Effects of citric acid supplementation on growth performance, intestinal morphology and microbiota, and blood parameters of geese from 1 to 28 days of age

J. J. Xue,[*] X. F. Huang,[*] Z. L. Liu,[*] Y. Chen,[*] Y. K. Zhang,[†] Y. Luo,[*] B. W. Wang,[‡] Q. G. Wang,[*,§] and C. Wang[*,§,1]

[*]*Poultry Science Institute, Chongqing Academy of Animal Sciences, Chongqing 402460, China;* [†]*College of Animal Science and Technology, Southwest University, Chongqing 402460, China;* [‡]*Department of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, China; and* [§]*Scientific Observation and Experiment Station of Livestock Equipment Engineering in Southwest, Ministry of Agriculture, Chongqing 402460, China*

ABSTRACT This study was conducted to investigate the effects of citric acid (**CA**) supplementation on growth performance, intestinal morphology, intestinal microbiota, and blood parameters of geese from 1 to 28 d of age and evaluate the optimum additional level of citric acid. A total of 180 one-day-old male goslings were randomly allotted to 5 treatment groups of 36 birds with 6 replications. The control group was fed a basal diet, and the other groups were fed the basal diet supplemented with 0.25, 0.50, 1.00, and 2.00% of citric acid, respectively. The results showed that goslings fed the diet supplemented with 1.00% CA had higher final body weight (**FBW**) and average daily gain (**ADG**) than other groups ($P < 0.05$). The CA supplementation at 0.25 to 1.00% improved the morphology of duodenum or jejunum ($P < 0.05$). The jejunal content pH value was significantly reduced with the addition of CA compared with the control group ($P < 0.05$). As citric acid levels increased, the IgA concentrations in plasma increased and then decreased, and the goslings fed 1.00% CA supplementation had the highest IgA concentrations ($P < 0.05$). The supplementation of 1.00% and 2.00% CA in diet significantly reduced the malondialdehyde (**MDA**) concentration in plasma ($P < 0.05$). No significant difference was found on some indices related to liver function in plasma ($P > 0.05$), while creatinine significantly increased by the 2.00% CA supplementation ($P < 0.05$). Besides, the higher *Coliform* level in cecal content and worse intestinal morphology were observed when CA supplementation was up to 2.00%. Hence, the dietary CA supplementation (especially 1.00%) in goslings improves the growth performance, intestinal morphology, immunity and antioxidant, while excessive CA addition may cause negative effects. According to the quadratic polynomial model, the addition of CA in diet for obtaining maximum average daily feed intake (**ADFI**) should be 1.09% (10.9 g/kg diet) for goslings from 1 to 28 d of age.

Key words: goose, citric acid, growth performance, intestinal morphology, blood parameters

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INTRODUCTION

Organic acids as an alternative to antibiotic growth promoters play an important role in enhancing profitability and are safe for use in poultry production (Adil et al., 2010; Kamal and Ragaa, 2014; Fikry et al., 2021). The use of organic acids has demonstrated positive effects in poultry production such as sanitizing feed to prevent pathogenic microorganism infections (Thompson and Hinton, 1997), improving nutrient utilization and enhancing the growth (Tollba, 2010; Islam, 2012).

Citric acid (2-hydroxy-1, 2, 3-propane-tricarboxylic acid) is a weak organic acid and a natural preservative and that is found in all animal tissues as an intermediary substance in oxidative metabolism (Abdel-Salam et al., 2014). Studies showed the addition of CA in poultry diet contributed to decreasing the counts of pathogenic intestinal bacteria (Tollba, 2010; Elnaggar and Abo El-Maaty, 2017; Fikry et al., 2021). Besides the antimicrobial activity, CA also reduce the pH of gastro-intestinal tract content (Nourmohammadi et al., 2011; Fikry et al., 2021), improve the morphometry of small intestine (Nourmohammadi and Afzali, 2013), and

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[1]Corresponding author: wangccq@foxmail.com

enhance the feed nutrients digestibility (Nourmohammadi et al., 2012; Fikry et al., 2021). Furthermore, supplementing CA in low nutrient diets may compensate for the performance losses of broilers (Das et al., 2012; Islam et al., 2021).

Goose is an important economic poultry in many countries. Its meat is rich in unsaturated fatty acids and essential fatty acids as well as low cholesterol, and provides the high-quality protein for humans (Schmid, 2011). Hence, there is a growing interest in improving worldwide goose production (Liu et al., 2020). In 2020, more than 700 million geese were used globally for meat production and about 86% of them are from China (Hou and Liu, 2021). There are many studies about the supplementation of CA in broiler, duck or quail (Nourmohammadi et al., 2012; Elnaggar and Abo El-Maaty, 2017; Fikry et al., 2021; Islam et al., 2021), but little is known in gosling. Therefore, the objective of this study was to investigate the effects of citric acid supplementation on growth performance, intestinal morphology, intestinal microbiota, and blood parameters of geese from 1 to 28 d of age and evaluate the optimum additional level of citric acid.

MATERIAL AND METHODS

Experimental Design, Birds, and Management

The experiment was approved by the Animal Care and Welfare Committee of the Chongqing Academy of Animal Science (**CAAS**), China. All geese used in this study were obtained from goose-breeding center of the CAAS.

