine HCl	0.19	Zinc (mg/kg)	45
Choline chloride	0.1	Analyzed	
Vitamine premix[2]	0.1	Crude protein	23.75
Mineral premix[3]	0.05	Calcium	1.1
Sugar + zinc	0.05	Phosphorus, total	0.57
L-Threonine	0.03		

[1] Aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid (HMTBA).
[2] Vitamine premix supplied/kg diet: Vitamin A, 12 000 IU; Vitamin D3, 2 500 IU; Vitamin E, 15 mg; Vitamin K3, 2mg; Vitamin B1, 2.25mg; Vitamin B2, 7.5 mg; Vitamin B6, 3.5mg; Vitamin B12, 0.020mg; folic acid, 1.5mg; pantothenic acid, 12.5mg.
[3] Mineral premix supplied/kg diet: Cu, 8mg as CuSO4.5H2O; Mn, 100mg as MnO; Fe, 80mg as FeSO4.H2O; I, 1mg as ethylenediamine dihydroiodide (EDDI); Se, 0.15mg as Na2SeO3.
[4] Data on dry matter.
[5] ME: Metabolisable energy.

Standards as well as samples collected from the IAC (20 µl), were injected into a HPLC and eluted isocratically with a mobile phase of 12.5 mN acetic acid:acetonitrile (1:1, v/v, pH=3.5) at a flow rate of 1ml/min. Aflatoxins were fluorometrically detected and identified using a fluorescence detector (Waters model 470); the excitation and emission wavelengths were 338 and 425 nm, respectively. Aflatoxins (standards and samples), were analyzed by HPLC without derivatization. Aflatoxins were identified by their retention time (Rt), compared with those for a pure aflatoxin standard solution under identical conditions.

ANIMALS

For the experiment, 300 one-day-old Ross broiler chicks were divided into four experimental groups and one reference group (such that the average weight of the animals in the groups differed by less than 1 g). Twenty birds of mixed sex (three replicates) were housed in plastic cages, 113 cm (l) 90 cm (w) 60 cm (h), in a light-cycled room (12 h cycle), maintained within the temperature range of 30–32 ºC with free access to food and water. In each cage, local heat sources from IR lamps of 250W were used to maintain the body temperature of the birds between 39 and 42 ºC. The floor was covered with 5 cm deep wood shavings and two 2 L capacity chick cup drinkers were placed per cage. When birds were 14 days old, the cup drinkers were replaced by trough drinkers. During the first seven days of age, chicks were fed in a tray feeder, over which a 1 cm mesh plastic screen was placed to prevent feed wastage. After seven days of age, feed was offered in trough feeders 91.5 cm (l) 11.5 cm (w) 5.4 cm (d).

COLLECTION OF SAMPLES AND MEASUREMENTS

Feed and water were provided *ad libitum* during the whole period of the experiment (28 d). Broilers were

individually weighed at the beginning of the experiment, then at weekly intervals until the end of the experiment. Live body weight (LBW), feed consumption (FC), and feed conversion ratio (FCR) were recorded during these periods. After 28 days, blood was drawn by cardiac puncture under anesthesia (the bird was exposed for one minute to 40% carbon dioxide, 30% oxygen, and 30% nitrogen) from 15 randomly selected birds from each treatment, and serum prepared. Total protein and albumin were determined using commercially available kits (Wiener Lab, Rosario Argentina). The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to Reitman and Franked (1970). The bled animals were then exposed to 80% carbon dioxide, 5% oxygen, and 15% nitrogen for euthanasia (Coenen *et al* 2000). Proventriculus, gizzard, liver plus gall bladder, spleen and bursa were excised, washed in cold saline and their relative percentages estimated. The intestinal weight was also considered and pH values in different parts of the GIT (proventriculus, gizzard, duodenum, jejunum and ileum) were also registered immediately by using a digital pH meter (HANNA, model HI 99163, Romania).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was conducted as a completely randomized design with three replicates. Data were assessed by analysis of variance (ANOVA) and means were separated by the Dunnet procedure using the Statistical Analysis System (SAS 1998). A significance value of (= 0.05) was used to distinguish significant differences between treatments.

RESULTS

Table 2 shows the result of the aflatoxin analysis. Results showed that feed contained 39 ± 0.8 ng/g of total aflatoxins. However, in the CA supplemented diets, the

Table 2. Efficiency of aflatoxin degradation of aqueous citric acid and pH in the diet.
Eficiencia del ácido cítrico acuoso en la degradación de las aflatoxinas y valores de pH de la dieta.

