

EFFECT OF ADDING CHLOROGENIC ACID ON BROILER PERFORMANCE, SOME BLOOD BIOCHEMICAL PARAMETERS AND GUT HEALTH

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ABSTRACT

This study was conducted to evaluate the effectiveness of chlorogenic acid on productive performance, blood biochemistry and intestinal histomorphology of broiler chickens. A total of 256-day old Ross 308 broiler chicks were divided into 4 treatments with 4 replications, 16 chicks per replicate. Treatments were arranged as follow: control group was left without any additive, whereas group 2-4 supplemented with 1, 1.5 and 2 mL of chlorogenic acid per liter of drinking water respectively. Results of this study showed that the body weight gain of birds received chlorogenic acid significantly increased when compared to control group. Lowest feed intake ($P<0.05$) was recorded for the birds received 1.5ml of chlorogenic acid than other experimental groups. Better feed conversion ratio was found in birds offered 1 and 1.5 mL chlorogenic acid compared to the control and those offered 2 mL /L of chlorogenic acid. The chlorogenic acid significantly increased villous heights and reduced crypt depths. The higher villous surface area was recorded for the birds received 1 mL of chlorogenic acid ($P<0.05$) than other groups. The birds in control group significantly increased the serum content of globulin over all treatments. It can be concluded that chlorogenic acid had a positive effect on performance and gut health of broiler chickens.

Key words: acid, chickens, gut histomorphology, blood biochemistry

حشطان وآخرون

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تأثير اضافة حامض كلوروجين في الاداء الانتاجي و بعض معايير الدم الكيمو حيوية و صحة الامعاء لدى فروج اللحم
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المستخلص

أجريت هذه الدراسة لتقييم فاعلية حامض الكلوروجينيك على الأداء الإنتاجي والكيمو حيوية في الدم والتشكيل النسيجي المعوي لفروج اللحم. تم استخدام 265 فرخ Ross308 بعمر يوم واحد وقسمت الى 4 معاملات من 4 مكررات (كل مكرر يحتوي على 16 فرخ). تم ترتيب المعاملات على النحو التالي: السيطرة عدم وجود مواد مضافة، الكلور وجينيك 1 مل / لتر من مياه الشرب، الكلور وجينيك 1.5 مل / لتر من مياه الشرب و 2 مل / لتر من مياه الشرب. أظهرت نتائج هذه الدراسة أن جميع مستويات حمض الكلوروجينيك أدت إلى زيادة معنوية في زيادة وزن الجسم عند مقارنتها بمجموعة السيطرة. تم تسجيل أقل استهلاك علف ($P < 0.05$) للطيور التي اضيفت لها 1.5 مل من حمض الكلوروجينيك عن المجموعات التجريبية الأخرى. تم الحصول على اعلى معدل تحويل في الطيور التي اضيفت لها 1 و 1.5 مل من حمض الكلوروجينيك مقارنة مع مجموع السيطرة وتلك التي اضيفت لها 2 مل / لتر من حمض الكلوروجينيك. زاد حمض الكلوروجينيك بشكل كبير من ارتفاعات الزغابات وخفض أعماق القبول. الحصول على اعلى معدل المساحة السطحية الزغابية ($P < 0.05$) للطيور التي اضيفت لها 1 مل من حمض الكلور وجينيك عن المجموعات الأخرى. زادت الطيور في المجموعة الضابطة زيادة معنوية في محتوى الجلوبولين في الدم في جميع العلاجات. يمكن الاستنتاج أن حمض الكلوروجينيك له تأثير إيجابي على أداء وصحة أمعاء الدجاج اللاحم.

