



Role of Organic Acids (Acetic and Citric Acids) in Enhancing Blood Parameters, Oxidative Stress Indicators, and Immune Response in Broiler

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Abstract

The experiment was conducted at the Agricultural Research and Experiment Station, College of Agriculture, Al-Muthanna University, from February 1, 2025, to March 7, 2025. 315 one-day-old, unsexed Ross 308 hybrid broiler chicks, weighing 38 grams each, were used. The chicks were randomly distributed into 7 experimental treatments, with 45 chicks per treatment and three replicates per treatment (15 chicks/replicate). The experimental treatments were as follows: T1: Control treatment with no additives. T2 and T3 add of citric acid at a concentration of 1 and 2 gm/kg of diet. T4 and T5 add of acetic acid at a concentration of 1 and 2 gm/kg of diet. T6 and T7 T6: Add of citric acid + acetic acid at a concentration of 0.5 and 1 gm/kg for each to the diet. The results showed a significant increase ($P \leq 0.05$) in T7 on some biochemical parameters, namely albumin, globulin, and total protein concentrations in blood plasma, accompanied by a significant decrease ($P \leq 0.05$) on cholesterol, glucose, and triglyceride concentrations. A significant improvement ($P \leq 0.05$) on the concentrations of antioxidants (malondialdehyde (MDA), glutathione (GSH-px), and catalase), in treatments that included both citric and acetic acids at a concentration of 1 gm/kg of feed for each, compared to the other experimental treatments and the control treatment. The immune response, as measured by cellular immunity (DHT), the volumetric standard for antibodies against Newcastle disease (ELISA), relative weight of the Bursas gland, and Bursa index, was significantly better ($P \leq 0.05$) during the 3-5 week post-infancy period for T7 compared to the other experimental treatments and the control treatment.

Keywords: organic acids, Citric acid, Acetic acid, blood parameters, oxidative indicators, immune response, broiler.

Introduction

The use of organic acids and their salts in poultry feed as efficient feed alternatives. They do not leave residues in the meat or eggs when consumed by birds, unlike antibiotics, which may leave drug residues in bird tissues. Their use as growth promoters is prohibited in many countries due to the emergence of antibiotic-resistant bacterial strains and genera (Al Salman and Al-Gharawi, 2019; Nguyen and Kim, 2020).

Organic acids offer an opportunity to reduce antibiotic dependence (Al-Gharawi, 2012; Alao *et al.*, 2024). Organic acids are widely recognized for their ability to improve overall performance, by reducing the total microbial load and competition among microbes for nutrients within the digestive tract, the risk of infection decreases, leading to improved food digestion and reduced ATP requirements in intestinal-associated tissues (Khan and Javid, 2016; Al-Salman and Al-Gharawi, 2023).

Organic acids are defined as additives with antibiotic-like properties, that improve animal health and productivity, by significantly contributing to intestinal health and development. Organic acids are characterized by their antimicrobial effect. They are considered safe substances and are used as feed substitutes, known as GRAS (Generally Recognized as Safe). They are approved by governmental and international organizations such as the World Health Organization (WHO), the European Union (EC), the Food and Agriculture Organization of the United Nations (FAO), and the U.S. Food and Drug Administration (Chukwudi *et al.*, 2025).

Organic acids are weak acids that help to adjust the pH of the intestines, when used correctly and in accordance with good nutritional and management practices. However, they should be added to animal feed within specific limits. (Adhikari *et al.*, 2020).

Organic acids, such as formic acid, acetic acid, propionic acid, and butyric acid, are important functional additives that improve poultry performance and the efficiency of nutrient digestion and absorption. Other positive effects of organic acids include increased immunity and disease resistance (Hu *et al.*, 2023). An acidic environment stimulates the production of mucus and digestive enzymes, which aids in the digestion of feed components and increases nutrient absorption more effectively. This enables poultry to extract more energy and nutrients, contributing to their growth and overall health (Zhu *et al.*, 2021). Adding organic acids to poultry feed provides numerous advantages, such as improved growth rates and reduced disease incidence. The most suitable period for using organic acids is during the early stages of broiler rearing (Leeson *et al.*, 2005).

The current study aims to demonstrate the role of organic acids, specifically acetic and citric acids added to feed, in certain biochemical blood parameters, oxidative stress indicators, and the immune response of broiler.

