

Dietary citric acid enhances growth performance, nutrient digestibility, intestinal microbiota, antioxidant status, and immunity of Japanese quails

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ABSTRACT A total of 300 un-sexed Japanese quail chicks (1-wk-old) were randomly allotted to 5 experimental groups to study the effect of citric acid (CA) on performance development, carcass estimates, blood measurements, antioxidant and immune measurements, digestive enzymes, and cecum microbiology traits of growing Japanese quail. The chicks were fed a basal diet supplemented with CA (5, 10, 15, and 20 g/kg in diet) had significantly ($P < 0.05$) greater live body weights at 3 and 5 wk of age and increased weight gain across all experimental periods (1–3, 3–5 and 1–5 wks of age) compared to the control group. No significant difference was found in any of the measured carcass traits. The digestion coefficients of crude protein, ether extract, crude fiber, and nitrogen free extract as well as

metabolizable energy significantly improved ($P < 0.05$) in all treatment groups compared to the control. CA supplementation from 10 to 20 g/kg had increased digestive enzyme activities (amylase and lipase). All treatment groups had higher ($P < 0.05$) albumin and globulin concentrations than the control group. A significant ($P < 0.05$) decrease in phosphorus (P) concentrations in the plasma was observed in all treatment groups. The IgG levels were greater ($P < 0.05$) in the 5 or 10 g/kg groups than the control group. Chicks fed CA at different levels had significantly decreased caecal content of TBC, *coliform*, *E. coli*, and *Salmonella*. We concluded that the inclusion of CA (especially 10 g/kg diet) in growing Japanese quail diets improved growth performance, immune response, and health.

Key words: citric acid, performance, digestive enzyme, blood, quail

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INTRODUCTION

Widespread Japanese quail breeding in developing countries may help overcome the current gap in meat supply (Minvielle 2004; Alagawany et al., 2021a; Reda et al., 2021). Quails are economically important as a source of meat and eggs, but quail production has not yet reached its full potential in the poultry industry (Genchev et al., 2008). The growth period of quail life is an important phase in realizing the long-term great performance (Elnesr et al., 2019). The use of feed additives has been an essential part of poultry production success and could be beneficial in quail diets. Familiar poultry diet supplements include antimicrobials, antioxidants, emulsifiers, binders, pH control agents, and coenzymes (Yilmaz et al., 2006; Alagawany et al., 2018). There is a widespread use of antibiotic alternatives such as organic acids, probiotics, and medicinal plants; despite their

higher price, these products are highly supported by consumers (Ipu et al., 2006; Abd El-Hack et al., 2020a; Elgeddawy et al., 2020; Elnesr et al., 2020). The poultry industry is dependent on antibiotics as a feed supplement to improve growth and feed efficiency, as well as for better control of pathogenic microorganisms (Islam et al., 2008; Sultan et al., 2015). The overuse of antibiotics has led to the development of antibiotic-resistant bacteria, and has been shown to cause residual effects in poultry, which has resulted in the ban of antibiotics in many countries. Functional feed additives are promising natural alternatives to antibiotics (Abd El-Hack et al., 2020b; Alagawany et al., 2020a; Rehman et al., 2020;).

Organic acids play a major role in enhancing profitability and are safe for use in poultry production. In fact, organic acids are routinely incorporated into the monogastric animal's diet in Europe as acidifiers and preservatives to replace antibiotics as promoters of growth and to prevent or control pathogens (Sugiharto, 2016; Alagawany et al., 2020b). Furthermore, organic acids reduce microorganism growth in the gastrointestinal tract, change intestinal pH, and enhance feed utilization (Skinner et al., 1991; Adams, 1999). This study investigates the impact of citric acid (CA)

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supplementation on performance, carcass traits, blood measurements, antioxidant and immune parameters, digestive enzymes, and cecum microbiology traits of growing Japanese quail.