كلمات مفتاحية: أفراخ، مواد مضافة، أحماض، معدل تحويل

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INTRODUCTION

Incorporation of organic acids and phytobiotics into the diets of broiler chickens has the ability to impede the growth of pathogenic bacteria in the intestinal tract and enhance the efficacy of feed conversion. The process of acidification has been observed to decrease the colonization of pathogens within the gastrointestinal tract, thereby impeding the potential harm inflicted upon the epithelial cell (3, 12). It has been shown that organic acids increase the protein and amino acid digestibility (1), and minerals such as calcium, zinc, phosphorus, and magnesium in broiler chickens (12). According to the reports, adding of butyric acid and its sodium salt has been found to have a beneficial impact on the productivity and gut health of broiler chickens (21, 13). Moreover, acid and phytobiotics have the potential to serve as an energy substrate for the intestinal epithelium, thereby promoting the growth of intestinal epithelial cells. (4, 12). Chlorogenic acid (CGA) can be defined as phenolic compound that is typically created through the esterification of caffeic acid and quinic acid. The term "CGA" primarily refers to 5-O-caffeoylquinic acid. This particular isomer of CGA has been the subject of extensive research due to its early availability in a pure crystal form for commercial use. However, it is worth noting that the nomenclature of 3-O-caffeoylquinic acid, and 5-O-caffeoylquinic acid have been a source of confusion in the field. (9). The synthesis of CGA is observed in various plant species, for instance, potato, sweet potato leaves, coffee beans, tubers, *Eucommia ulmoides* leaves and wide range of fruits, vegetables, and *Lonicerae Flos* (9). It has been proven the biological functions of these compounds on the broiler chickens health. These functions include anti-tumor properties, hypoglycemic, cholesterol-reducing, immunomodulatory, anti-inflammatory, antioxidant, antimicrobial and cholesterol-reducing, (14). Moreover, the CGA exhibits neuroprotective, cardiovascular protective, gastrointestinal protective, reno-protective, and hepatoprotective properties (19). Regarding domestic animals, previous study has indicated that the adding of CGA in their diet has the potential to enhance various

aspects such as gut microbiota, antioxidant capacity, intestinal development, growth performance, digestion, barrier function, absorption capacity, and gut microbiota in weaned piglets (2, 7). The literature on broilers has shown that CGA has been found to mitigate the growth retardation and intestinal damage caused by *Clostridium perfringens* or coccidia challenges in broiler chickens. This is achieved through the inhibition of inflammation response, maintenance of redox status, and improvement of intestinal barrier function (5). Additionally, administration of chlorogenic acid (CGA) or CGA-enriched extract derived from *Eucommia ulmoides* leaves can effectively enhance the meat quality of broiler chickens exposed to heat stress and oxidative stress. This improvement is achieved by augmenting the antioxidant capacity and modulating the transcription of genes associated with antioxidant defense mechanisms (6). To best of our knowledge there are limited information about the effect of CGA on broiler chickens. Thus, the purpose of this study was to examine the impact of different concentrations of CGA in drinking water on growth performance, serum biochemical indices, and intestinal morphology of broiler chickens.

MATERIALS AND METHODS

Experimental design : A total of 256 one-day old chicks were purchased from Arshad Hatchery and subsequently transported to the Poultry Farm/Sumel. The birds randomly distributed to four treatment groups, with four replicates of 16 chicks. Upon the arrival of the chicks, initial body weights were recorded. The chicks were provided with a 5% sugar solution. Treatments were arranged as follows: control group was left without any additive, whereas group 2-4 supplemented with 1, 1.5 and 2 mL of chlorogenic acid per liter of drinking water, respectively. Live body weight, feed intake, Feed conversion ratio (FCR) and mortality were recorded at days 10, 24 and 35. Throughout the experiment, the temperature, lighting program were adjusted according to the Ross 308 guide.

Blood biochemical parameters and Newcastle disease antibody (NDV)

On day 24, 2 birds per replicate were selected randomly and slaughtered. Roughly about 5

mL of blood were collected from jugular vein for blood biochemical parameters. The serum was obtained by centrifuging blood for 15 minutes, and subsequently stored at -20 °C until it used for analysis. The serum biochemical parameters, including triglycerides, albumin, cholesterol, total protein, and high-density lipoprotein which were analyzed using colorimetric enzymatic methods. These methods were performed according to the protocols outlined in the corresponding commercial kits obtained from Randox Laboratories Limited, United Kingdom. For the NDV titer measurement, the serum analyzed according to Oberlander (17) and Reisinger (18).

Jejunum histology

On day 24, tissue samples of proximal jejunum were collected from 2 birds per replicate, flushed with buffered saline and fixed in 10% of buffered formaldehyde for micromorphological measurement. Samples were imbedded in paraffin wax, stained with eosin and hematoxylin stain and sectioned. Sections of the sample were photographed using a digital camera mounted on a microscope (Dino-Eye-Microscope Eye-piece Camera) at a magnification of 10 times. The Dino-eye program was used to determine the morphometric indicators. Digitization of image processing the depth of the crypt (m) (from the villus/crypt junction to the muscular section) and the height of the villus (m) (from the tip of the villus to the villus/crypt junction) were calculated. A total of 7–10 well-oriented villi for each jejunum were measured (10).

Feed preparation

Experimental diets (starter, grower, and finisher) were obtained from the Badi feed factory in Duhok province. The treatments were as follows: control group was left without any additive, whereas groups 2-4 were supplemented with 1, 1.5, and 2 mL of CGA per liter of drinking water respectively for 35 days.

vaccination programs

According to the commercial farm vaccination protocol followed in Duhok, chicks were immunized. Birds were sprayed with the Infectious Bronchitis (IB) + Newcastle (B1) (Intervet SP product) on the first day.