A total of 180 one-day-old male White Yuzhou goslings were randomly allotted to 5 treatment groups with 6 replicates per group and 6 birds per replicate. All pens had similar average initial body weight (83.23 ± 0.41 g) at the start of the experiment. The control group was fed a basal diet, and the other groups were fed the basal diet supplemented with 0.25, 0.50, 1.00, and 2.00% of citric acid, respectively. Citric acid supplementation was in anhydrous form with 99.5% purity (Shandong Ensign Industry Co. Ltd., Weifang, China). Ingredient composition and nutrient content of the basal diet is shown in Table 1. And the diet provided was in a pelleted form. Geese were reared in the same house and had ad libitum access to water and pelleted feed during the entire experimental period. The temperature was kept at 31°C from 1 to 3 d of age and then decreased 1°C each 2 d until a temperature of 26°C was reached. The lighting was continuous from 1 to 7 d of age and then it was reduced gradually to 16L (light): 8D (dark).

Table 1. Composition and nutrient levels of basal diet for 1 to 28 d of age (as-fed basis).

Items	%
Ingredients	
Maize	54.5
Soybean meal	26.0
Wheat bran	15.0
Limestone	1.30
Calcium hydrogen phosphate	1.90
Sodium chloride	0.40
DL-Methionine	0.30
Tryptophan	0.10
Threonine	0.10
Choline chloride	0.10
Mineral and vitamin premix[1]	0.30
Total	100.00
Nutrient levels[2]	
Metabolizable energy (MJ/kg)	11.90
Crude protein	18.07
Calcium	1.05
Total phosphorus	0.75
Lysine	1.04
Methionine	0.46

[1]Premix provided the following per kg of diet: Cu ($CuSO_4 \cdot 5H_2O$) 8 mg; Fe ($FeSO_4 \cdot H_2O$) 96 mg; Zn ($ZnSO_4 \cdot H_2O$) 80 mg; Mn ($MnSO_4 \cdot H_2O$) 100 mg; Se (Na_2SeO_3) 0.3 mg; I (KI) 0.4 mg; Vitamin A 6000 IU; Vitamin D_3 1,500 IU; Vitamin E 10 IU; Vitamin K_3 2.4 mg; Vitamin B_1 1.5 mg; Vitamin B_2 5 mg; Vitamin B_6 3 mg; Vitamin B_{12} 0.02 mg; Pantothenic acid 10 mg; Nicotinic acid 50 mg; Folic acid 0.5 mg; Biotin 0.15 mg.

[2]Analyzed values except for metabolizable energy, lysine, and methionine.

Growth Performance and Carcass Traits

At 28 d of age, the final body weight (**FBW**), average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed/gain ratio (**F/G**) were measured on a pen basis. ADFI and F/G were corrected for mortality. Two birds were selected from each pen according to the average body weight of corresponding pen and were slaughtered for carcass assessments. The percentage of breast meat, thigh meat, abdominal fat, liver, and glandular-gizzard stomach were calculated based on live body weight.

Blood Parameters

At the end of trial, one bird per pen with a weight close to the average weight of the pen was selected and blood samples were collected from the jugular vein by using the anticoagulation vacuum tube. Whole blood was centrifuged at 3,000 × g for 20 min to separate the plasma, and were then stored at −70°C until analyzed.

Alanine amino transferase (**ALT**), aspartate amino transferase (**AST**), total protein (**TP**), albumin (**ALB**), globulin (**GLO**), creatinine (**CRE**), urea, and uric acid (**UA**) in plasma were determined as described by Liu et al. (2022b). IgA and IgG in plasma were determined by using corresponding commercial analytical ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), monitoring the change of absorbance at 450 nm with the microplate reader (SpectraMAX Plus384, Molecular Devices, San Francisco, CA) according to the manufacturer's recommendations. Plasma levels of total antioxidant capacity (**T-AOC**), malondialdehyde (**MDA**), glutathione peroxidase (**GSH-Px**), superoxide dismutase (**SOD**), and catalase (**CAT**) were determined by using commercial analytical kits according to the manufacture's recommendations (Jian Cheng Bioengineering Institute, Nanjing, China).

Intestinal Morphology

After collecting blood, the gosling was slaughtered, and excised intestinal segments. The duodenum, jejunum and ileum segments were collected and analyzed according to Liu et al (2022a). Briefly, 1 cm sections from the middle portion of the duodenum, jejunum and ileum tissues were fixed in 10% formaldehyde phosphate buffer after washed with 0.1 M phosphate buffered saline, and the fixed sections were processed, dehydrated, and embedded in paraffin wax, and sectioned at 5 μm and stained with the hematoxylin-eosin. Histological sections were examined with villus height (**VH**), crypt depth (**CD**), and muscularis thickness (**MT**), which were performed on 10 well-oriented villi chosen and 10 muscularis thicknesses from each segment, using a digital camera microscope (BA400 Digital, McAudi Industrial Group Co., Ltd., Xiamen, China) and the Motic Advanced 3.2 digital image analysis system. The ratio of villus height to crypt depth (**VH/CD**) was calculated subsequently.

