2020, Scienceline Publication
World's Veterinary Journal
World Vet J, 10(3): 362-374, September 25, 2020
ISSN 2322-4568
DOI: https://dx.doi.org/10.36380/scil.2020.wvj45

Effect of Different Dietary Crude Protein Levels and Citric Acid on Broiler Chickens' Performance, Carcass Characteristics, Intestinal Morphology, and Blood Components

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ORIGINAL ARTICLE
pii: S232245682000045-10
Received: 19 Jul 2020
Accepted: 28 Aug 2020

ABSTRACT

The present study was conducted to investigate the effect of dietary protein levels and citric acid on the growth performance, carcass yield, abdominal fat, chemical composition of meat, intestinal morphology, and blood parameters of broiler chickens. A total of 160 Cobb 500 unsexed one-day-old broilers were assigned to 4 dietary treatments, 4 replicates of 10 chickens each. A factorial design arrangement 2×2 was used, including two protein levels, 100% (optimal level) and 90% (low level) of recommended dietary crude protein for cobb 500 broiler chickens, each protein level supplemented with or without 20-gram citric acid /kg. The results showed that chickens fed the diet containing 100% required Crude Protein (CP) supplemented with citric acid which could significantly improve body weight gain, feed conversion ratio, carcass yield, abdominal fat, fat content in meat, intestinal morphology, cecal microbial content, and blood parameters (Albumin, haemoglobin Ac1, fructosamine, and cholesterol). Chickens fed the low CP diet supplemented with citric acid could compensate for the growth performance equivalent to those fed the optimal CP diet. Both required protein level and citric acid were significantly improved blood albumin and reduced haemoglobin Ac1 and fructosamine, which could serve as indicators of the blood protein glycation. In conclusion, citric acid addition could alleviate the negative effect of feeding broiler chickens on low CP diets through its beneficial impact on intestinal morphology, cecal bacterial counts, blood cholesterol reduction, and glycated proteins.

Keywords: Broiler, Citric Acid, Glycation, Performance, Protein level

INTRODUCTION

It is of utmost significance for the livestock industry to benefit from inexpensive, highly efficient, and safe diets. Inclusion of low crude protein content in poultry diets can be considered as one of the effective strategies to reduce ammonia emission from poultry wastes, environmental impact, and diet costs (Belloir et al., 2017 and Barekatain et al., 2019). However, such diets can result in negative consequences, including poor growth performance and carcass yield of broilers (Bregendahl et al., 2002; Liu et al., 2017; Allameh and Toghyani, 2019). The negative effect of a low protein diet on chickens' growth performance might be attributed to the effects of protein deficiency on intestinal morphological changes (Yu et al., 2019). Therefore, nutritionists have developed strategies to lessen the negative effects of the low CP diets on broiler chickens' growth performance (Kermani et al., 2017; Goodarzi Boroojeni et al., 2018 and Sharifuzzaman et al., 2020).

Many endogenous factors limited protein utilization in the chickens' diet, such as gastrointestinal tract physiology, oxidative stress, and glycation. Glycation is one of the non-enzymatic reactions observed in avian species, which might reduce amino acids' nutritional value, therefore low glycation of protein and amino acids could enhance poultry performance (Makino et al., 2015). On the other hand, the low dietary protein could increase the glycated amino acids in chicken plasma (Honma et al., 2017). Therefore, diet adjustments or food additives could be considered as early prevention against glycation (Guilbaud et al., 2016).

As reported, human consumption of several compounds, such as organic acids, minerals, and probiotics induce anti-glycation activity. These compounds, especially organic acids, had been used as alternatives for antibiotics (Polycarpo et al., 2017) and growth promoters in poultry diets (Sabour et al., 2019 and Adhikari et al., 2020). Several studies lend support to the effects of organic acids, especially citric acid, on the enhancement of growth performance (Islam et al., 2011 and Tanzin et al., 2015), carcass yield (Lakshmi et al., 2016), and intestinal histology (Khosravinia et al., 2015). Therefore, the current study aimed to evaluate the impact of adding citric acid to broiler diets with protein levels on growth performance, carcass characteristics, intestinal morphometry, microbiota, and blood biochemical parameters.

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MATERIALS AND METHODS

This six-week study was conducted between March and April 2019 at the Poultry Research Unit, Regional Center for Food and Feed (RCFF), Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The analyses were carried out at laboratories of RCFF.

Ethical approval

This experiment was performed according to all ethics and animal rights of Agriculture Research Center, Egypt.

Housing and management

The present study consisted of 160 Cobb 500 unsexed one-day-old broiler chicks with 40 gram (g) initial weight. The chickens were randomly assigned into four different groups, four replicates of 10 chickens each, and were housed in a semi-closed house in mesh wire-floored cages equipped with automatic drinkers and pan feeders. The chicks were kept under a similar veterinary control and management system in all treatments. The house temperature was maintained at 32 º C for the first 5 days, and then gradually decreased to 24 º C at the age of 21 days. The chickens were received continuous light for the first 24 hours, and then 23 hours light and one hour of darkness for the remainder of the experiment. All chicks were monitored for general health twice daily. Feed and water were offered *ad-libitum* throughout the experimental period.