Statistical analysis

Using SAS software (SAS, 2010), one-way ANOVA analysis of variance was used to statistically examine all the results. Duncan's multiple range tests were used to identify differences among means at ($P < 0.05$).

RESULTS AND DISCUSSION

Performance: The performance data are presented in Table 1. The result showed that there were no significant differences among treatments for feed intake, live body weight and FCR at days 10 and 24 of bird's age. However, when assessed over the 35 days of experimental period birds that received 1 and 1.5 ml/L of CGA significantly ($P < 0.05$) increased the live body weight when compared to bird received control or 2 ml/l of CGA. The birds received 1.5 ml/l CGAs had lower feed intake overall treatments. Birds that received 1.5 and 1 ml/l of CGA significantly reduced FCR compared to control and 2 ml/l CGA groups. The study conducted by Zhang (24) found that broiler chickens that were fed with CGA exhibited a significantly greater daily gain compared to the control group. The findings presented in this study were in contrast to the results reported by Zhang (25), who observed that the inclusion of CGA in the diet improved the growth performance of broiler chickens. The utilization of chlorogenic acid as a feed additive in poultry production has been demonstrated their anti-inflammatory, antibacterial, antioxidant and antiviral properties. The efficacy of dietary herbal plants and their extracts in improving broiler performance has been scientifically validated. These substances have been found to enhance feed consumption, stimulate and activate digestive enzymes, and promote intestinal digestion and improved nutrient utilization (11).

Table 1. Effect of different levels of chlorogenic acid on broiler chickens' performance

Treatment	Body weight			Feed intake			Feed conversion ratio		
	10	24	35	10	24	35	10	24	35
Control	259.40	1085 a	1831.59 c	278.38	1366.74	2977.70 a	1.07	1.26	1.62 a
CGA 1ml/L	264.78	1117.8	2004.33 a	273.00	1347.40	2931.91 a	1.03	1.21	1.46 b
CGA 1.5ml/L	261.34	1156.2	1936.13ab	278.44	1364.08	2802.55 b	1.06	1.18	1.45 b
CGA 2 ml/L	267.78	1132.6	1886.39 bc	281.03	1336.94	2950.63 a	1.06	1.17	1.56 a
Pooled SEM	2.319	19.135	20.410	1.82	19.922	25.119	0.009	0.022	0.023
P-Value	0.635	0.656	0.005	0.50	0.956	0.043	0.385	0.609	0.003

abc Means with the same superscripts are not statistically different from each other at ($P < 0.05$).

SEM= Standard error of means, CGA= Chlorogenic acid

Histomorphology

The jejunum histomorphology data are shown in table 2. All birds received CGA had higher villous height and lower crypt depth ($p < 0.001$) compared to birds in control group. Birds that were received 1.5 ml/l of CGA had higher villous height and lower crypt depth ($p < 0.001$) compared to birds received 2 ml/l CGA. No significant difference was detected between treatments for muscle thickness. The birds received 1 ml/L CGA had significantly higher villous tip width and villous area overall treatments. The base width of birds in control and 1 ml/l CGA significantly increased compared to 1.5 and 2 ml/L CGA. The results agreed with Liu (8), who reported that broilers fed diets supplemented with chlorogenic acid exhibited greater villi height compared to those fed control diets. In comparison to the control group. The groups administered with chlorogenic acid reduced crypt depth and thinner intestinal muscle. Consequently, enhanced villus height or an increased ratio of villus height to crypt depth are commonly correlated with effective nutrient absorption and enhanced performance. The CGA employed in the present study exhibited a notable enhancement in the histomorphological characteristics of the intestinal tract in broiler chickens. The results are consistent with the findings of Naveed (16), which demonstrated notable enhancements in the intestinal morphology of

broilers when incorporating phytochemical additives into their diets. The measurements of villus height, crypt depth, and V/C value have been found to serve as indicators of the overall integrity, developmental status, and nutrient absorption capacity of the intestinal tract in animals (8, 15, 21, 22, 23). Furthermore, our study revealed that the administration of CGA resulted in an increase in villus height. This finding aligns with the previous report by Zahang (25), which demonstrated that CGA supplementation in the diet improved villus height and preserved intestinal integrity in broilers afflicted with necrotic enteritis.

Blood biochemical parameters

The serum biochemistry data are shown in Table 3. There were no significant differences between treatments that were recorded for serum cholesterol, ALT, protein, and albumin. However, birds in control group had significantly higher globulin overall treatments. The amount of albumin and globulin that make up total serum protein, which is primarily made up of these two proteins, is a good indicator of the body's immune system and protein metabolism. The ratio of albumin to globulin shows the health of the immune system in the body. If the ratio falls, this refers to higher globulin synthesis and improved immunological function (20). This was not the case of our study when chlorogenic acid reduced the serum globulin compared to non-treated group.