Gastrointestinal Tract Content pH Value

After the selected two goslings per pen were slaughtered, the gastrointestinal tract (proventriculus, gizzard, duodenum, jejunum, ileum, and cecum) contents were separately collected. The corresponding segments contents of two birds were mixed. The pH of mixed digesta was determined with a pH meter (Mettler Toledo Inc., Shanghai, China) according to the method described by Chaveerach et al. (2004).

Microbiology and Volatile Fatty Acid

The one gram mixture of cecal contents from two goslings per pen was collected and transferred to 9 mL sterile phosphate buffer solution and well mixed, then serially diluted up to 10^7. Spread plate count method was used to count the total bacterial count (TBC), Coliforms, Staphylococcus aureus, and Lactobacillus. The TBC and Staphylococcus aureus were counted using nutrient agar and mannitol salt agar, respectively. The Coliforms were counted according to Sheiha et al. (2020) using MacConkey agar medium. The Lactobacillus were counted using MRS medium under anaerobic condition at 36°C for 48 h. The number of colony-forming units (**CFU**) of all bacteria were expressed as $\log_{10}$ colony-forming units per gram digesta.

The remaining mixture of cecal contents were immediately put into liquid nitrogen and then stored at −70°C used for the measurement of volatile fatty acid (**VFA**). The concentration of VFA was estimated using a method of Lan et al. (2021) with some modifications. 0.5 gram of cecal content was dissolved in 1 mL ultrapure water, and the supernatant was extracted after 2 centrifugations to mix with 5% formic acid. Following filtering, the levels of VFA were estimated by Gas Chromatography (Shimadzu, GC-2014C).

Statistical Analysis

Data were subjected to one-way ANOVA using the GLM procedure of SAS (SAS Institute, 2003), with pen used as the experimental unit for analysis. When difference among groups was significant ($P < 0.05$), means were compared by Duncan's multiple comparison procedure of SAS (SAS Institute, 2003).

The optimum additional level of CA was evaluated by quadratic polynomial model using nonlinear procedure of SAS (SAS Institute, 2003). The quadratic polynomial model was provided as follows:

$$y = ax^2 + bx + c$$

where y = response criteria (ADFI), x = supplemental level of CA (%), a, b, c are the coefficients of equation.

RESULTS AND DISCUSSION

Growth Performance

The effects of citric acid on the growth performance of goslings are presented in Table 2. Birds fed the diet supplemented with 1.00% CA had higher FBW and ADG than other groups ($P < 0.05$), but no significant effects were observed on ADFI and F/G ($P > 0.05$). In the present study, some results were in agreement with a previous ducklings study by ELnaggar and Abo EL-Maaty (2017), who reported that diets supplemented with 2 or 3% CA improved the FBW and ADG compared with the control group. Fikry et al. (2021) got similar results in Japanese quails that the groups fed the 5 to 20 g/kg CA-supplemented diet had higher FBW and ADG compared with the control group. It is noteworthy that the growth performance is not improved further or even repressed when the excessive CA is supplemented in diet. In current study, the FBW and ADG of group with 2.00% CA was close to the control group, while they were higher in group with 1.00% CA (Table 2). Study about broilers found that groups treated with 1.50% CA had the higher FBW at 6 wk of age and ADG

Table 2. Effects of citric acid on growth performance of geese from 1 to 28 d of age.[1]

	Citric acid level %						
Items[2]	0	0.25	0.50	1.00	2.00	SEM	P value
FBW (g/bird)	1316[b]	1290[b]	1322[b]	1385[a]	1329[b]	18.47	0.017
ADG (g/bird per day)	44.0[b]	43.1[b]	44.3[b]	46.5[a]	44.5[b]	0.661	0.018
ADFI (g/bird per day)	83.8	85.8	90.0	91.5	85.9	2.802	0.283
F/G (g/g)	1.90	1.99	2.03	1.97	1.93	0.055	0.456

[a,b]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).

[1]Each value represents the mean of 6 replicates.

[2]FBW, final body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed/gain.

from 3 to 6 wk of age than control group, but there was no further raising when the CA was up to 3.00% (Abdel-Fattah et al., 2008). Nourmohammadi and Khosravinia (2015) reported that the addition of CA at 60 g/kg in broilers resulted in the worse WG and ADFI than control group or CA at 30 g/kg. Fikry et al. (2021) also demonstrated that FBW and ADG of groups fed 15 or 20 g/kg CA in Japanese quails decreased compared with group fed 10 g/kg CA. Effects of CA on ADFI and F/G in birds at different growth stages have not been unanimous conclusion (Rafacz-Livingston et al., 2005; Centeno et al., 2007; Islam et al., 2011; Elnaggar and Abo El-Maaty, 2017). In our study, although there was no statistically significant difference, ADFI increased and then decreased as dietary CA increased, and this response provided a significant fit to a quadratic polynomial model {y (ADFI) = 83.42 + 15.22x (dietary CA) − $6.98x^2$; $R^2 = 0.9631$, $P = 0.0369$}. According to the quadratic polynomial model, the addition of CA in diet for obtaining maximum ADFI should be 1.09% (10.9 g/kg diet) for goslings from 1 to 28 d of age.