The experimental diets and design

The experiment was designed in a 2 (levels of protein) × 2 (levels of Citric Acid, CA) factorial design arrangement during starter (1-14 days old), grower (15-28 days old), and finisher (29-42 days old) periods. The experimental diets included the protein levels of 100% (optimal level) or 90% (low level) of the requirements, and the CA levels of 0 or 20 g/kg diet. The experimental diets were formulated to meet the nutritional recommendations according to Cobb 500 guide with the exception of CP in low protein diets (Table 1).

Broiler performance

The chickens and diets in each pen were weighed weekly, and feed efficiency was adjusted for mortality on a pen basis. The Initial Body Weight (IBW) and Final Body Weight (FBW) of each period were used to calculate Body Weight Gain (BWG). Feed Intake (FI) was recorded at the end of each period during the experiment. Feed Conversion Ratio (FCR) was calculated according to the following equation:

FCR = FI (g) / BWG (g)

BWG, FI, and FCR were calculated cumulatively at the end of the experiment.

The mortality was daily recorded throughout the experiment period.

Carcass measurements

At the end of the experiment, eight chickens from each treatment with approximately similar body weight were chosen for carcass measurements. Prior to slaughter, the selected chicks were deprived of feed for eight hours, individually weighed, and then slaughtered. After complete bleeding, the chicks were plucked by dry-plucking, and their weights were recorded. Head and shanks were removed, then the chicks were eviscerated, and different organs (i.e., intestine, gizzard, lungs, spleen, liver, and heart) were separated. The carcass giblets (i.e., gizzard, liver, and heart), and organs related to the immune system (i.e., spleen, and bursa of Fabricius) were separately weighed.

The proportional weight of giblets or carcass (dressing, %) to live weight was calculated using the following equations:

Giblets weight (%) = [GW / LBW] × 100

Dressing (%) = [DW / LBW] × 100

Where LBW denotes live body weight, GW refers to giblets weight, and DW is dressed weight

Meat composition

The carcass was stored in an airtight polyethylene bag at −18°C for the later evaluation of the whole-body chemical composition (dry matter, CP, fat, and ash).

Intestinal morphology

Small intestines were immediately removed from slaughtered chickens, excised, and flushed with distilled water for content removal. Segments of 2cm in length from the midpoint of the duodenum fixed in a 10% buffered formalin. The samples were evaluated in terms of the Villus Height (VH) and Width (VW), Crypt Depth (CD), Villus Height to Crypt Depth ratio (VH/CD). The surface area of the villus was calculated as the product of the height multiplied by the width (Allameh and Toghyani, 2019).

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Bacteriological analysis of caecal content

At the end of the experimental period, eight birds from each experimental group were selected and slaughtered for bacteriological analysis. The caecum from each chicken was removed aseptically and the contents were transferred into a sterile test tube. The samples of caecum contents were examined for caecum microflora, including *Lactobacillus* bacteria, *E- coil*, and *Clostridium perfringens*.

Blood parameters

At the time of slaughter, eight blood samples were collected from experimental groups. Commercial diagnostic kits (Diamond Diagnostics Company, Egypt) used to measure total protein, albumin, cholesterol, renal function, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). The globulin concentration was calculated as the difference between total protein and albumin according to Coles (1974). Haemoglobin (Hgb) was measured as mentioned by Van Kampen and Zillstra (1983) and plasma glucose was analyzed based on Trinder's (1969) method. Hemoglobin A1c (HbA1c) values were determined using a Variant II Kit analyzer (Bio-Rad, Hercules, California, USA). Fructosamine was calculated according to the following equation (Cohen et al., 2003):

Fructosamine = (HbA1c–1.61) ×58.82

Statistical analysis

The general linear model was employed for data analysis using SAS software (SAS Institute, USA, 2004). Duncan's multiple range test (Duncan, 1955) was performed to detect significant differences of means. The p-value less than 0.05 was considered statistically significant. The employed model was as following:

$Y_{ij}= \mu + P_i + C_j + (PC)_{ij} + e_{ij}$

Where, Y_{ijis} refers to the observation of the parameter measured, μ is overall mean, P_i denotes the fixed effect of protein level, C_j counts for the fixed effect of CA level, $(PC)_{ij}$ is the fixed effect of interaction between levels of CP and CA, and e_{ij} stands for a random error.

Table 1. Composition and calculated analysis of the experimental diets for broiler during starter (1-14 days old), grower (15-28 days old), and finisher (29-42 days old) periods at the Poultry Research Unit, Giza, Egypt.