Materials and Methods

This experiment was conducted at the poultry farm of the Agricultural Research and Experiment Station, College of Agriculture, Al-Muthanna University, from February 1, 2025, to March 7, 2025, to demonstrate the effect of adding different levels of citric acid, acetic acid, and their mixture to the feed on some intestinal characteristics of broiler chickens. 315 day-old Ross 308 broiler chicks, ready for market, were used. The chicks were raised in four-tiered batteries, each tier containing a cage measuring 1.5 × 1 m. The chicks were randomly distributed into seven experimental treatments, with 45 chicks per treatment and three replicates per treatment (15 chicks/replicate), as follows:

T1: (Control treatment without additives).

T2: Add citric acid at a concentration of 1 gm/kg of diet.

T3: Add citric acid at a concentration of 2 gm/kg of diet.

T4: Add acetic acid at a concentration of 1 gm/kg of diet.

T5: Add acetic acid at a concentration of 2 gm/kg of diet.

T6: Add citric acid at a concentration of 0.5 gm/kg of diet+ acetic acid at a concentration of 0.5 gm/kg of diet.

T7: Add citric acid at a concentration of 1 gm/kg of feed, along with acetic acid at a concentration of 1 gm/kg of diet.

The organic acids acetic acid and citric acid were obtained from a chemical import office in Baghdad (Bab Al-Muadham). The two organic acids were added to the experimental feed in the specified quantities. The feed was spread on a clean, waxed plastic sheet. The organic acids were then mixed with the feed, stirring to ensure even distribution of the feed pieces. The mixed feed was stored in 50 kg woven polypropylene bags.

Blood samples were collected at the end of the fifth week by taking blood from the brachial (winged) vein of six birds from each treatment group. Blood was collected in 10 ml glass tubes without anticoagulant and placed horizontally to remove the fibrinogen proteins. The blood was centrifuged at 3000 rpm for 15 minutes, and the serum was collected in separate sterile tubes at -18°C. The serum was analyzed for cholesterol, triglycerides, glucose, protein, albumin, globulin, and oxidative stress markers, malondialdehyde (MDA), glutathione peroxidase (GSH-px), and catalase). As for the immune response, Enzyme Linked Immunosorbent Assay (ELISA), Delayed type hypersensitivity test (DTH), relative Bursa weight, and Bursa index were all studied.

Results And Discussion

Table (1) shows the effect of adding different levels of citric and acetic acid on the concentration of cholesterol, triglycerides, and glucose in the blood serum of broiler chickens.

Regarding cholesterol concentration, a significant decrease ($P \leq 0.05$) was observed in T7 and T6 compared to T5 and T3, which in turn showed a significant decrease ($P \leq 0.05$) in T4 and T2, which were themselves significantly lower ($P \leq 0.05$) compared to the control treatment. However, T7 and T6 did not show a significant effect compared to T5 and T3, nor did T4 and T2 show a significant effect compared to the control treatment. The cholesterol concentrations in broiler blood serum were 289.89, 278.96, 275.37, 274.09, 277.71, 271.20, and 269.72 mg/100 ml for treatments T1, T2, T3, T4, T5, T6, and T7, respectively.

Regarding triglyceride concentrations in broiler blood serum, Table (1) shows a significant decrease ($P \leq 0.05$) in T7 compared to T6, which in turn showed a significant decrease ($P \leq 0.05$) compared to T5 and T3. These concentrations were also significantly lower ($P \leq 0.05$) compared to T4 and T2, and compared to the control group. Table (1) shows no significant difference between T5 and T3 on the one hand, and T4 and T2 on the other. The mean triglyceride concentration was 145.19, 147.20, 143.06, 145.22, 142.96, 139.04, and 135.84 mg/100 ml of broiler serum for treatments T1, T2, T3, T4, T5, T6, and T7, respectively.

Regarding glucose concentration, a significant decrease ($P \leq 0.05$) was observed in T7, compared to T6, which also showed a significant decrease ($P \leq 0.05$). This was in contrast to T5 and T3, which showed a significant decrease ($P \leq 0.05$) compared to T4 and T2, which also showed a significant decrease ($P \leq 0.05$) compared to the control treatment. Table (1) shows no significant differences among treatments T1, T2, T3, T4, and T5. The glucose concentrations in broiler blood serum were 211.32, 208.91, 203.72, 207.40, 203.12, 195.60, and 192.74 mg/100 ml, respectively, for treatments T1, T2, T3, T4, T5, T6, and T7.