Carcass Traits

The results of the carcass traits are given in Table 3. With the increasing of citric acid levels, the proventriculus-gizzard percentage increased firstly and then decreased. The goslings fed diet supplemented with 0.50% CA had significantly greater proventriculus-gizzard percentage than the group fed basal diet or 2.00% CA supplemented diet ($P < 0.05$). No significant effects were observed on breast muscle percentage, thigh muscle percentage, abdominal fat percentage, and liver percentage ($P > 0.05$).

In our study, 0.5% CA supplementation in goslings exhibited a significant increase in proventriculus-gizzard percentage. This result was in line with studies in broilers (Nourmohammadi et al., 2010; Nourmohammadi and Khosravinia, 2015). Contrary to the present study, some researches about other poultry showed that the CA supplementation in diet had no significant effects on the relative weight of proventriculus or gizzard (Haq et al., 2014; Elnaggar and Abo El-Maaty, 2017; Fikry et al., 2021). However, Dehghani Tafti and Jahanian. (2016) found that the gizzard percentage was decreased by 2.5 g/kg CA supplementation in broilers. The CA supplementation of various levels in goslings showed no significant effects on breast meat and thigh meat percentage in the present study, which was agreement with Haq et al. (2014). Abdominal fat and liver displayed no significant change among groups in our study, which was supported by some previous studies (Ebrahimnezhad et al., 2008; Nourmohammadi et al., 2010; Fikry et al., 2021). Abdel-Fattah et al. (2008) and Haq et al. (2014) reported that the CA supplementation did not impact abdominal fat, while significantly enhanced the liver percentage. A similar trend was seen in another study about ducks (Elnaggar and Abo El-Maaty, 2017). Nevertheless, Dehghani Tafi and Jahanian (2016) found broilers fed 2.5 g/kg CA supplementation diet had lower liver percentage accompanied with the unchanged abdominal fat percentage. The disagreement mentioned above all may be due to the differences on species, age or CA supplementation levels in diet.

Table 3. Effects of citric acid on carcass traits of geese at 28 d of age.[1]

Items[2]	Citric acid level % 0	0.25	0.50	1.00	2.00	SEM	P value
Breast meat (%)	1.19	1.21	1.22	1.21	1.20	0.051	0.992
Thigh meat (%)	11.3	12.2	11.9	11.8	11.8	0.272	0.285
Abdominal fat (%)	1.16	1.19	1.26	1.12	0.96	0.108	0.314
Liver (%)	3.27	3.16	3.14	3.16	3.18	0.140	0.966
Proventriculus-gizzard (%)	6.71[bc]	7.28[ab]	7.47[a]	7.23[ab]	6.49[c]	0.237	0.024

[a,b,c]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).

[1]Each value represents the mean of 6 replicates.

[2]Calculated as a percentage of live body weight before slaughter.

Intestinal Morphology

The effects of citric acid on intestinal morphology of goslings are showed in Table 4. The groups receiving CA-supplemented diet had higher duodenum VH and VH/CD than the control group ($P < 0.05$). The jejunum VH/CD and MT were increased by supplementing the 1.00% CA ($P < 0.05$). However, the 2.00% CA group had the lower duodenum VH/CD and MT and jejunum MT than the 1.00% CA group ($P < 0.05$). There were no significantly changes in ileum VH, CD, VH/CD, and MT ($P > 0.05$).

The present results indicated that the CA supplementation promoted the development of small intestine,

Table 4. Effects of citric acid on intestinal morphology of geese at 28 d of age.[1]

Items[2]	Citric acid level % 0	0.25	0.50	1.00	2.00	SEM	P value
Duodenum							
VH (μm)	826.1[b]	962.5[a]	965.9[a]	1024.5[a]	953.7[a]	23.07	<0.0001
CD (μm)	182.4	165.0	164.9	153.7	165.4	6.880	0.095
VH/CD	4.55[c]	5.88[ab]	5.95[ab]	6.72[a]	5.79[b]	0.289	0.005
MT (μm)	335.2[ab]	364.5[a]	340.4[a]	357.4[a]	301.2[b]	13.76	0.023
Jejunum							
VH (μm)	1,194	1,200	1,209	1,250	1,199	53.17	0.932
CD (μm)	167.4	154.4	150.5	133.2	144.0	11.59	0.300
VH/CD	7.16[b]	8.00[b]	8.15[ab]	9.38[a]	8.45[ab]	0.436	0.018
MT (μm)	282.0[b]	315.0[ab]	310.5[ab]	329.7[a]	273.2[b]	13.44	0.039
Ileum							
VH (μm)	914.0	901.1	841.9	845.6	801.2	49.35	0.387
CD (μm)	130.7	135.2	134.0	117.4	116.4	6.421	0.082
VH/CD	6.99	6.75	6.37	7.22	6.84	0.347	0.478
MT (μm)	316.3	298.7	337.6	302.2	318.4	17.90	0.572

[a,b,c]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).

[1]Each value represents the mean of 6 replicates.

[2]VH, villus height; CD, crypt depth; MT, muscularis thickness; VH/CD, villus height to crypt depth ratio.