Stage / Ingredient	Starter (1-14days old)				Grower (15-28days old)				Finishers (29- 42days old)			
Yellow Corn (8.2% CP)	56.46	53.23	61.30	57.89	59.60	56.00	65.07	61.67	63.92	60.37	69.04	65.34
Soybean Meal (44.5% CP)	31.66	31.557	29.00	29.00	31.85	32.47	27.30	27.30	27.50	27.90	23.40	23.95
Corn Gluten Meal (59.4% CP)	6.00	6.50	4.04	4.50	2.00	2.00	1.65	2.082	1.55	1.62	1.00	1.00
Vegetable Oil	1.82	2.75	1.55	2.50	2.75	3.83	2.05	3.05	3.467	4.60	2.85	4.00
Vitamins & Minerals premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Common Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-lysine-HCl	0.17	0.17	0.25	0.25	0.10	0.09	0.205	0.21	0.11	0.11	0.23	0.23
DL-Methionine	0.10	0.1	0.111	0.111	0.125	0.125	0.145	0.145	0.12	0.12	0.15	0.15
Di-calcium Phosphate	2.10	2.05	2.107	2.107	1.96	1.90	1.90	1.91	1.75	1.75	1.70	1.70
Limestone	0.91	0.86	0.86	0.86	0.83	0.80	0.90	0.85	0.80	0.75	0.85	0.85
Choline chloride	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
Citric acid	0.00	2.00	0.00	2.00	0.00	2.00	0.00	2.00	0.00	2.00	0.00	2.00
Total	100	100	100	100	100	100	100	100	100	100	100	100
Composition, calculated**												
Metaboizable E k.cal/kg	3000	3000	3003	3000	3053	3053	3052	3053	3154	3159	3153	3155
Crude protein %	22.3	22.3	20.3	20.3	20.3	20.2	18.5	18.5	18.4	18. 3	16.7	16.6
Calcium %	0.93	0.90	0.90	0.90	0.86	0.84	0.86	0.85	0.79	0.77	0.78	0.79
Available phosphors %	0.46	0.45	0.46	0.46	0.44	0.43	0.42	0.42	0.40	0.39	0.38	0.38
Lysine %	1.23	1.22	1.23	1.22	1.13	1.13	1.12	1.12	1.03	1.03	1.05	1.05
Methionine %	0.49	0.49	0.46	0.46	0.46	0.46	0.45	0.45	0.43	0.42	0.43	0.43
Methionine+ Cysteine %	0.86	0.86	0.81	0.81	0.80	0.80	0.77	0.76	0.74	0.74	0.72	0.72

CP: Crude Protein.*Vitamin and mineral premix each 2Kg contains: Vitamin A 10000000 IU, Vitamin D_3 3000000 IU, Vitamin E 25000 mg, Vitamin K 2000 mg, Vitamin B_1 1500mg; Vitamin B_2 5000mg, Vitamin B_6 1500mg, Vitamin B_{12} 15000mcg, Niacin 30000 mg, folic acid 1000mg, Pantothenic acid 10000mg, Biotin 75000mcg, copper 50000mg, iron 40000mg, manganese 60000mg, zinc (Oxide) 60000mg, iodine 1000mg, selenium 300mg, cobalt 200 mg, BHT (Antioxidant) 250mg and Carrier: Calcium Carbonate up to 2 Kg .**Values were calculated from the data according to NRC (1994). Calculated values of crude protein are based on determined values for corn (8.2% CP), soybean meal (44.5% CP), and corn gluten meal (59.4% CP).

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RESULTS AND DISCUSSION

Growth performance

The effect of adding CA in low protein broiler diet on FI, BWG, and FCR at day 42 of age is presented in table 2. The reduction in dietary CP percentage could significantly increase FI, while BWG and FCR were negatively affected. Although CA addition had no significant effect on FI, it could significantly improve BWG and FCR values ($p \leq 0.05$). The best BWG and FCR values were recorded for the chickens fed 100% CP (optimal level) with the addition of CA. The low protein diet supplemented with CA showed improvements in BWG and FCR of chickens, compared to those fed a low protein diet without CA addition. Regarding citric acid, the BWG and FCR values (2312 g and 1.59, respectively) in chickens receiving a low protein with CA diet were as ameliorated as those fed the required CP (2314 g and 1.57, respectively). Consequently, significant differences were observed in the investigated treatments due to CP and CA interaction.

The obtained results of the present study addressing feed consumption elevation due to low protein levels were in line with the results obtained by Aletor et al. (2000) and Barekatain et al. (2019) indicating increased FI caused by low protein use in the broiler diet. However, Roy et al. (2010) and Park and Kim (2019) stated that feed consumption of broilers was not significantly influenced by dietary protein level. On the other hand, Malomo et al. (2013) and Allameh and Toghyani (2019) concluded that the FI of broiler chickens decreased significantly as the CP level reduced. Several reasons could explain the increase in feed consumption for the low protein diets fed with broilers, including the effect of CP on the appetite of fed broilers (Srilatha et al., 2018), deficiency of some critical amino acids, and the differences in the digestibility of amino acids in the diets (Kamran et al., 2010), and the birds might increase the feed intake to get their CP requirements (Selim, 2015; Belloir et al., 2017 and Mahmoud et al., 2017).