(g CA/kg)	Total aflatoxins (ng/g)*		Disappearance of fluorescence (%)	Final pH of diet
	Before CA addition	After CA addition		
6.25	40 ± 1.5[a]	4 ± 0.7[a]	90	5.6 ± 3.4[-4a]
12.5	37 ± 1.2[a]	3 ± 0.6[a]	92	5.1 ± 4.3[-4b]
25	42 ± 1.9[a]	3 ± 0.9[a]	93	4.5 ± 6.3[-4c]
50	38 ± 0.7[a]	3 ± 0.3[a]	92	3.9 ± 3.6[-4d]
0	39 ± 0.4[a]	42 ± 2.6[b]	-	6.2 ± 4.2[-4e]

Mean of three replicates ± s.e.
Column means with common superscripts do not differ (P > 0.05).
* AFB1 and AFB2.

post-reaction aflatoxin content was 3 ± 0.3 ng/g. This reduction in the aflatoxin content represents approximately 92%. The chromatograms of the HPLC (not presented) indicate that the toxins in the feed were AFB_2 and AFB_1, with concentrations of 3 and 36 ng/g, respectively. In extracts of CA-supplemented diets, the fluorescence of AFB_2 was not detected, while AFB_1 fluorescence was much weaker than in the untreated samples. Table 2 also shows the pH values for the ration; as the CA concentration increased, the pH value was decreased. Samples with no acid presented an average pH value of 6.2; while the lowest pH value (3.9) was observed in the ration prepared with the addition of 50 g CA/kg.

The effects of dietary CA supplementation on growth performance of broiler chickens are summarized in table 3. The results indicated that LBW was significantly improved in birds fed diet supplemented with 12.5, 25 and 50 g CA/kg, with average values of 913, 892 and 1013 g, respectively. In general, LBW was slightly higher in animals fed with the addition of CA in their diet, as compared to the control. Additionally, FC did not significantly differ between groups; the average value was 57 g/bird/day. Birds fed supplemental CA had significantly better FCR values compared to the control diet (1.99 g feed/g gain). However, no difference was found in birds fed with the addition of 6.25, 12.5 and 25 CA/kg; in those groups FCR values were 1.81, 1.81 and 1.82 g feed/g gain,

respectively. The lowest value in FCR was registered in birds fed a diet containing 50 g CA/kg (1.55 g feed/g gain).

Dietary supplementation of CA did not significantly affect the values of hematocrite and the serum concentrations of total protein and albumin (table 4). However, significant differences were found for both serum AST and ALT activities levels. As the CA concentration increased, higher AST values were registered. The animals fed diet supplemented with 50 g CA/kg, presented the highest AST value (45 U/L), as compared to the control (23 U/L). On the contrary, supplementation of CA lowered the values of the ALT activity.

Organ weights were not affected due to the addition of CA to the ration (table 5). Moreover, the effect of dietary acidification on pH values of different GIT segments are presented in table 6. The results indicated that CA supplementation had no effect on the proventriculus, gizzard, duodenum, jejunum and ileum pH values.

DISCUSSION

Sorghum is considered the fifth most important crop in the world after wheat, rice, maize, and barley, due to its resistance to drought and high temperatures (ICRISAT/FAO 1996). Its nutritional value is quite similar to maize and it has been used as an ingredient for inclusion in foods and feeds. Unfortunately, sorghum

Table 3. Effect of dietary citric acid on growth performance of broiler chickens.
Efecto del ácido cítrico sobre el desempeño del pollo de engorda.

Parameter	Dietary organic acid supplementation (g CA/kg)				
	6.25	12.5	25	50	0 (control)
LBW (g)	851 ± 13.6[ab]	913 ±15.4[bc]	892 ± 15.5[bc]	1013 ± 47.8[c]	786 ± 11.4[a]
FC (g/bird/day)	55 ± 2.3[a]	59 ± 2.7[a]	58 ± 2.9[a]	56 ± 3.8[a]	56 ± 2.0[a]
FCR (g feed/g gain)	1.81 ± 0.02[b]	1.81 ± 0.02[b]	1.82 ± 0.02[b]	1.55 ± 0.03[c]	1.99 ± 0.02[a]

Mean ± s.e.
Row means with common superscripts do not differ (P > 0.05).
LBW = live body weight; FC = feed consumption; FCR = feed conversion ratio.
n = 60.

Table 4. Effect of dietary citric acid on some serum constituents in broiler chickens.
Efecto del ácido cítrico sobre algunos constituyentes del suero sanguíneo de los pollos de engorda.