Table 5. Effects of citric acid on pH values of gastrointestinal tract of geese at 28 d of age.[1]

	Citric acid level %						
Segments	0	0.25	0.50	1.00	2.00	SEM	*P* value
Proventriculus	2.89	2.89	2.89	2.82	2.82	0.181	0.992
Gizzard	2.80	2.66	2.68	2.60	2.65	0.138	0.852
Duodenum	5.57	5.60	5.54	5.52	5.54	0.256	0.999
Jejunum	7.17[a]	6.94[b]	6.84[bc]	6.73[c]	6.67[c]	0.076	0.002
Ileum	7.81	7.62	7.69	7.78	7.79	0.180	0.846
Cecum	7.13	7.11	6.98	7.02	7.07	0.247	0.999

[a,b,c]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).
[1]Each value represents the mean of 6 replicates.

while the excess CA supplementation had no further positive effects on it, and the appropriate level in diet is 1.00%. These results were in agreement with Nourmohammadi and Khosravinia (2015), who found that 6 g CA/kg supplementation in broilers had adversely affected villi height/crypt depth ratio in the duodenum. The improved intestinal development may be the factor of motivating the gosling performance.

Gastrointestinal Tract Content pH Value

As shown in Table 5, no significant differences were noted among all groups in pH value in content of proventriculus, gizzard, duodenum, ileum and cecum ($P > 0.05$). Whereas, jejunal content pH value was significantly reduced with the addition of CA compared with the control group ($P < 0.05$). The present results partly confirmed those of Abdel-Fattah et al. (2008) who found that diet supplemented with 1.50% or 3.00% CA had no effect on the pH value of content of gizzard, duodenum, jejunum and ileum. Study in broilers revealed that the pH value of crop, gizzard, duodenum and ileum were not affected by 3.00% CA supplementation, while the pH value of jejunal content significantly decreased (Nourmohammadi and Khosravinia, 2015). Hence, the jejunal content pH value may be more sensitive to CA supplement than other segments. In Japanese quails, Fikry et al. (2021) found that cecal content pH values decreased with the addition of CA at 5 g/kg to 20 g/kg. The disagreement might be due to the different species.

Bacteriological Count and Volatile Fatty Acid Analysis

The effects of CA supplementation on cecal microbiota (total bacterial count, *Coliform*, *Staphylococcus aureus*, and *Lactobacillus*) are presented in Table 6. Significant differences were found in total bacterial count ($P < 0.05$), which was the highest with the 0.50% CA supplementation, while no significant differences were observed on *Staphylococcus aureus* and *Lactobacillus* ($P > 0.05$). Additionally, the goslings fed 2.00% CA displayed significantly increase in *Coliform* compared with other groups ($P < 0.05$). In ducks and Japanese quails, studies showed the inclusion of CA reduced the total bacterial count and *E. coli* (ELnaggar and Abo EL-Maaty, 2017; Fikry et al., 2021) and increased *Lactobacillus* (Fikry et al., 2021). But, improvements in bacteriological count have not been confirmed by our study, which may be due to unchanged pH value in cecal content, because the reduced pH value has been considered a contributor to increasing favorable bacteria and inhibiting the pathogenic bacteria (Abdel-Fattah et al., 2008; Fikry et al., 2021). Meanwhile, a negative effect caused by high CA addition in diet was observed in our study, because the highest CA supplementation level led to the highest *Coliform*.

Table 6. Effects of citric acid on microbiota and VFA of geese at 28 d of age.[1]

	Citric acid level %						
Items	0	0.25	0.50	1.00	2.00	SEM	*P* value
Microbiota ($\log_{10}$ (CFU/g))							
Total bacterial count	7.08[c]	7.12[bc]	7.53[a]	7.22[bc]	7.38[ab]	0.094	0.013
Coliform	6.58[b]	6.62[b]	6.64[b]	6.65[b]	6.84[a]	0.068	0.004
Staphylococcus aureus	6.48	6.32	6.41	6.30	6.41	0.177	0.847
Lactobacillus	6.50	6.47	6.35	6.45	6.43	0.150	0.946
VFA[2] (μg/g)							
Acetic acid	723.3	713.4	725.3	666.9	641.4	34.30	0.250
Propionic acid	474.3	467.5	474.8	462.6	470.1	7.752	0.742
Butyric acid	405.7	400.9	407.1	390.2	397.0	10.04	0.705

[a,b,c]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).
[1]Each value represents the mean of 6 replicates.
[2]VFA, volatile fatty acid.

In addition, the results in Table 6 revealed the inclusion of CA in diet had no significant impact on VFA (acetic acid, propionic acid and butyric acid) levels in cecal contents ($P > 0.05$). Quantifying VFA concentrations may have important implications for poultry production, because studies indicate that VFA contributes to inhibiting the growth of harmful microorganisms, improving immune responses and repairing intestinal epithelial cells (Mccafferty et al., 2019; Lan et al., 2021). Little information has been published regarding the effects of citric acid supplementation on VFA in intestinal content of poultry. But study showed that commercial organic acid supplementation had no influence on VFA levels in cecal contents of young broilers (Chaveerach et al., 2004), which are in agreement with our results. Therefore, the effects of CA supplementation in diet on cecum may be limited in goslings considering the unimproved bacteriological count and VFA levels.