Table 2. Effect of adding of chlorogenic acid to the drinking water on jejunum histomorphology of broiler chickens at day 24

Treatment	Villous height	Crypt	Muscle	Villous Tip	Villous Base	Villi/crypt ratio	Villous area
Control	1370.63 C	199.900a	214.500	157.35 b	250.75 a	7.4122 b	27360 b
CGA 1ml/L	1594.25 ab	153.950 c	196.775	228.25 a	256.75 a	10.8159 a	381715a
CGA1.5ml/L	1624.80 a	178.800b	204.000	157.75 b	175.80 c	9.7004 a	271899b
CGA 2 ml/L	1507.20 b	150.750 c	194.775	156.30 b	203.25 b	10.2871 a	273542b
Pooled SEM	18.28692	3.50713	3.36168	4.80522	4.55223	0.23059	6368.87
P-Value	<.0001	<.0001	0.1520	<.0001	<.0001	<.0001	<.0001

abc Means with the same superscripts are not statistically different from each other at ($P < 0.05$).

SEM= Standard error of means, CGA= Chlorogenic acid

Table 3. effect of adding chlorogenic acid to the drinking water on blood biochemical parameters of broiler chickens on day 24.

Treatment	Cholesterol	ALT	Protein	Albumin	Globulin
Control	8.600	5.175	28.465	11.970	16.495 a
CGA 1ml/L	8.800	4.550	23.450	12.960	10.490 b
CGA 1.5 ml/L	8.600	5.750	21.363	15.430	5.933 b
CGA 2 ml/L	4.650	5.400	21.400	11.847	9.553 b
Pooled SEM	0.810	2.135	1.686	0.479	1.328
P-Value	0.3270	0.180	0.128	0.2523	0.0139

abc Means with the same superscripts are not statistically different from each other at ($P < 0.05$).

SEM= Standard error of means, CGA= Chlorogenic acid, ALT= Alanine transaminase

Newcastle disease virus antibody

The NDV data was presented in figure 1. The results showed that the CGA significantly

increase the NDV antibody when compared to control group.

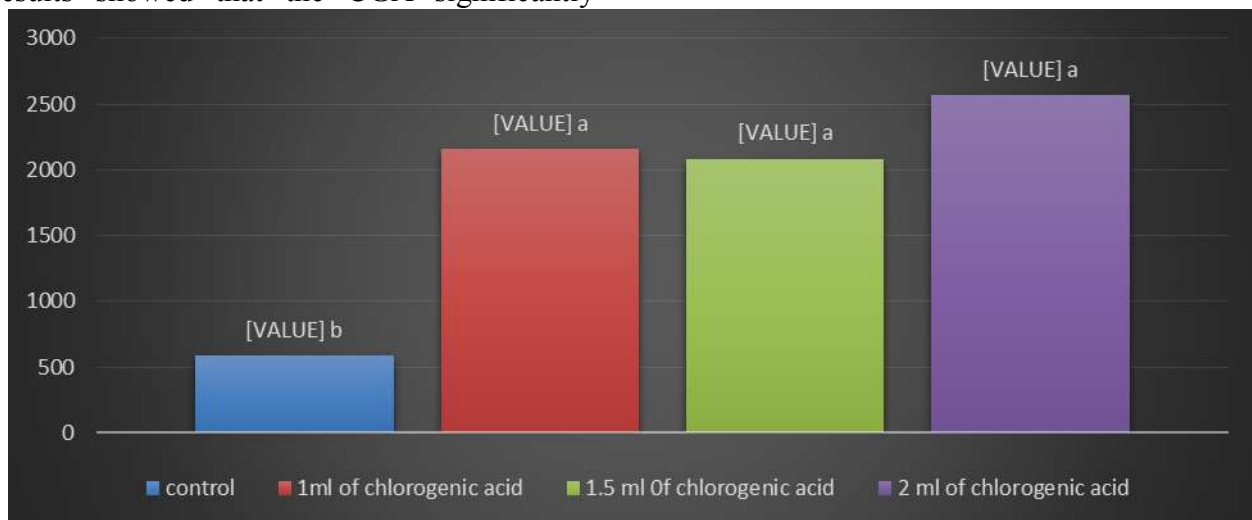


Figure 1. Effect of chlorogenic acid on Newcastle disease antibody

CONCLUSION

It could be concluded that chlorogenic acid was effective in improving performance. The data also demonstrates that chlorogenic acid improved gut integrity by increasing VH and VH:CD ratio and decreasing CD. Administration of chlorogenic acid has a positive effect on improving performance and gut health in broiler chickens.

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