Parameter	Dietary organic acid supplementation (g CA/kg)				
	6.25	12.5	25	50	0 (control)
Hematocrite (%)	63 ± 1.7[a]	67 ± 1.2[a]	67 ± 2.7[a]	66 ± 1.0[a]	63 ± 1.8[a]
Total protein (g/L)	3.8 ± 0.1[a]	3.6 ± 0.1[a]	3.6 ± 0.1[a]	3.6 ± 0.1[a]	3.6 ± 0.1[a]
Albumin (g/L)	1.1 ± 0.1[a]	1.2 ± 0.1[a]	1.3 ± 0.1[a]	1.2 ± 0.1[a]	1.2 ± 0.1[a]
AST (U/L)	30 ± 0.9[b]	32 ± 1.0[b]	38 ± 1.2[c]	45 ± 0.8[d]	23 ± 1.7[a]
ALT (U/L)	5 ± 0.3[b]	6 ± 0.9[b]	6 ± 1.3[b]	8 ± 0.3[b]	21 ± 0.8[a]

Mean of 15 replicates ± s.e.
Row means with common superscripts do not differ (P > 0.05).
AST = aspartate aminotransferase; ALT = alanine aminotransferase.

is invaded by fungi during its development and harvest in the field, as well as during the process of transport and storage. Therefore, one of the factors affecting the sanitary quality of feeds prepared with this grain is aflatoxin contamination produced by certain species of fungi, among them strains of *Aspergillus flavus*. In this research, the aflatoxin quantification/identification method indicates that the toxins in the diet were only AFB_2 and AFB_1, with a total concentration of 39 ng/g. These total aflatoxin concentration are commonly found in commercial grains of underdeveloped countries being one of the major causes of low productivity in animals. Animals which consume high levels of these toxins may develop various health problems, depending on their susceptibility. In the case of poultry, aflatoxicosis is characterized by weakness, anorexia with lower growth rate, poor utilization, decreased weight gain, decreased egg production, increased susceptibility to environmental/microbial stress and increased mortality (Leeson *et al* 1995, Bailey *et al* 1998, Kubena *et al* 1998, Dershant-Li *et al* 2003, Kermanshahi *et al* 2009).

Supplementation with aqueous CA substantially reduced the pH of the diets according to the added content (table 2). This effect is interesting because the acidic treatment reduced the aflatoxin concentration in the feed up to 92%. The chromatograms of acidified samples may provide support for detoxification activity. It also suggests that the molecule structure in post-treated aflatoxin contaminated samples changes, the lactone ring may be opened. Thus, detoxification initially involves the formation of the β-keto acid structure (catalyzed by de acidic medium), followed by hydrolysis of the lactone ring yielding the AFD_1 molecule, derivated from decarboxylation of the lactone ring-opened form of AFB_1 (Méndez-Albores *et al* 2005), which is 130 times less mutagenic in the Ames test and presenting 20-fold toxicity decrease (Schroeder *et al* 1985). Therefore, the fluorescence strength varies in the HPLC chromatograms at the same retention time values.

Organic acids are also extremely efficient against bacteria in poultry, and their antimicrobial effect increases with concentration/length of the carbon chain (Van Immerseel *et al* 2006). At a pH below 3.5, almost all organic acids are effective in controlling bacterial growth. As an alpha-hydroxy-carboxylic acid, CA has higher dissociation capacity and its carboxylic group links with ammonia in equimolar quantity (one molecule of CA saturated three molecules of ammonia), thus CA possesses inhibiting, bacteriostatic, and neutralizing effects against ammonifying bacteria, *E. coli,* and *Salmonellae* (Ivanov 2001).