MATERIAL AND METHODS

A total of 300 un-sexed Japanese quail chicks (1-wk-old) were randomly allotted to 5 treatment groups of 60 birds with 5 replications. The first group was fed a basal diet and served as the control group. The remaining groups were fed the control diet supplemented with 5, 10, 15, and 20 g/kg CA. The basal diet covered the nutritional demands of broiler chickens during the growth period (d 1–35) as recommended by [NRC \(1994\)](#), and its structure and chemical composition are presented in [Table 1](#). All chicks were floor brooded, healthy and bred in identical conditions. Diet and water were supplied ad-libitum. Day light except an hour was preserved during the whole experimental period. The experimental procedures were performed according to the Local Experimental Animal Care Committee, Zagazig University.

Growth and Carcass Traits

Individual quail weights were recorded at 1, 3, and 5 wk of age to calculate the body weight (g) and weight gain (g/day) during the experimental period. Feed intake (g) was recorded and converted to g feed/g gain throughout the experiment. At 35 d, 5 birds were

Table 1. Ingredients and nutrient contents of basal diet of growing Japanese quail.

Items	(g/kg)
Ingredient	
Maize 8.5%	518.0
Soybean meal 44%	367.0
Maize gluten meal 62%	52.1
Soybean oil	29.0
Limestone	7.0
Di-calcium phosphate	16.5
Salt	3.0
Premix ¹	3.0
L-Lysine	1.3
DL-Methionine	1.1
Choline chloride	2.0
Total	1000
Calculated composition ²	
Metabolizable energy (MJ/kg)	12.53
Crude protein (g/kg)	240.0
Calcium (g/kg)	8.0
Nonphytate phosphorus (g/kg)	4.5
Lysine (g/kg)	13.0
Total sulphur amino acids (g/kg)	9.2

¹Provides per kg of diet: Vitamin A, 12,000 I.U.; Vitamin D3, 5000 I.U.; Vitamin E, 130.0 mg; Vitamin K3, 3.605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 4.950 mg; Vitamin B12, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18.333 mg; Folic acid, 2.083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.

²Calculated according to [NRC \(1994\)](#).

randomly selected from each group and were slaughtered for carcass assessments. Also, the pH of cecal contents was estimated using a digital pH meter (Model 507; Crison Instruments S.A., Barcelona, Spain) ([Reda et al., 2020a,b](#)).

Digestibility

Ten additional, 5-wk-old quails were obtained to determine the digestibility of each dietary alimentary and to determine the metabolizable energy (ME). These quails were housed in individual cages and fed competent experimental rations. The chicks were allowed to acclimate for 3 d, then the feed consumption was recorded and feces were collected every 24 h for 5 d. Once collected, all quail feathers were removed from the excreta, and the cleaned samples were weighed and dried in ovens at 70°C for 36 h. Diet and fecal analyses were carried out directly according to [AOAC \(2006\)](#). Nutritive values were calculated as ME. The ME was 4.2 Kcal per gram total digestible nutrients (TDN).

Blood Parameters

At the end of the experiment, 10 birds were chosen randomly from each group, then weighed and slaughtered to collect blood samples. Blood samples were collected in test tubes using EDTA to investigate the possible effect of the treatments on blood. The test tubes were sealed with rubber stoppers then gently shook to mix the anticoagulants and the blood. Afterward, leukocytes (WBCs; $\times 10^3/\text{mm}^3$), lymphocytes (%), monocytes (%), and heterocytes (%) were estimated per [Campbell \(1988\)](#).

Whole blood was centrifuged at $3,000 \times g$ for 20 min to obtain isolated plasma, and then stored at -20°C until analyzed. The Biuret method of [Armstrong and Carr \(1964\)](#) was used to determine total protein (g/dL). Albumin concentrations (g/dL) were calorimetrically estimated according to [Hill \(1985\)](#). Albumin concentrations were subtracted from the total protein concentrations to obtain the globulin concentrations (g/dL). Triglycerides and total cholesterol were determined according to [Allain et al. \(1974\)](#). The method described by [Myers et al. \(1994\)](#) was used to estimate high-intensity lipoprotein levels (HDL). Low-intensity lipoprotein (LDL) levels were determined according to [Friedewald et al. \(1972\)](#). Plasma mineral concentrations (Ca and P) were estimated according to [Reitman and Frankel \(1957\)](#). Liver enzyme activities (alanine-aminotransferase [ALT] and aspartate-aminotransferase [AST]) were also determined using commercial kits. Amylase enzyme activities were determined according to [Somogyi \(1960\)](#), while, lipase enzyme activity was determined per [Tietz and Fiereck \(1966\)](#). The method by [Lynn and Clevette-Radford \(1984\)](#) was used to measure the activity of protease enzymes. Total antioxidant capacity (TAC) was determined according to [Koracevic et al. \(2001\)](#). The spectrophotometric method