Several studies reported the inferior effects of low dietary protein on broiler growth performance (Hernández et al., 2012; Folorunso et al., 2014 and Liu et al., 2017). In the same vein, Allameh and Toghyani (2019) concluded that the weight of broiler chickens significantly decreased when the dietary CP reduced from 20.4% to 17.9%. Moreover, they concluded that feeding 85% of CP requirements had negative effects on the daily weight gain of broilers. Recently, Hilliar et al. (2020) and Macelline et al. (2020) concluded that birds were fed low protein diets had a low BWG, compared to chicks that were fed high protein diets. However, Tarsewics et al. (2006) found that diets with different protein levels had no significant effect on the BWG of birds.

The FCR results of the current study were in accordance with the findings of a study conducted by Gheorghe (2013) concluding that the highest FCR value in a group fed sub-optimal protein diet might be due to the increase in the feed consumption with low body weight gain. Selim (2015) stated that the different amounts of feed consumed on a 22% CP diet could result in less rapid passage of feed in the intestine, compared to a diet with low CP (20%) with a high rapid passage of consumed feed. This issue could result in a decrease in nutrients utilization, which could have a negative effect on the BWG. The results of the current study suggested that CA had no contributory role in FI which was also supported by Abdel-Fattah et al. (2008) and Dehghani-Tafti and Jahanian (2016). On the contrary, Haque et al. (2010) reported that the CA had a significant effect on the FI of broiler chickens.

More notably, the outcomes of the current study underline the improvement of BW and FCR values due to the use of CA. In the same vein, Panda et al. (2009) asserted that FCR was reduced in treatments with organic acids and had no effect on FI. Moreover, Islam et al. (2011) reported that birds fed CA diets had high body weights of 2.2, 4.68, and 5.03% for the 0.25, 0.75, and 1.25% of CA groups, respectively, compared to the control birds. In addition, Nourmohammadi and Khosravinia (2015) concluded that 3% of CA in the broiler diet led to an increase in BWG around 4.16%. Lakshmi et al. (2016) announced that adding CA (2 %) to the broiler diet improved the broiler performance by 10%. Nourmohammadi and Khosravinia (2015) stated that adding CA (3%) had positive effects on BWG, FI, and FCR of broilers since it could ameliorate nutrients digestibility as a result of proper intestinal conditions. However, Biggs and Parsons (2008) found that using 3% of CA had no significant effect on the broiler's performance.

Carcass measurements

The effects of adding CA to a low protein broiler diet on the whole body chemical composition and abdominal fat percentage are shown in table 3. A significant ($p < 0.05$) increase was found in DM, fat, and abdominal fat percentage due to a low protein level, compared to the optimal CP level. On the other hand, it should be mentioned that the protein level had no significant effect on CP and ash percentage regarding the whole body. Citric acid improved the chemical composition of meat (DM, Fat, and CP %) and abdominal fat percentage compared to non-supplemented groups. The protein level and CA interactions showed significant differences ($p < 0.05$) in DM, CP, fat, and abdominal fat percentage. Citric acid added to a low protein diet significantly ($p < 0.05$) decreased fat and abdominal fat and increased CP percentage in broiler carcass.

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The negative effect of sub-optimal dietary CP on carcass composition in the present study was in agreement with the findings of studies performed by Aletor et al. (2000); Namroud et al. (2008); Sigolo et al. (2017), and Srilatha et al. (2018). Likewise, Javaid et al. (2012) construed the effect of dietary protein on meat DM through the depression of liver lipogenesis leading to a reduction in fat deposition in the muscles and subsequently a reduction in the DM of the meat. Furthermore, the negative correlation between protein levels and fat deposition could initially be due to the increase in feed consumption for birds to meet growth requirements, as a response to any nutrient deficiency (Kamran, 2010) could increase fat deposition (Gous et al., 1990). Second, high Metabolic Energy to CP ratio in a low protein diet promotes lipogenesis (Sigolo et al., 2017) because of the more energy intake per unit of CP intake (Srilatha et al., 2018). Third, the increase of abdominal fat by low CP diets could be due to the reducing in the heat increment which demands the transamination and deamination of superfluous amino acids. Therefore, higher energy was deposited as abdominal fat (Namroud et al., 2008 and Allameh and Toghyani, 2019).