Immune Indices and Antioxidant Property

The effects of citric acid on immune indices and antioxidant property are showed in Table 7. As citric acid levels increased, the IgA concentrations increased firstly and then decreased, and the goslings fed 1.00% CA supplementation had the highest IgA concentrations. The supplementation of 0.50% and 1.00% CA in diet significantly improved IgA concentrations in plasma than control group and 2.00% CA

Table 7. Effects of citric acid on immune indices and antioxidant property of geese at 28 d of age.[1]

Items[2]	Citric acid level %					SEM	P value
	0	0.25	0.50	1.00	2.00		
IgA (g/L)	2.05[c]	2.23[bc]	2.46[ab]	2.59[a]	2.00[c]	0.106	0.002
IgG (g/L)	18.40	18.21	18.76	19.39	17.95	1.046	0.858
SOD (U/mL)	64.79	72.66	66.05	69.55	71.07	4.026	0.569
T-AOC (U/mL)	8.68	8.15	8.20	8.55	8.58	0.728	0.972
CAT (μmol/mL)	35.76	33.45	38.91	36.76	38.43	2.824	0.663
GSH-Px (U/mL)	372.2	375.9	401.6	397.3	384.5	16.25	0.581
MDA (nmol/mL)	5.52[a]	5.16[ab]	4.90[abc]	4.08[c]	4.22[bc]	0.324	0.020

[a,b,c]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).
[1]Each value represents the mean of 6 replicates.
[2]T-AOC, total antioxidant capacity; CAT, catalase; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde.

group ($P < 0.05$), while no significant effect was observed on IgG concentrations ($P > 0.05$). Immunoglobulin plays a vital role in identifying and neutralizing foreign objects such as pathogenic bacteria and viruses, and the level of it indicated the immunity of poultry. In current study, the increased IgA in 0.50% and 1.00% CA groups meant that the immunity of goslings was enhanced. The enhancement of immunity was supported by ELnaggar and Abo EL-Maaty (2017), who reported that duckling fed the diet supplemented with citric acid had greater IgA and IgG. In Japanese quails, Fikry et al. (2021) found that IgG level was greater in groups receiving 5 and 10 g CA/kg than in the control group. However, the IgA levels reduced markedly, which was equal to the levels in control group, when the citric acid supplementation increased from 1.00% to 2.00% (Table 7). This result illustrated that the excess CA supplementation had no positive effects on the immunity, which concurred with Fikry et al. (2021).

In addition, the supplementation of 1.00% and 2.00% CA in diet significantly reduced the MDA concentration in plasma ($P < 0.05$), though the SOD, T-AOC, CAT and GSH-Px were not affected ($P > 0.05$). The present result partly agreed with Fikry et al. (2021), who found the MAD concentration reduced in groups receiving 10 to 20 g CA/kg compared with the control group. Reactive oxygen species (**ROS**), a family of oxygen derivatives, have positive effects on many biological process, but excessive ROS leads to oxidative stress (Zhang et al., 2013; Xue et al., 2021). The MDA is the primary final product of lipid peroxidation, and the high level of MDA usually indicated the occurrence of oxidative damage (Simsek et al., 2009). Hence, the CA supplementation in diet reduce the lipid peroxidation in poultry, which may result from the decreased polymorphonuclear cell degranulation and the attenuated release of myeloperoxidase (Abdel-Salam et al., 2014). Antioxidant enzymes such as CAT, SOD, and GSH-Px, is an important part of antioxidant defense system, and can prevent oxidative stress by scavenging formed ROS (Seven et al., 2009; Zhang et al., 2013). Several previous studies reported that the inclusion of CA in poultry feed increased SOD, GSH-Px and T-AOC (Elnaggar and Abo El-Maaty, 2017; Fikry et al., 2021), but they were not changed in present study, and the disagreement warrants further investigation.

Blood Biochemistry

The results of blood biochemistry parameters were presented in Table 8. Analysis of variance revealed no significant differences among the groups in plasma ALT, AST, TP, GLO, and ALB ($P > 0.05$), which were consistent with some previous researches (Nourmohammadi et al., 2010; El-Haliem et al., 2018). There were no significant differences in urea and uric acid ($P > 0.05$), while creatinine significantly increased by the 2.00% CA supplementation ($P < 0.05$). Similarly, some studies about broilers showed the urea or uric acid was not affected by the inclusion of CA (Abdel-Fattah et al., 2008; Nourmohammadi et al., 2010; Nourmohammadi and Khosravinia, 2015; Dehghani-Tafti and Jahanian, 2016). However, ELnaggar and Abo EL-Maaty. (2017) reported that the broilers receiving diet with 2.00% and 3.00% CA supplementation had the lower urea and creatinine than the control group. Fikry et al. (2021) found the urea decreased firstly and then increased by the increasing of CA supplementation

Table 8. Effects of citric acid on blood biochemistry of geese at 28 d of age.[1]