Table (1) Effect of adding different levels of citric acid, acetic acid to the diet on the concentration of cholesterol, triglycerides and glucose in the blood serum of broiler (mean ± standard error).

Treatments	Cholesterol (mg/ 100 ml)	Triglycerides (mg/ 100 ml)	Glucose (mg/ 100 ml)
T1	0.85 ± 289.89 a	0.33 ± 147.20 a	0.41 ± 211.32 a
T2	0.82 ± 278.96 b	0.04 ± 145.19 b	0.69 ± 208.91 b
T3	0.22 ± 275.37 c	0.14 ± 143.06 c	0.35 ± 203.72 c
T4	0.47 ± 277.71 b	0.33 ± 145.22 b	0.97 ± 207.40 b
T5	0.20 ± 274.09 c	0.33 ± 142.96 c	0.39 ± 203.12 c
T6	0.48 ± 271.20 d	0.46 ± 139.04 d	0.60 ± 195.60 d
T7	0.23 ± 269.72 d	0.42 ± 135.84 e	0.77 ± 192.74 e
Sig.	*	*	*

Table (1) shows a significant decrease in cholesterol and triglyceride concentrations in the citric and acetic acid synergistic mixture treatments compared to all experimental and control treatments. This may be attributed to the ability of organic acids to increase bile salt secretion, which aids in the elimination of excess cholesterol, and to inhibit the activity of HMG-CoA reductase, the main enzyme responsible for cholesterol synthesis in the liver (Abdel-Fattah *et al.*, 2008).

The same table also shows a significant decrease in glucose concentration in the citric and acetic acid synergistic mixture treatments compared to all experimental and control treatments. A slight decrease or stabilization of serum glucose concentration was observed. This decrease may be due to the role of organic acids. Improving energy utilization efficiency and carbohydrate metabolism, which ensures the provision of energy necessary for vital processes and rapid growth without metabolic disturbances (Kamal and Ragaa, 2014).

Table (2) shows the effect of adding different levels of citric acid, acetic acid, and their mixture to the feed on the concentration of albumin, globulin, and protein in broiler blood serum.

The table indicates a significant increase ($P \leq 0.05$) in broiler blood serum albumin concentration with T7 compared to T6. T7 also showed a significant increase ($P \leq 0.05$) in broiler blood serum albumin concentration, which was significantly higher ($P \leq 0.05$) than T4 and T2. T4 and T2, in turn, showed a significant increase ($P \leq 0.05$) compared to the control treatment. No significant differences were found between T5 and T3 and T4 and T2. The mean albumin concentration was 2.02, 2.06, 2.14, 2.06, 2.16, 2.39, and 2.43 mg/100 ml of broiler serum for treatments T1, T2, T3, T4, T5, T6, and T7, respectively.

Regarding globulin concentration, a significant effect ($P \leq 0.05$) was observed in T7 compared to T6, and it was significantly higher ($P \leq 0.05$) than T5 and T3. T5 and T3 were significantly higher ($P \leq 0.05$) than T4 and T2, respectively. These treatments showed a significant increase compared to the control treatment. From the same table, no significant differences were observed between T5 and T3, or between T4 and T2. The mean globulin concentration was 1.79, 1.94, 2.02, 1.95, 2.04, 2.15, and 2.19 mg/100 ml of broiler blood serum.

Regarding the total protein concentration in broiler blood serum, the data in the table showed that birds in T7 ($P \leq 0.05$) were superior to birds in T6, and both treatments (T5 and T3) were significantly superior ($P \leq 0.05$) to T4 and T2. These treatments were also significantly superior ($P \leq 0.05$) to the control treatment. However, no significant differences were observed between T5 and T3, or between T4 and T2. The mean total protein concentration was 3.82, 4.00, 4.17, 4.02, 4.21, 4.54, 4.65 mg/100 ml of broiler serum for treatments T1, T2, T3, T4, T5, T6, T7 respectively.

Table (2) Effect of adding different levels of citric acid, acetic acid to the diet on the concentration of albumin, globulin and total protein in the blood serum of broiler (mean \pm standard error).