In agreement with our results, Abdel-Fattah et al. (2008) found that 3% of CA dietary supplementation could significantly decrease the abdominal fat. Furthermore, Samanta et al. (2008) stated that the mixture of organic acids induced non-significant increase in meat CP and a decrease in meat fat. However, Dehghani-Tafti and Jahanian (2016) reported that organic acids had no significant effect on the abdominal fat deposition. The effects of CA on the percentage of fat and abdominal fat could be due to the role of dietary organic acids in inhibiting glycolysis and stimulating glycogenesis (Sabour et al., 2019).

Table 4 summarized the effects of adding CA to a low protein broiler diet on the percentage of dressing and the relative weight of the lymphoid organs (Bursa and Spleen). Protein levels had no significant effect on the relative weight of lymphoid organs. However, the low protein level decreased the dressing (from 74.93% for optimal protein levels to 73.86% for low protein levels). The percentage of dressing and relative weight of bursa was significantly higher in groups supplemented with CA. The results showed that adding CA to a low protein diet improved the percentage of dressing percentage and had no significant effect on the relative weight of bursa and spleen. The highest value for the percentage of dressing (75.86%) was recorded by a diet containing 100% of required CP + CA while the lowest value (73%) was for the group fed on 90% of required CP- CA. The addition of CA to a low protein diet had improved the percentage of dressing to be approximately the same as the control group. The negative effects of the low protein diets on dressing percentage in the present study supported by Selim (2015) and Dehghani-Tafti and Jahanian (2016), who found that reducing dietary CP levels decreased the carcass yield of chickens.

The percentage of dressing was significantly higher in CA groups. The findings of the current study are in line with the results of the previous studies (Islam et al., 2011; Dehghani-Tafti and Jahanian, 2016 and Sultan et al., 2018). In the current study, the results showed that the relative weight of bursa of Fabricius and the spleen are not affected by low protein diets. However, CA resulted in the improved relative weight of bursa of Fabricius, there were no differences regarding the spleen percentage. The spleen and bursa of Fabricius are a part of the immunity system (Abdel-Fattah et al., 2008). An immunological response could be expected via the improved in relative lymphoid organs (Katanbaf et al., 1989). The lack of protein levels effect on lymphoid organs was agreed with Golian et al. (2010), while Jahanian (2009) and Barekatain et al. (2019) found that reducing dietary CP had decreased the weights of lymphoid organs.

Intestinal morphology

The effect of adding CA to a low protein broiler diets on VH, VW, CD, VH/CD ratio, and absorption area is presented in table 5. The current study showed that low dietary protein had a significant effect on the intestinal morphology. The decrease in dietary CP content significantly decreased VH, VW, and VW/CD, and absorption area, compared to an optimal protein level. Citric acid addition improved ($p \leq 0.05$) VH, VH/CD, and increased absorption surface. The interaction between protein level and CA showed that the chickens' group fed on CP 100 % + CA had the highest values for VH, VW, VH/CD, and absorption area, while CP 90% - CA recorded the lowest values. The intestinal morphology parameters of the chickens' group fed low dietary protein (CP90) rose to a comparable level to that of the required protein fed (CP100) when CA was included.

Khosravinia et al. (2015) suggested that the increase in digestion and absorption was correlated with the increase in the VH/CD ratio. As a result, the ratio was a marker of the digestive capacity of the small intestine (Allameh and Toghyani, 2019).

The decrease in dietary CP content caused a significant decrease in VH, VW, VW/CD, and absorption area, which lend support to a study conducted by Mahmoud et al. (2017) reporting a slight decrease in the VH of broilers fed with 98% of required CP, compared to 100% of required CP. Moreover**,** Allameh and Toghyani (2019) stated that the reduction of dietary CP (85% of required CP) resulted in a negative impact on VH in the jejunum. However, Laudadio et al. (2012) found an increase in VH in the duodenum of broilers that consumed low dietary protein (20.5% CP), compared to those consumed high dietary protein (22.5% CP).

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The improvement of VH, VH/ CD, and the enlargement in villus surfaces area of the broilers fed diets supplemented with CA in the present study was also observed by Khosravinia et al. (2015), who found 3% dietary CA increased VH of the broiler. Moreover, Paul et al. (2007) and Samanta et al. (2010) stated that the use of acidifiers in the chickens' diet could increase VH. The improvement in VH resulted in an enlarged surface area for nutrients absorption and thus to higher growth performance (Mohammadagheri et al., 2016).

The amelioration of body gain and FCR by dietary supplementation with CA could be due to the improvement in VH and enlarged absorptive surface area (Dehghani and Jahanian, 2012). The positive influence of organic acids on VH and VH/ CD ratios suggested that CA might improve the rate of nutrient absorption (Nourmohammadi and Afzali, 2013).