per Nishikimi et al. (1972) was used to determine superoxide dismutase (SOD). The method described by Mihara and Uohiyama (1978) was used to measure malondialdehyde (MDA). The method described by Bianchi et al. (1995) was used to estimate gamma immunoglobulin (IgG).

Microbiology Traits

Total anaerobic count, aerobic plate counts (APC), and total coliform counts were carried out according to the American Public Health Association (Reda et al., 2020a,b).

Statistical Analysis

Data were analyzed using SAS (SAS 2001) and a completely randomized design. The significance level was set at ($P < 0.05$) according to the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$ where Y_{ij} = the observation, μ = the overall mean, T_i = the treatment effect and e_{ij} = random error. All data (were analysed with one-way ANOVA using the Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Growth Performance

The averages of growth performance traits including live body weight (LBW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) are listed in Table 2. The chicks fed the CA-supplemented diet had significantly ($P < 0.05$) higher LBWs at 3 and 5 wks of age, as well as higher BWG through all experimental periods (1–3, 3–5, and 1–5 wks of age) compared to the control. CA-supplemented birds had lower FI and better FCR during different intervals. The addition of CA at 10 g/kg may be the preferable supplementation level, as appeared from the given growth performance results. In general, LBW, BWG, and FCR

were improved in all treatment groups, and all treatment groups consumed less food across all experimental periods.

These results concur with those of previous studies. Abd-El-Hlim et al. (2018), who reported that broiler chicks fed the recommended amount of protein + 1.5% CA diets, had improved ($P < 0.05$) body weights during the starter phase. ELnaggar and Abo EL-Maaty, 2017 showed that ducklings fed 2 or 3% CA-supplemented diets had significantly greater LBW, BWG, and FCR compared to the control. Nourmohammadi and Khosravinia (2015) demonstrated that weight gain and daily FI were significantly improved in broiler chicks supplemented with 30 g CA/kg, but repressed when CA was increased to 60 g/kg. In another study, 1.5% commercial acetic acid improved broiler performance, but increased CA levels of up to 3% resulted in no further improvements (Abdel Fattah et al., 2008). In contrast to the above data, PinWu and Chen (2016) and Abd-El-Hlim et al. (2018) demonstrated that diets supplemented with 1.0% CA did not change turkey growth performance. The growth parameters of broiler chicks were also not significantly influenced by dietary 2.0 to 4.0% CA (Rafacz-Livingston et al., 2005). The improvements in growth performance seen in this study were likely due to the decrease in the pH of gut content, CA's positive impact on gut morphometry and size, as well as enhanced nutrient digestion (Rehman et al., 2016).

Digestion Coefficients and Digestive Enzyme

Improved ($P < 0.05$) digestion coefficients for NFE, EE, CP, CF, and ME were found in all treatment groups compared to the control (Table 3). A similar trend was seen in another study, in which the inclusion of organic acids improved digestibility coefficients of CP, CF, and EE (Ndelekwute et al., 2019). In this study, the impact of CA dietary supplementation on digestive enzyme activity is presented in Table 3. Birds fed 10 to 20 g/kg CA recorded higher digestive enzyme activities (amylase

Table 2. Growth performance of broiler chicks as affected by different levels of bile salts.