Organic acids also have a positive effect on growth performance; since dietary acidification increases gastric proteolysis and protein/amino acid digestibility by enhancing digestive enzyme activities (Langhout 2000). The reason why protein is better used when CA is added to diet is due to the fact that pepsinogen is converted to pepsin, which increases pepsin activity and improves protein digestibility. Moreover, peptides arising from pepsin proteolysis trigger the release of hormones (including gastrin and cholecystokinin) which regulate the digestion and absorption of protein. The results of LBW, FC and FCR obtained in this experiment confirm those reported by Khosravi *et al* (2008) in broilers fed diet supplemented with 2 g/kg propionic acid during 21 d period. In those birds, LBW, FC and FCR registered values of 612 g, 48 g and 1.5, respectively. The improvement of FCR and the increase of LBW have already been demonstrated in broilers fed a diet supplemented with acidifiers (Denli *et al* 2003). However, test results for the inclusion of certain organic acids in broiler rations remain limited and controversial. Yesilbag and Colpan (2006) investigated the effect of three levels of an organic acid mixture (formic/propionic/ammonium salts) on performance, egg production/quality and serum parameters in laying hens. In general, growth performance (LBW and FC), and egg quality parameters were not significantly affected by supplementing diets with 0.5, 1 or 2% dietary organic acid mixture. Cave (1984) and Jacob *et al* (1990) reported a decrease in food consumption in broiler chickens fed with a diet containing 3% and 7.5% lactic acid, respectively. It is well known that organic acids have a low tendency to free their H^+ ions and the strong taste is associated with them. Consequently, high amounts of organic acid supplementation may reduce the feed consumption, because of changes in diet palatability. In this research, the highest addition of CA to diets (50 g/kg) was successfully tolerated by the chickens (table 3). In summary, the better LBW and FCR values in some acidified diets may be due to the effect of CA by reducing the aflatoxin content, or either by controlling pathogenic bacteria, or maintaining the health of GIT improving the broilers performance.

Among the different serum parameters measured, hematocrite, serum total protein, and albumin concentrations were not significantly affected at the end of the experiment in broilers receiving the four dosages of CA (table 4). These results are consistent with those obtained in broiler chicks due to acetic acid inclusion (Abdo 2004). Abdel-Fattah *et al* (2008) also reported that total protein and albumin were not affected due to the inclusion of 1.5 or 3% CA in broiler chickens fed during 42 d period.

While the increment of the enzymatic values in birds varies with the different species, the elevation of the enzymatic activity has been correlated with hepatocellullar damage. The most frequent cause of the elevation of the AST activity in birds is hepatic disease; birds with AST values in the upper 230 U/L range are considered abnormal (Campbell and Coles 1986). A moderate increase (2–4 fold) in the AST enzyme is observed when there is soft weave injury, whereas in the hepatic necrosis, a more

remarkable elevation is caused. In this research, the results demonstrate that the inclusion of different concentrations of CA in the feed, significantly affects the AST activity; however, the maximum increase in the AST activity (1.9 fold), was observed in birds fed a diet with 50 g CA/kg (table 4). In the case of ALT activity, lower values were registered due to the inclusion of CA. These results are in agreement with those reported by Brenes *et al* (2003) who reported ALT values of 1 and 1.5 U/L in broiler chickens fed a diet containing 20 g CA/kg at different levels of available phosphorous (2.5 and 3.5 g/kg, respectively). Aktaç *et al* 2003a reported that CA (LD_{25} = 480 mg/kg.bw) applied intraperitoneally to mice increases serum AST level (from 177.8 to 307.2 UI/L), and decrease ALT activity (from 695 to 101 UI/l). Microscopic examination of liver showed histopathological changes such as tissue degeneration, cytoplasmic vacuolisations, nuclear membrane invaginations, picnotic nucleus and necrosis of hepatocytes, in part attributed to acidosis and calcium deficiency. Those findings are in close agreement with the results found in the present investigation; however, the effect of CA on transaminase activity might be related to differences in gender and lines of the animals.

As shown in table 5, no statistical differences were noted among all treatments in the relative proventriculus, gizzard, liver, intestine, spleen and bursa percentages. These results confirmed those of Denli *et al* (2003) who found that dietary organic acids had no effect on carcass yield and liver weight of broiler chickens at 42 d old. Yesilbag and Colpan (2006) also concluded that dietary supplementation with organic acids did not affect interne organ weights (hearth, liver, and spleen) in laying hens fed with a diet containing up 1.5% organic acid mixture during 18 week period.

The effect of dietary acidification on pH values of different GIT segments are presented in table 6. The results indicate that CA supplementation slightly reduced proventriculus, gizzard, duodenum, jejunum and ileum pH values, compared with the control group. However, the differences are not significant. These results are consistent with Denli *et al* (2003) who reported that giving broilers an organic acid mixture showed no significant reduction in the intestinal pH. Hernández *et al* (2006) reported no effect on intestinal pH with the use of a product containing a combination of propionic/formic acid. These authors attributed this insignificant effect to the strong buffering action of the GIT in broiler chickens. Prior to birth, the GIT of birds is free of any strains of microbial populations, and bacteria from the diet, water, excreta and environment begin to colonize the GIT almost shortly.

Table 5. Effect of supplemental citric acid on some organs as a percentage of body weight in broiler chickens.

Efecto de la suplementación de ácido cítrico en algunos órganos expresado como porcentaje del peso corporal de los pollos de engorda.