Bacteriological analysis of caecal content

The effect of adding CA to a low protein broiler diets on the cecum microbial counts (log^{-1} CFU/g), including *Lactobacillus*, *E. coli*, and *Clostridium perfringens* is shown in table 6. Dietary protein level had no significant effect on cecum microbial counts. However, CA addition had significantly improved *Lactobacillus* counts and reduced *E. coli* and *Clostridium perfringens* counts. The interaction between protein and CA showed significant differences in the bacterial populations (*Lactobacillus*, *Escherichia coli*, and *Clostridium perfringens*). Citric acid caused a significant increase in *Lactobacillus*, compared to non-supplemented groups. Furthermore, smaller numbers of *E- coil* and *Clostridium perfringens* were attributed to CA addition.

The obtained results of the current study addressing the effect of the protein levels on cecum microbial counts were in line with the findings of a study carried out by Ravangard et al. (2017), in which they reported that there were no significant differences in *Lactobacillus* and *Escherichia* bacteria counts in broiler chickens fed on the either low or required dietary protein levels.

Citric acid supplementation resulted in a significant improvement in *Lactobacillus* counts and reduction in *E. coli* and *Clostridium perfringens* counts, which resemble the results of EL-Afifi et al. (2001) and Ghazalah et al. (2011). Furthermore**,** Ahmad et al. (2018) found that CA (40g/kg) increased beneficial (*Lactobacillus*) bacteria, while reduced *E. coli* count, compared to the control group. *Lactobacillaceae* have an important role in host metabolism (Zhu et al., 2015). Similar to the findings of the current study, Cesare et al. (2019) observed a positive relation between *Lactobacillaceae* and amelioration of FCR.

Blood Parameters

The effect of adding CA and dietary protein level on blood components of broilers is presented in table 7. The protein level showed no significant effect on glucose, uric acid (UA), and AST, while the low protein level led to a decrease ($p < 0.05$) in albumin, total protein, and increase in hemoglobin A1c, fructosamine (glycation indicators), and cholesterol. Citric acid addition was increased albumin and decreased hemoglobin A1c, fructosamine, cholesterol, and uric acid. The results showed that the interaction between CP level and CA had no effect on blood glucose, hemoglobin, UA, and AST. The optimal protein level groups recorded the highest values ($p < 0.05$) for total protein, albumin, and globulin, and lowest values for hemoglobin A1C, fructosamine, and cholesterol.

The results of the present study related to the effect of the CP level on the blood parameters were in accordance with the findings of Swennen et al. (2005), Kamran et al. (2010), and Mohamed et al. (2012) concluding that the level of dietary protein did not alter glucose concentration. In addition, Alam et al. (2004) and Mohamed et al. (2012) indicated that the dietary protein level had no significant effect on the haemoglobin concentration of broilers. In addition, Mohamed et al. (2012) stated that the dietary protein caused only the globin section of the haemoglobin to increase with no effect on the haem section.

A low level of albumin is related to the glycation of plasma proteins and HbA1c (Bhonsle et al., 2012). According to Honma et al. (2017), protein levels in chicken diets could modify blood albumin levels and low dietary protein could increase the glycated amino acids in chicken plasma. Haemoglobin A1c reflects non-enzymatic glycosylation and fructosamine examined for glycation, associated primarily with albumin (Anguizola et al., 2013). The *In vivo* experiment conducted by Bhonsle et al. (2012) indicated that blood albumin could control the plasma protein glycation. Moreover, Tiwari et al. (2015) found an inverse correlation between plasma albumin and HbA1c.

The present results for CA were consistent with those obtained by Abdel-Fattah et al. (2008) indicating that dietary acidifiers (e.g. CA, acetic acid, and lactic acid) significantly decreased total lipids, and cholesterol, in the blood.

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Furthermore, Al-Saad et al. (2014) reported that there was no effect of organic acid in the blood hemoglobin and glucose. Ur Rehman et al. (2016) found that organic acids had no significant effect on the hemoglobin of broiler chickens. In the same vein, Nosrati et al. (2017) concluded that organic acid had no effect on the blood total protein and glucose of the broiler.

The positive effects of dietary protein and CA on the glycation reaction may be due to its effects on albumin levels. Moreover, the dietary protein might alter plasma amino compounds (Honma et al., 2017). In the study performed by Ali et al. (2013), it was found that there was a correlation between intestinal microflora and HbA1c, which could explain the effect of CA on HbA1c through its effect on the intestinal microflora. More studies are needed to clarify the correlation between CA, intestinal microflora, and glycation in poultry.

Table 2. The effect of low protein broiler diets supplemented with citric acid on broilers' performance during 1-42 days of age at the Poultry Research Unit, Giza, Egypt.