Items	Citric acid level (g/kg diet)					SEM	P value
	0	5	10	15	20		
Body weight (g)							
wk 1	30.46	30.48	30.37	30.41	30.48	0.097	0.9099
wk 3	109.00 ^d	123.44 ^{ab}	124.50 ^a	118.00 ^c	121.34 ^b	0.655	<0.0001
wk 5	189.33 ^d	194.00 ^c	210.72 ^a	207.21 ^b	196.33 ^c	0.835	<0.0001
Body weight gain (g / day)							
1–3 wk	5.61 ^d	6.64 ^{ab}	6.73 ^a	6.25 ^c	6.49 ^b	0.047	<0.0001
3–5 wk	5.74 ^b	5.04 ^d	6.16 ^a	6.37 ^a	5.36 ^c	0.072	<0.0001
1–5 wk	5.67 ^d	5.84 ^c	6.44 ^a	6.31 ^b	5.92 ^c	0.031	<0.0001
Feed intake (g / day)							
1–3 wk	14.75 ^a	13.81 ^{bc}	13.71 ^c	13.10 ^d	14.25 ^b	0.148	0.0002
3–5 wk	26.08 ^a	22.63 ^d	25.21 ^b	26.29 ^a	23.67 ^c	0.076	<0.0001
1–5 wk	20.42 ^a	18.22 ^d	19.46 ^b	19.69 ^b	18.96 ^c	0.113	<0.0001
Feed conversion ratio (g feed/ g gain)							
1–3 wk	2.63 ^a	2.08 ^c	2.04 ^c	2.09 ^c	2.19 ^b	0.015	<0.0001
3–5 wk	4.55 ^a	4.49 ^a	4.09 ^b	4.13 ^b	4.42 ^a	0.072	0.0071
1–5 wk	3.60 ^a	3.12 ^{bc}	3.02 ^c	3.12 ^{bc}	3.20 ^b	0.031	<0.0001

Means in the same row with no superscript letters or with a common superscript letter are not significantly different ($P < 0.05$).

Table 3. Digestion coefficient and nutritive values affected by different levels of citric acid.

Items ¹	Citric acid level (g/kg diet)					SEM	P value
	0	5	10	15	20		
CP	85.26 ^d	86.11 ^c	88.41 ^a	87.54 ^b	87.15 ^b	0.162	<0.0001
EE	70.60 ^c	74.46 ^b	78.21 ^a	77.46 ^a	74.85 ^b	0.603	<0.0001
CF	24.75 ^c	28.43 ^a	26.89 ^b	26.17 ^b	27.04 ^b	0.418	0.0016
NFE	77.83 ^d	79.62 ^{cd}	83.70 ^a	81.30 ^{bc}	82.47 ^{ab}	0.621	0.0005
ME	3021 ^c	3110 ^{bc}	3306 ^a	3169 ^b	3167 ^b	30.01	0.0040
Digestive enzymes (unit)							
Amylase	12.60 ^c	13.45 ^c	15.45 ^b	18.10 ^a	17.87 ^a	0.604	0.0002
Lipase	8.50 ^b	8.03 ^b	12.00 ^a	11.50 ^a	10.00 ^{ab}	0.596	0.0037

Means in the same row with no superscript letters or with a common superscript letter are not significantly different ($P < 0.05$).

¹CF, Curd fiber; CP, Curd protein; EE, Ether extract; ME, Metabolic energy; NFE, Nitrogen free extract.

and lipase). FCR improvement may be attributed to organic acids, because the acidic anions promote the absorption of minerals like Ca, P, Mg, and Zn (Abdel-Latif et al., 2020; Pearlin et al., 2020). Diets enriched with organic acidifiers induced significant improvements in nutrient absorption by increasing the intestinal villus height in chickens (Xia et al., 2004). Diet acidifier stimulation directly impacts the gut cell proliferation linked with short chain fatty acids. Similarly, Denli et al. (2003) concluded that probiotic, antibiotic, and organic acid supplementation improved intestinal length and weight on d 42. In the same context, chymotrypsin and trypsin activities were higher in chickens who consumed rations enriched with organic acids (Liu et al., 2017). The improvement in nutrient digestibility in this study may be due to the increased digestive enzyme activities. Increased activity of intestinal digestive enzymes in birds may be an indicator for improving nutrient digestibility and increasing the productive performance (Alagawany et al., 2021b).