Treatments	Albumin (gm/ 100 ml)	Globulin (gm/ 100 ml)	Total protein (gm/ 100 ml)
T1	0.008 \pm 2.02 e	0.012 \pm 1.79 e	0.010 \pm 3.82 e
T2	0.008 \pm 2.06 d	0.006 \pm 1.94 d	0.012 \pm 4.00 d
T3	0.012 \pm 2.14 c	0.003 \pm 2.02 c	0.014 \pm 4.17 c
T4	0.008 \pm 2.06 d	0.006 \pm 1.95 d	0.005 \pm 4.02 d
T5	0.006 \pm 2.16 c	0.003 \pm 2.04 c	0.005 \pm 4.21 c
T6	0.008 \pm 2.39 b	0.011 \pm 2.15 b	0.018 \pm 4.54 b
T7	0.008 \pm 2.43 a	0.013 \pm 2.19 a	0.021 \pm 4.65 a
Sig.	*	*	*

Table (2) shows a significant increase in the concentration of albumin, globulin, and total protein in the organic acid synergistic mixture treatments. The best results were obtained compared to the groups treated with only one organic acid in addition to the control group. This significant increase is attributed to the increased efficiency of protein digestion and amino acid absorption resulting from the reduction in the pH of the digestive tract. This, in turn, stimulates digestive enzymes, most importantly peptidase and proteinase. The improved albumin-to-globulin ratio reflects the birds' health status. This is due to the synergistic effect of these acids in improving the intestinal environment and reducing the bacterial burden (Abdel-Fattah *et al.*, 2008). These results are consistent with those of Abdul Aziz (2006) regarding the addition of citric and acetic acids, respectively.

Their findings indicated that the added organic acids may improve the birds' immune response. Globulin levels are used as an indicator of immune responses and are an important source for antibody production. An elevated total protein concentration in blood plasma within normal levels is a good indicator of health status. It plays an important role in maintaining fluid volume balance between the blood and tissues. It transports many nutrients to all body tissues and balances the osmotic pressure between the blood and tissues (Abdul Aziz, 2006).

Table (3) shows the effect of adding different levels of citric acid, acetic acid, and their mixture to the feed on the concentration of antioxidants, namely Malondialdehyd (MDA), glutathione (GSH-px), and catalase enzyme, in the blood serum of broiler chickens.

The results showed a significant decrease ($P \leq 0.05$) in the concentration of MDA in the blood serum of broiler chickens in T7 compared to T6. T7 also showed a significant decrease ($P \leq 0.05$) compared to T5, T4, T3, and T2, which were themselves significantly lower ($P \leq 0.05$) compared to the control treatment. No significant differences were found between treatments T5, T4, T3, and T2. The mean serum concentration of MDA was 5.44, 5.04, 4.94,

5.03, 4.91, 4.67, and 4.27 gm/100 ml in broiler chickens for treatments T1, T2, T3, T4, T5, T6, T7, T8, and T9, respectively.

Regarding serum glutathione (GSH-px) concentration in broiler chickens, a significant increase ($P \leq 0.05$) was observed in T7 compared to T6, which was significantly higher ($P \leq 0.05$) compared to T5 and T3. T4 and T2 were superior to T4 and T2, which were themselves significantly higher ($P \leq 0.05$) compared to the control group. No significant differences were found between T5 and T3 and T4 and T2. The mean serum glutathione concentration in broiler chickens was 31.85, 32.20, 32.96, 32.22, 32.97, 34.14, and 34.70 U/L for treatments T1, T2, T3, T4, T5, T6, T7, T8, and T9, respectively.

Regarding serum catalase concentration in broiler, a significant effect ($P \leq 0.05$) was observed for T7 compared to T6, which was significantly higher ($P \leq 0.05$) than T5 and T3. T5 and T3 were also significantly higher ($P \leq 0.05$) than T4 and T2, which were themselves significantly higher ($P \leq 0.05$) than the control treatment. There were no significant differences between T5 and T3, or treatments T4 and T2. The mean serum concentration of catalase in broiler chickens was 44.46, 44.92, 45.31, 44.97, 45.36, 45.86, and 46.27 IU for treatments T1, T2, T3, T4, T5, T6, T7, T8, and T9, respectively.

Table (3) Effect of adding different levels of citric acid, acetic acid to the diet on the concentration of Malondialdehyd (MDA), glutathione (GSH-px) and catalase in the blood serum of broiler (mean \pm standard error).