Treatments		Parameters		
CP	**CA**	**Feed intake (g/chicken)**	**BW gain (g/chicken)**	**FCR (g feed: g gain)**
Main effect of CP				
100%	-----	3631^{b}	2376^{a}	1.53^{b}
90%	-----	3682^{a}	2278^{b}	1.62^{a}
Main effect of CA				
-----	0%	3655	2280^{b}	1.60^{a}
-----	2%	3658	2375^{a}	1.54^{b}
CP×CA effect				
100%	0%	3629^{b}	2314^{b}	1.57^{b}
100%	2%	3633^{b}	2438^{a}	1.49^{c}
90%	0%	3681^{a}	2245^{c}	1.64^{a}
90%	2%	3683^{a}	2312^{b}	1.59^{b}
SEM		7.57	16.43	0.01
p-value				
CP		<0.0001	0.0009	<0.0001
CA		0.38	0.001	0.001
CP×CA		<0.0001	<0.0001	<0.0001

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. The beginning weight was 40g. BWG: Body weight gain, FCR: Feed conversion ratio, CP: Crude protein, CA: Citric acid, SEM: Standard error of the mean.

Table 3. The effect of low protein broiler diets supplemented with citric acid on broilers' whole body composition and abdominal fat percentage at 42 days of age at the Poultry Research Unit, Giza, Egypt.

Treatments		Parameters				
CP	**CA**	**Whole-body composition (%)**				**Abdominal fat (%)**
		Dry matter	**Fat**	**Protein**	**Ash**	
Main effect of CP						
100%	---------	27.05^{b}	7.06^{b}	22.55	4.13	1.16^{b}
90%	---------	27.76^{a}	8.26^{a}	22.39	4.05	1.39^{a}
Main effect of CA						
---------	0.00	27.11^{b}	8.57^{a}	21.89^{b}	4.11	1.43^{a}
---------	2.00	27.70^{a}	6.75^{b}	23.05^{a}	4.08	1.13^{b}
CP×CA effect						
100%	0%	26.50^{b}	7.60^{b}	21.83^{c}	4.29	1.32^{b}
100%	2%	27.59^{a}	6.52^{c}	23.27^{a}	3.97	1.00^{d}
90%	0%	27.71^{a}	9.54^{a}	21.95^{c}	3.92	1.53^{a}
90%	2%	27.80^{a}	6.98^{cb}	22.82^{b}	4.18	1.25^{c}
SEM		0.12	0.98	0.43	0.01	0.03
p-value						
CP		0.002	0.02	0.78	0.64	0.0001
CA		0.01	0.0002	0.0011	0.79	<0.0001
CP×CA		<0.0001	<0.0001	<0.0001	0.38	<0.0001

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. CP: Crude protein, CA: Citric acid, SEM: Standard error of the mean

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Table 4. The effect of low protein broiler diets supplemented with citric acid on the percentage of dressing and relative weight of lymphoid organs at 42 days of age at the Poultry Research Unit, Giza, Egypt.

Treatments		Parameters		
CP	CA	Dressing (%)	Relative weight of lymphoid organs (%)	
			Spleen	Bursa
Main effect of CP				
100%	----------	74.93^{a}	0.14	0.22
90%	----------	73.86^{b}	0.13	0.21
Main effect of CA				
----------	0.00	73.50^{b}	0.12	0.19^{b}
----------	2%	75.29^{a}	0.14	0.24^{a}
CP×CA effect				
100%	0%	74.00^{b}	0.13	0.20
100%	2%	75.86^{a}	0.14	0.24
90%	0%	73.00^{c}	0.11	0.18
90%	2%	74.72^{b}	0.14	0.23
SEM		0.22	0.01	0.01
P-value				
CP		0.01	0.10	0.80
CA		<0.0001	0.13	0.007
CP×CA		<0.0001	0.50	0.058

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. CP: Crude Protein, CA: Citric Acid, SEM: Standard Error of the Mean

Table 5. The effect of low protein broiler diets supplemented with citric acid on intestinal morphology at 42 days of age at the Poultry Research Unit, Giza, Egypt.

Treatments		Parameters				
CP	CA	Villus height (μm)	Villus Width (μm)	Crypt depth (μm)	VH/CD	Absorption area/1000
Main effect of CP						
100%	--------	1905^{a}	157.33^{a}	152.13	12.57^{a}	300.00^{a}
90%	--------	1759^{b}	132.72^{b}	153.46	11.58^{b}	233.45^{b}
Main effect of CA						
--------	0.00	1770^{b}	142.56	154.89	11.53^{b}	252.30^{b}
--------	2%	1894^{a}	147.49	150.70	12.62^{a}	279.39^{a}
CP×CA effect						
100%	0%	1858^{b}	155.31^{a}	151.74	12.37^{a}	288.56^{b}
100%	2%	1952^{a}	159.34^{a}	152.51	12.77^{a}	311.03^{a}
90%	0%	1682^{c}	129.80^{b}	158.04	10.56^{b}	218.30^{d}
90%	2%	1835^{b}	135.64^{b}	148.88	12.47^{a}	248.90^{c}
SEM		14.53	2.09	1.5	0.18	5.48
p-value						
CP		<0.0001	<0.0001	0.67	0.004	0.01
CA		0.002	0.22	0.17	0.001	<0.0001
CP×CA		<0.0001	<0.0001	0.21	<0.0001	<0.0001

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. VH: Villus height, CD: Crypt depth, CP: Crude protein, CA: Citric acid, SEM: Standard error of the mean.