Carcass Trait and Cecal Content pH

The results of carcass traits and cecal content pH are listed in Table 4. Statistical analyses showed no significant changes in the measured carcass traits. Brzóška et al. (2013) had similar results; chicks fed 3, 6, and 9 g/kg CA displayed no significant change in their carcass traits. Additionally, Nourmohammadi et al. (2010) found that 3 or 6% CA supplementation had no significant effect on abdominal fat weight. In contrast, Ahsan-ul-Haq et al. (2014) concluded that the relative breast

meat and giblet weight of broiler chicks were not significantly affected by CA supplementation, but that dressing percentage was significantly improved and abdominal fat weight was reduced with increasing CA levels (0.5, 1.0, and 1.5%). Nourmohammadi and Khosravinia (2015) found that 30 and 60 g/kg CA significantly increased the relative weights of the proventriculus, gizzard, and ileum. ELnaggar and Abo EL-Maaty (2017) found that broiler chick fed 2 or 3% CA had significantly enhanced dressing and relative weight of the total edible parts, but decreased abdominal fat compared to the control.

Caecal content pH values were reduced ($P < 0.05$) in the treatment groups compared to the control (Table 4). Similar results were found in broiler chicks supplemented at 30 or 60 g/kg CA significantly ($P < 0.01$) reduced the pH value of gut segment contents (Nourmohammadi and Khosravinia, 2015).

Hematological Parameters

The hematological parameters are presented in Table 5. The concentration of WBCs, MID and HCT % significantly ($P < 0.05$) increased, while significant decreases in the concentration of LYM % were found in birds fed 5, 10, and 15 g CA/kg compared to the control and the 20 g CA/kg diet, which were relatively equal. ELnaggar and Abo EL-Maaty (2017) found that, diets supplemented with 2.0 or 3.0% CA significantly increased RBCs, hemoglobin, PCV, MCV, MCH, WBCs, lymphocytes, and monocytes compared to the control group. Moreover, ducks fed diets supplemented

Table 4. Relative weights of carcass traits as affected by different level of bile salts.

Items	Citric acid level (g/kg diet)					SEM	P value
	0	5	10	15	20		
Carcass %	77.65	76.07	77.36	75.28	74.21	1.078	0.3118
Liver %	2.21	3.07	2.48	2.94	2.96	0.248	0.1926
Gizzard %	2.01	2.48	2.60	2.61	2.11	0.226	0.3903
Heart %	0.89	0.90	0.99	0.92	0.90	0.093	0.9495
Giblets %	5.10	6.46	6.07	6.47	5.96	0.474	0.3758
Dressing %	82.75	82.53	83.43	81.74	80.18	0.940	0.2590
Caecal content pH	6.89 ^a	6.31 ^{bc}	6.16 ^c	6.25 ^{bc}	6.38 ^b	0.059	<0.0001

Means in the same row with no superscript letters or with a common superscript letter are not significantly different ($P < 0.05$).

Table 5. Hematology of Japanese quail as affected by different levels of citric acid.

Items ¹	Citric acid level (g/kg diet)					SEM	P value
	0	5	10	15	20		
WBCs (10 ³ /μL)	185.24 ^b	275.23 ^{ab}	265.09 ^a	267.86 ^a	180.77 ^b	8.603	<0.0001
LYM (%)	92.43 ^a	88.31 ^b	88.39 ^b	88.46 ^b	92.80 ^a	0.596	0.0089
MID (%)	6.90 ^b	10.49 ^a	9.66 ^a	10.26 ^a	6.67 ^b	0.389	0.0011
GRA (%)	0.62	1.21	1.95	1.27	0.53	0.204	0.1316
RBCs (10 ⁶ /μL)	2.06	2.72	1.83	3.29	2.03	0.276	0.1475
HGB (g/dL)	12.40	14.26	12.77	13.95	10.87	0.820	0.2060
HCT (%)	21.63 ^b	27.67 ^a	24.68 ^{ab}	27.95 ^a	19.88 ^b	1.288	0.0180
MCV (μm ³)	119.4	100.01	103.39	100.05	104.58	7.165	0.6454
MCH (pg)	69.23	51.62	69.63	49.95	57.35	6.560	0.4262
PLT (10 ³ /μL)	26.01	13.08	21.47	16.00	19.00	2.852	0.1251

Means in the same row with no superscript letters or with a common superscript letter are not significantly different ($P < 0.05$)

¹GRA, granulocytes; HGB, hemoglobin; HCT, hematocrit; LYM, lymphocytes; MCV, Mean corpuscular volume; MID, mid-range; MCH, Mean corpuscular hemoglobin; PLT, Platelet count; RBCs, red blood cells; WBCs, white blood cells.

with 3% CA had significantly higher RBCs, hemoglobin, PCV, and MCH than the other groups.