Items[2]	Citric acid level %					SEM	P value
	0	0.25	0.50	1.00	2.00		
ALT (U/L)	14.17	13.75	14.50	15.00	14.00	1.688	0.986
AST (U/L)	34.00	28.75	28.00	30.50	32.50	4.116	0.800
TP (g/L)	31.73	28.28	29.24	33.30	28.60	1.876	0.203
GLO (g/L)	15.42	13.52	13.68	16.55	13.62	1.068	0.139
ALB (g/L)	16.32	14.77	15.56	16.75	14.98	0.872	0.363
CRE (μmol/L)	0.67[b]	0.53[b]	0.70[b]	0.72[b]	1.80[a]	0.196	0.004
Urea (mmol/L)	0.95	1.09	1.01	0.99	0.97	0.064	0.580
UA (μmol/L)	239.0	237.7	224.4	233.3	205.3	39.32	0.963

[a,b]In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), while with common or no letter superscripts mean no significant difference ($P > 0.05$).
[1]Each value represents the mean of 6 replicates.
[2]ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; GLO, globulin; ALB, albumin; CRE, creatinine; UA, uric acid.

from 5 to 20 g/kg. Additionally, study showed a large dose of citric acid caused the renal toxicity in mice (Chen et al., 2015). Hence, we conclude that the excess CA supplementation in goslings may injure the kidney function considering the increased creatinine.

CONCLUSIONS

In conclusion, dietary citric acid supplementation (especially 1.00%) in goslings increases the growth performance, improves intestinal morphology, diminishes the pH value of jejunal contents, reduces oxidative damage and enhances immunity, while excessive CA addition may cause negative effects. According to the quadratic polynomial model, the addition of CA in diet for obtaining maximum ADFI should be 1.09% (10.9 g/kg diet) for goslings from 1 to 28 d of age.

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DISCLOSURES

There are no conflicts of interest with any individual or organization.

REFERENCES

Abdel-Fattah, S. A., M. H. El-Sanhoury, N. M. El-Mednay, and F. Abdel-Azeem. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7:678–687.
Abdel-Salam, O., E. R. Youness, N. A. Mohammed, S. Morsy, E. A. Omara, and A. A. Sleem. 2014. Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. J. Med. Food. 17:588–598.
Adil, S., T. Banday, G. A. Bhat, M. S. Mir, and M. Rehman. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int. 2010:479485.
Centeno, C., I. Arija, A. Viveros, and A. Brenes. 2007. Effects of citric acid and microbial phytase on amino acid digestibility in broiler chickens. Br. Poultry Sci. 48:469–479.
Chaveerach, P., D. A. Keuzenkamp, L. J. A. Lipman, and F. Van Knapen. 2004. Effect of organic acids in drinking water for young broilers on campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes. Poult. Sci. 83:330–334.
Chen, X. G., Q. X. Lv, W. Deng, and Y. M. Liu. 2015. Effects of the food additive, citric acid, on kidney cells of mice. Biotech. Histochem. 90:38–44.
Das, S. K., K. M. Islam, and M. Islam. 2012. Performance and immunity of broiler due to addition of citric acid in low nutrient diet. Indian J. Anim. Sci. 82:629–633.
Dehghani Tafti, A., and R. Jahanian. 2016. Effect of supplemental organic acids on performance, carcass characteristics, and serum biochemical metabolites in broilers fed diets containing different crude protein levels. Anim. Feed. Sci. Tech. 211:109–116.
Ebrahimnezhad, Y., M. Shivazad, R. Taherkhani, and K. Nazeradl. 2008. Effects of citric acid and microbial phytase supplementation on performance and phytate phosphorus utilization in broiler chicks. J. Poult. Sci. 45:20–24.
El-Haliem, H. A., F. A. M. Attia, H. S. Saber, and I. H. Hermes. 2018. Effect of dietary levels of crude protein and specific organic acids on broilers performance. Egyptian. J. Anim. Prod. 55:15–27.
Elnaggar, S. A., and H. Abo El-Maaty. 2017. Impact of using organic acids on growth performance, blood biochemical and hematological traits and immune response of ducks (cairina moschata). Egypt. Poult. Sci. 37:907–925.
Fikry, A. M., A. I. Attia, I. E. Ismail, M. Alagawany, and F. M. Reda. 2021. Dietary citric acid enhances growth performance, nutrient digestibility, intestinal microbiota, antioxidant status, and immunity of Japanese quails. Poult. Sci. 100:101326.
Haq, A., M. Ch Tabassum, F. Ahmad, J. Shafi, M. Ashraf, M. Javed, and S. Rehman. 2014. Effect of dietary acidification with citric acid on carcass characteristics, haemogram and serum metabolite values of broiler chicken. Pak. J. Life Soc. Sci. 12:36–41.
Hou, S. S., and L. Z. Liu. 2021. The current situation, prospect and suggestions on Waterfowl industry in 2020. Chin. J. Anim. Sci. 57:235–239.
Islam, K. M. 2012. Use of citric acid in broiler diets. Worlds. Poult. Sci. J. 68:104–118.
Islam, K. M., M. Debi, R. Haque, and M. M. Uddin. 2021. Effect of citric acid in low nutrient diet on growth and bone mineral metabolism of broiler. Bang. J. Anim. Sci. 50:36–42.
Islam, K. M. S., H. Schaeublin, C. Wenk, M. Wanner, and A. Liesegang. 2011. Effect of dietary citric acid on the performance and mineral metabolism of broiler. J. Anim. Physiol. Anim. Nutr. 96:808–817.
Kamal, A., and N. Ragaa. 2014. Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. Nat. Sci. 12:38–45.
Lan, J., G. Chen, G. Cao, J. Tang, Q. Li, B. Zhang, and C. Yang. 2021. Effects of α-glyceryl monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens. Poult. Sci. 100:100875.
Liu, Z. L., Y. Chen, J. J. Xue, X. F. Huang, Z. P. Chen, Q. G. Wang, and C. Wang. 2022a. Effects of ambient temperature on the growth performance, fat deposition, and intestinal morphology of geese from 28 to 49 days of age. Poult. Sci. 101:101814.
Liu, Z. L., Z. P. Chen, J. J. Xue, X. F. Huang, Y. Chen, B. W. Wang, Q. G. Wang, and C. Wang. 2022b. Effects of ambient temperature on growth performance, blood parameter, and fat deposition of geese from 14 to 28 days of age. Poult. Sci. 101:101758.
Liu, Z. L., J. J. Xue, X. F. Huang, Y. Luo, and C. Wang. 2020. Effect of feeding frequency on the growth performance, carcass traits, and apparent nutrient digestibility in geese. Poult. Sci. 99:4818–4823.
Mccafferty, K. W., M. R. Bedford, B. J. Kerr, and D. Iii. 2019. Effects of age and supplemental xylanase in corn- and wheat-based diets on cecal volatile fatty acid concentrations of broilers - ScienceDirect. Pout. Sci. 98:4787–4800.
Nourmohammadi, R., and N. Afzali. 2013. Effect of citric acid and microbial phytase on small intestinal morphology in broiler chicken. Ital. J. Anim. Sci. 12:44–47.
Nourmohammadi, R., M. Hosseini, H. Saraee, and A. Arab. 2011. Plasma thyroid hormone concentrations and ph values of some gi-tract segments of broilers fed on different dietary citric acid and microbial phytase levels. Am. J. Anim. Vet. Sci. 6:1–6.
Nourmohammadi, R., S. M. Hosseini, and H. Farhangfar. 2010. Effect of dietary acidification on some blood parameters and weekly performance of broiler chickens. J. Anim. Vet. Adv. 9:3092–3097.
Nourmohammadi, R., S. M. Hosseini, H. Farhangfar, and M. Bashtani. 2012. Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed corn-soybean meal diets. Ital. J. Anim. Sci. 11:36–40.
Nourmohammadi, R., and H. Khosravinia. 2015. Acidic stress caused by dietary administration of citric acid in broiler chickens. Arch. Anim. Breed. 58:309–315.
Rafacz-Livingston, K. A., C. Martinez-Amezcua, C. M. Parsons, D. H. Baker, and J. Snow. 2005. Citric acid improves phytate phosphorus utilization in crossbred and commercial broiler chicks. Poult. Sci. 84:1370–1375.
SAS Institute. 2003. SAS User's Guide: Statistics. Version 9.0. SAS Institute, Inc., Cary, NC.