Table 6. The effect of low protein broiler diets supplemented with citric acid on caecal bacterial counts

Treatments		Parameters		
CP	CA	*Lactobacillus*	*Escherichia coli*	*Clostridium perfringens*
Main effect of CP				
100%	--------	3.49	7.34	11.32
90%	--------	3.36	7.59	10.92
Main effect of CA				
--------	0.00	3.16^{b}	7.66^{a}	11.54^{a}
--------	2%	3.71^{a}	7.28^{b}	10.71^{b}
CP×CA effect				
100%	0%	3.17^{c}	7.55^{ab}	11.63^{a}
100%	2%	3.82^{a}	7.14^{c}	11.02^{c}
90%	0%	3.14^{c}	7.77^{a}	11.45^{b}
90%	2%	3.59^{b}	7.42^{bc}	10.39^{d}
SEM		0.09	0.08	0.14
p-value				
CP		0.46	0.12	0.17
CA		<0.0001	0.009	0.0003
CP×CA		<0.0001	0.01	<0.0001

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. CP: Crude Protein, CA: Citric Acid, SEM: Standard Error of the Mean

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Table 7. The effect of low protein broiler diets supplemented with citric acid on blood parameters at 42 days of age

Treatments		**Parameters**										
CP	**CA**	**Glu. (mg/dl)**	**TP (g/dl)**	**Alb. (g/dl)**	**Glob. (g/dl)**	**Hgb (g/dl)**	**HbA1c**	**FA.**	**TC (mg/dl)**	**UA (mg/dl)**	**AST (u/l)**	**ALT (u/l)**
Main effect of CP												
100%	--------	241.98	4.37^{a}	1.55^{a}	2.81^{a}	8.47	3.31^{b}	100.35^{b}	121.01^{b}	3.98	163.33	40.00^{a}
90%	--------	241.23	3.59^{b}	1.35^{b}	2.24^{b}	8.38	3.71^{a}	123.87^{a}	132.66^{a}	3.97	167.50	30.50^{b}
Main effect of CA												
--------	0.00	240.53	3.80	1.35^{b}	2.45	8.45	3.72^{a}	123.87^{a}	134.33^{a}	4.19^{a}	167.50	33.50
--------	2%	242.68	4.16	1.55^{a}	2.60	8.41	3.32^{b}	100.35^{b}	119.35^{b}	3.76^{b}	163.50	37.00
CP×CA effect												
100%	0%	241.53	4.17^{ab}	1.45^{b}	2.72^{ab}	8.63	3.50^{b}	111.13^{b}	129.33^{b}	4.16	167.00	37.00^{ab}
100%	2%	242.43	4.57^{a}	1.65^{a}	2.92^{a}	8.32	3.13^{c}	89.57^{c}	112.70^{c}	3.80	159.67	43.00^{a}
90%	0%	239.53	3.43^{c}	1.25^{c}	2.18^{b}	8.27	3.93^{a}	136.61^{a}	139.33^{a}	4.23	168.00	30.00^{b}
90%	2%	242.93	3.75^{bc}	1.45^{b}	2.30^{b}	8.50	3.50^{b}	111.13^{b}	126.00^{b}	3.73	167.33	31.00^{b}
SEM		2.7	0.13	0.40	0.11	0.07	0.09	5.06	2.53	0.09	7.99	1.88
P-value												
CP		0.80	0.0004	0.01	0.004	0.50	0.01	0.01	0.015	0.69	0.89	0.006
CA		0.67	0.17	0.01	0.40	0.77	0.01	0.01	0.0006	0.006	0.12	0.37
CP×CA		0.17	0.0008	0.001	0.038	0.18	0.001	0.001	0.0001	0.06	0.18	0.03

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. CP: Crude Protein, CA: Citric Acid, Glu: Glucose, TP: Total Protein, Alb: Albumin, Glob: Globulin, Hgb: Hemoglobin, HbA1c: Hemoglobin A1c, FA: Fructosamine, TC: Total Cholesterol, UA: Uric Acid, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SEM: Standard error of the mean.

CONCLUSION

Broiler chickens fed a low protein diet supplemented with citric acid addition had the same performance as those with the required protein. The beneficial effect of citric acid on nutrients utilization, growth performance, and carcass may be due to the effect of citric acid on intestinal villus height, absorption area, cecal microbial content, and blood chemistry.

DECLARATIONS

Authors' contribution

All authors have equally contributed to the design of the study, data collection, data analysis writing, and revision of the manuscript. In addition, all the authors approved and agreed to the publication of the manuscript.

Competing interests

The authors have declared no competing interest.

Acknowledgements

The authors would like to appreciate the Deanship of Regional Center for food and feed, Agricultural Research Center, Giza, Egypt, for funding this study.

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