Blood Biochemistry

The blood plasma content, liver, and kidney measurements are given in Table 6. The CA-supplemented groups had higher ($P < 0.05$) albumin and globulin concentrations than the control group, without significant difference between them. Furthermore, groups treated with 5 g CA/kg had higher A/G ($P < 0.05$) than the other groups or the control group. No significant effect was observed on the total protein concentrations (Table 5). Our findings agree with Nourmohammadi et al. (2010), who found that the

dietary inclusion of CA (3 and 6%) had no significant effect on total protein in broiler chicks. ELnaggar and Abo EL-Maaty (2017) reported that dietary CA supplementation at 2 to 3% increased serum globulin in ducks. Contradicting results were obtained by Ghazalah et al., 2011, who reported that 1.0 to 2.0% CA significantly increased blood serum total protein. ELnaggar and Abo EL-Maaty (2017) reported that dietary supplementation of CA at 2.0 to 3.0% significantly increased serum total protein in ducks.

Liver and Kidney Measurements

The results in Table 6 revealed no significant differences among the different treatment groups or control

Table 6. Blood chemistry as affected by different levels of citric acid.

Items ¹	Citric acid level (g/kg diet)					SEM	P value
	0	5	10	15	20		
Liver and kidney functions							
TP (g/dL)	2.62 ^b	3.09 ^a	3.13 ^a	3.24 ^a	3.18 ^a	0.073	0.0027
ALB (g/dL)	1.33 ^b	1.75 ^a	1.64 ^a	1.69 ^a	1.65 ^a	0.035	0.0005
G (g/dL)	1.29 ^c	1.34 ^{bc}	1.49 ^{ab}	1.56 ^a	1.53 ^a	0.046	0.0132
AG (%)	1.03 ^b	1.30 ^a	1.10 ^b	1.09 ^b	1.07 ^b	0.030	0.0019
AST (IU/L)	185.53	172.10	167.55	153.65	163.65	8.303	0.1795
ALT (IU/L)	13.65 ^a	12.12 ^{ab}	12.25 ^{ab}	10.70 ^b	13.60 ^a	0.512	0.0260
Creatinine (mg/dL)	0.54	0.60	0.51	0.52	0.48	0.029	0.2661
Urea (mg/dL)	0.76 ^a	0.58 ^{bc}	0.66 ^{ab}	0.47 ^c	0.71 ^{ab}	0.041	0.0066
Lipid profile							
TC (mg/dL)	211.37 ^a	199.40 ^a	179.00 ^b	174.74 ^b	206.52 ^a	3.616	0.0005
TG (mg/dL)	155.06	141.17	145.24	142.45	164.73	5.030	0.1355
HDL (mg/dL)	49.03 ^c	53.98 ^{ab}	54.91 ^{ab}	56.20 ^a	51.67 ^{bc}	1.154	0.0140
LDL (mg/dL)	131.33 ^a	117.19 ^a	95.05 ^b	90.05 ^b	121.91 ^a	4.341	0.0004
VLDL (mg/dL)	31.01	28.23	29.05	28.49	32.95	1.006	0.1355
Immunity and antioxidants							
IgG (mg/dL)	0.74 ^c	1.20 ^a	0.99 ^b	0.87 ^{bc}	0.79 ^c	0.054	0.0012
IgM (mg/dL)	0.46 ^b	0.52 ^b	0.92 ^a	1.01 ^a	0.63 ^b	0.060	0.0003
GPX (ng/mL)	0.21 ^c	0.22 ^c	0.25 ^{ab}	0.26 ^a	0.23 ^{bc}	0.008	0.0131
TAC (ng/mL)	0.17 ^c	0.20 ^b	0.28 ^a	0.21 ^b	0.19 ^b	0.008	<0.0001
SOD (U/mL)	0.13 ^b	0.26 ^a	0.29 ^a	0.23 ^a	0.15 ^b	0.020	0.0014
MDA (nmol/mL)	0.45 ^a	0.36 ^{ab}	0.23 ^c	0.28 ^{bc}	0.35 ^b	0.029	0.0040
Minerals							
Ca (mg/dL)	8.84	8.67	8.64	8.12	9.58	0.311	0.1814
P (mg/dL)	5.08 ^a	4.46 ^{bc}	4.23 ^c	4.33 ^c	4.65 ^b	0.070	0.0002