Parameter	Dietary organic acid supplementation (g CA/kg)				
	6.25	12.5	25	50	0 (control)
Proventriculus	0.5 ± 0.03^a	0.5 ± 0.01^a	0.6 ± 0.05^a	0.5 ± 0.02^a	0.6 ± 0.01^a
Gizzard	2.4 ± 0.10^a	2.3 ± 0.18^a	2.4 ± 0.14^a	2.4 ± 0.08^a	2.5 ± 0.27^a
Liver	2.8 ± 0.19^a	2.9 ± 0.14^a	2.5 ± 0.21^a	2.5 ± 0.17^a	2.9 ± 0.15^a
Intestine	5.1 ± 0.13^a	5.2 ± 0.31^a	5.3 ± 0.26^a	5.4 ± 0.16^a	6.0 ± 0.72^a
Spleen	0.08 ± 0.01^a	0.12 ± 0.03^a	0.08 ± 0.01^a	0.09 ± 0.01^a	0.10 ± 0.01^a
Bursa	0.23 ± 0.02^a	0.25 ± 0.02^a	0.23 ± 0.06^a	0.28 ± 0.02^a	0.24 ± 0.02^a

Mean of 15 replicates ± s.e.
Row means with common superscripts do not differ (P > 0.05).

Table 6. Effect of supplemental citric acid on pH values of some gastrointestinal tract segments in broiler chickens.

Efecto de la suplementación de ácido cítrico en los valores de pH de algunos segmentos del tracto gastrointestinal de los pollos de engorda.

Parameter	Dietary organic acid supplementation (g CA/kg)				
	6.25	12.5	25	50	0 (control)
Proventriculus	3.5 ± 0.11^a	3.4 ± 0.17^a	3.3 ± 0.11^a	3.3 ± 0.08^a	3.4 ± 0.08^a
Gizzard	2.4 ± 0.11^a	2.6 ± 0.03^a	2.9 ± 0.33^a	2.5 ± 0.14^a	2.6 ± 0.21^a
Duodenum	6.2 ± 0.04^a	6.1 ± 0.12^a	6.1 ± 0.08^a	6.4 ± 0.06^a	6.3 ± 0.05^a
Jejunum	6.3 ± 0.04^a	6.4 ± 0.06^a	6.3 ± 0.03^a	6.4 ± 0.04^a	6.4 ± 0.01^a
Ileum	6.6 ± 0.10^a	6.5 ± 0.11^a	6.4 ± 0.03^a	6.6 ± 0.06^a	6.5 ± 0.10^a

Mean of 15 replicates ± s.e.
Row means with common superscripts do not differ (P > 0.05).

Consequently, the pH level in specific areas of the GIT is a factor which establishes a specific microbial population, and also affects the digestibility and absorption of most nutrients. Taking into account that most pathogens grow at a pH close to 7, and that beneficial microorganisms live in an acidic media (5.8-6.2) in which they compete with pathogens, CA could be used as an alternative for immune system development as well as for increasing bird performance.

In conclusion, the current study showed that the aqueous CA treatment significantly reduce the aflatoxin content in the ration. CA supplementation also promotes growth performance of young broiler chickens; however, CA increases serum AST levels and decrease ALT activity. According to these results, it seems that CA may be beneficial when used in broilers chickens. Nevertheless, further studies are needed on the effect of these organic acid concentrations on the complete broiler growing cycle.

SUMMARY

This study was undertaken to investigate the effects of citric acid (CA) on aflatoxin degradation, growth performance and some serum constituents in broilers. 300 one-day-old Ross broiler chickens were randomly divided into five treatment groups of three replicates, 20 chicks each. Four groups received the diet supplemented with CA (6.25, 12.5, 25 and 50 g/kg), while the other served as a control. Diet was prepared following the NRC guidelines and the experiment was terminated when chicks were 28 d old. The results showed that aflatoxins in the diet, at a concentration of 39 ng/g were almost degraded (92%) by the acidification procedure. In general, live body weight (LBW) was slightly higher in animals fed with the addition of CA; diet containing the highest concentration (50 g CA/kg) resulted in a significant increase in LBW and improvement of feed conversion ratio. However, as the CA concentration increased, higher serum aspartate aminotransferase activity values were registered. On the contrary, hematocrite, total protein and albumin were not affected by any level of added CA. From these results, it is concluded that dietary CA supplementation can be used as an additive to degrade aflatoxins in the ration as well as to promote growth performance in young broiler chickens.

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