Schmid, A. 2011. The role of meat fat in the human diet. Crit. Rev. Food Sci. Nutr. 51:50–66.

Seven, P., S. Yilmaz, I. Seven, I. Cerci, M. Azman, and M. Yilmaz. 2009. Effects of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. Acta Vet. Brno. 78:75–83.

Sheiha, A. M., S. A. Abdelnour, M. El-Hack, A. F. Khafaga, and M. T. El-Saadony. 2020. Effects of dietary biological or chemical-synthesized nano-selenium supplementation on growing rabbits exposed to thermal stress. Animals (Basel) 10:430.

Simsek, U. G., B. Dalkilic, M. Ciftci, and A. Yuce. 2009. The influences of different stocking densities on some welfare indicators, lipid peroxidation (MDA) and antioxidant enzyme activities (GSH, GSHPx, CAT) in broiler chickens. J. Anim. Vet. Adv. 8:1568–1572.

Thompson, J. L., and M. Hinton. 1997. Antibacterial activity of formic and propionic acids in the diet of hens on Salmonellas in the crop. Br. Poult. Sci. 38:59–65.

Tollba, A. 2010. Reduction of broilers intestinal pathogenic microflora under normal or stressed condition. Egypt. Poult. Sci. 30:249–270.

Xue, J. J., Z. L. Liu, X. F. Huang, Y. Chen, Z. P. Chen, Q. G. Wang, B. W. Wang, and C. Wang. 2021. Estimates of stocking density of female geese in different growth stages. J. Appl. Poultry Res. 30:100215.

Zhang, H. Y., X. S. Piao, Q. Zhang, P. Li, J. Q. Yi, J. D. Liu, Q. Y. Li, and G. Q. Wang. 2013. The effects of Forsythia suspensa extract and berberine on growth performance, immunity, antioxidant activities, and intestinal microbiota in broilers under high stocking density. Poult. Sci. 92:1981–1988.