Means in the same row with no superscript letters or with a common superscript letter are not significantly different ($P < 0.05$).

¹Alb, albumin, A/G, albumin/ globulin ratio; AST, aspartate aminotransferase and ALT, alanine aminotransferase; GLOB, globulin; GSH, reduced glutathione; GPX, glutathione peroxidase; IgG and M, immunoglobulin G and M; HDL, high density lipoprotein; LDL, low density lipoprotein; MDA, malondialdehyde; SOD, superoxide dismutase; TC, total cholesterol; VLDL, very low density lipoprotein; TG, triglycerides; TAC, total antioxidant capacity; TP, total protein.

group in plasma AST and creatinine concentrations, while the concentrations of plasma ALT and urea were reduced ($P < 0.05$) in CA treated groups when compared to the control. Chicks that received 15 g CA/kg recorded the lowest values of ALT and urea concentrations compared to all other groups. These results agree with [ELnaggar and Abo EL-Maaty, 2017](#) who reported that ducks fed diets supplemented with 2 to 3% CA / kg had significantly lower urea, creatinine, AST and ALT concentrations compared to the control group. The reduced plasma urea levels in CA-supplemented birds could be an indication of the birds improved protein digestion abilities and by the fact that uric acid is the major end product of protein metabolism in poultry.

Plasma Minerals

The results illustrated in [Table 6](#) show that phosphorus (P) concentrations decreased ($P < 0.05$) in the plasma of quail chicks supplemented with CA. Plasma calcium (Ca) concentrations were insignificantly influenced by CA supplementation. These results agree with [Nourmohammadi et al. \(2010\)](#) who showed that the addition of 60 g CA/kg in broiler chick diets significantly decreased P concentrations in blood compared to the control. [Nourmohammadi and Khosravinia \(2015\)](#) illustrated that 3.0 and 6.0% CA supplementation had no significant effect on plasma Ca concentrations in broiler chicks. Moreover, [Ghazalah et al., 2011](#) demonstrated that 1 to 2% CA significantly increased blood serum P. [Abdel Fattah et al., 2008](#) reported that broiler chicks fed acidified diets (acetic, citric, or tactic acid) had significantly higher serum concentrations of Ca and P. In contrast, [Islam et al. \(2012\)](#) did not find any significant effects on mineral blood profiles when used CA at 0.75%. [PinWu and Chen \(2016\)](#) found no significant differences in serum Ca and P concentrations when turkey diets were supplemented with 1.0% CA. Citric acid enhanced the utilization of P by competitively chelating Ca, a consequence of reducing the formation of insoluble Ca phytate complexes in chicks ([Snow et al., 2004](#)).

Lipid Profile

The effect of dietary CA supplementation on lipid profiles (TC, TG, HDL, LDL, and VLDL) is given in [Table 6](#). The concentrations of TC and LDL decreased

($P > 0.05$), while the concentration of HDL significantly increased in the groups supplemented with 10 or 15 g CA/kg compared to the control ([Table 6](#)). The other experimental groups (5 and 20 g CA/kg) and the control were relatively equal. These findings agree with [ELnaggar and Abo EL-Maaty, 2017](#), who found dietary CA supplementation at 2 to 3% significantly decreased serum cholesterol and LDL compared to the control group. [Kamal and Ragaa \(2014\)](#) indicated that blood cholesterol and total lipids were also significantly reduced by dietary acidifiers. The useful role of organic acids in lowering total lipid and cholesterol may due to their ability to reduce microbial intracellular pH ([Abdel Fattah et al., 2008](#)).

Immune Indices and Antioxidants

[Table 6](#) shows the effect of dietary CA supplementation on immune indices (IgG and IgM) and antioxidant status (GPx, TAC, SOD, and MDA). Significant differences were observed among the trial groups in the plasma IgG and IgM contents. IgG levels were greater ($P < 0.05$) in groups receiving 5 or 10 g CA/kg than in the control group. The other CA levels (15 and 20 g/kg) were not significantly affected. Significant ($P < 0.05$) increases were recorded in IgM values in the birds fed 10 or 15 g CA/kg diet compared to the control. The other groups were not significantly different than the control group. These results coincide with those obtained in ducklings ([ELnaggar and Abo EL-Maaty, 2017](#)), who had noticeably higher concentration of globulin and total protein compared to the control group when supplemented with 2 to 3% CA. This indicated that ducklings fed acidifiers-supplemented diets had better disease resistance and immune responses. Thus, the addition of CA may improve the immune response, since immune responses and antibodies are linked by globulin levels ([Kamal and Ragaa, 2014](#)).

With regard to antioxidant status, [Table 6](#) shows that the values of GPx, TAC, and SOD were higher in CA treated groups than in the control. In contrast, plasma MDA contents were reduced ($P < 0.05$) in all CA treatment groups compared to the control. These results agree with [ELnaggar and Abo EL-Maaty, 2017](#) who reported that 2 to 3% organic acids supplementation in duckling diets significantly decreased serum levels of

Table 7. Bacteriology as affected by different levels of citric acid.

Items	Citric acid level (g/kg diet)					SEM	P value
	0	5	10	15	20		
Cecal bacterial count (Log CFU/g)							
Total bacterial count	8.65 ^a	8.57 ^b	8.64 ^a	8.59 ^b	8.56 ^b	0.009	0.0002
Lactobacillus	7.44 ^d	7.67 ^c	8.47 ^b	8.59 ^{ab}	8.70 ^a	0.045	<0.0001
Coliform	5.47 ^a	4.37 ^b	4.39 ^b	4.34 ^b	4.30 ^b	0.041	<0.0001
<i>E. coli</i>	4.54 ^a	3.59 ^b	3.47 ^c	3.42 ^{cd}	3.37 ^d	0.024	<0.0001
Salmonella	3.54 ^a	2.48 ^c	2.39 ^c	2.40 ^c	3.09 ^b	0.044	<0.0001

Means in the same row with no superscript letters or with a common superscript letter are not significantly different ($P < 0.05$).

TAC, GSH, GPx, and SOD compared to the control group.

Bacteriological Count

The impact of dietary CA supplementation on cecal microbiota (TBC, *Lactobacillus*, *Coliform*, *E. coli*, and *Salmonella*) is shown in Table 7. It was observed that CA-supplemented chicks exhibited a significant decrease in cecal TBC, *Coliform*, *E. coli*, and *Salmonella*, while *Lactobacillus* levels were significantly increased in CA-supplemented chicks compared to the control. Our results agree with ELnaggar and Abo EL-Maaty, 2017, who reported that growing ducks supplemented with 2 to 3% CA in their basal diets had significantly lower levels of TBC, *Salmonella*, *E. coli*, and *Proteus* spp. than the control group. The lower bacteria levels observed may be due to the lower cecum pH, as suggested by Abdel Fattah et al., 2008, who reported that reduced pH is conducive to increasing favorable bacteria and limiting the growth of pathogenic bacteria, which need a relatively higher pH to grow.

CONCLUSIONS

We concluded that the inclusion of CA (especially at 10 g/kg) in growing Japanese quail diets improved their growth performance, carcass traits, nutrient digestibility, immune response, and health. Chicks fed CA at different levels exhibited significant decreases in cecal content of pathogenic bacteria including *coliform*, *E. coli*, and *Salmonella*.

DISCLOSURES

There is no conflict of interest.

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