Treatments	Malondialdehyd (MDA) (mg/ 100 ml)	glutathione (GSH-px) (U/ L)	catalase (IU)
T1	0.108 \pm 5.44 a	0.145 \pm 31.85 e	0.067 \pm 44.46 e
T2	0.040 \pm 5.04 b	0.040 \pm 32.20 d	0.025 \pm 44.92 d
T3	0.006 \pm 4.94 b	0.168 \pm 32.96 c	0.023 \pm 45.31 c
T4	0.006 \pm 5.03 b	0.035 \pm 32.22 d	0.006 \pm 44.97 d
T5	0.003 \pm 4.91 b	0.023 \pm 32.97 c	0.037 \pm 45.36 c
T6	0.043 \pm 4.67 c	0.020 \pm 34.14 b	0.018 \pm 45.86 b
T7	0.028 \pm 4.27 d	0.054 \pm 34.70 a	0.029 \pm 46.27 a
Sig.	*	*	*

The decrease in malondialdehyde (MDA) levels and the increase in glutathione and catalase concentrations in broiler chickens following the synergistic mixture of organic acids, such as citric and acetic acids, resulted in a significant improvement in the antioxidant status of these birds, targeting oxidative stress pathways. This decrease coincided with a significant increase in catalase activity and glutathione levels. The synergistic effect is attributed to citric acid's role as a potent chelating agent for divalent metals, which are key catalysts for free radical production and lipid oxidation reactions. This inhibits the primary production of malondialdehyde (MDA). Meanwhile, acetic acid lowers the pH of the digestive tract, thereby increasing the number of beneficial bacteria, which thrive in acidic environments, and reducing the number of harmful bacteria, due to their inability to survive in acidic conditions. This, in turn, reduces the internal oxidative burden on the birds. (Khan and Javid, 2016).

Table (4) illustrates the effect of adding different levels of citric acid, acetic acid, and their mixture to the feed on the immune response of broiler chickens. The results indicate a significant advantage ($P \leq 0.05$) in T7 for all studied immune traits (Enzyme Linked Immunosorbent Assay (ELISA), Delayed type hypersensitivity test (DTH), relative Bursa weight, and Bursa index). This is compared to T6, which was significantly superior ($P \leq 0.05$) to T5 and T3, which in turn was significantly superior ($P \leq 0.05$) to T4 and T2, and ultimately significantly superior ($P \leq 0.05$) to the control treatment. While no significant differences were recorded for the T5, T3, T4, and T2 treatments. DTH rates were 0.355, 0.370, 0.384, 0.371, 0.386, 0.404, and 0.440. ELISA were 1867.61, 1949.90, 2038.20, 1950.93, 2041.50, 2124.33, and 2212.03. Relative Bursa weight were 0.092, 0.109, 0.119, 0.110, 0.120, 0.132, and 0.145. Bursa index was 1.00, 1.18, 1.29, 1.19, 1.30, 1.43, and 1.57 for treatments T1, T2, T3, T4, T5, T6, and T7 respectively.

Table (4) Effect of adding different levels of citric acid, acetic acid to the diet on immune response (Delayed type hypersensitivity test (DTH), Enzyme Linked Immunosorbent Assay (ELISA), relative Bursa weight, and Bursa index) in the blood serum of broiler (mean \pm standard error).

Treatments	DTH	ELISA	Relative Bursa weight	Bursa index
T1	0.0014 \pm 0.355 e	2.39 \pm 1867.61 e	0.0017 \pm 0.092 e	0.01 \pm 1.00 e
T2	0.0006 \pm 0.370 d	1.25 \pm 1949.90 d	0.0008 \pm 0.109 d	0.02 \pm 1.18 d
T3	0.0010 \pm 0.384 c	2.40 \pm 2038.20 c	0.0006 \pm 0.119 c	0.03 \pm 1.29 c
T4	0.0010 \pm 0.371 d	1.20 \pm 1950.93 d	0.0008 \pm 0.110 d	0.01 \pm 1.19 d
T5	0.0006 \pm 0.386 c	1.49 \pm 2041.50 c	0.0008 \pm 0.120 c	0.03 \pm 1.30 c
T6	0.0014 \pm 0.404 b	3.19 \pm 2124.33 b	0.0003 \pm 0.132 b	0.02 \pm 1.43 b

T7	0.0036±0.440 a	1.86±2212.03 a	0.0006±0.145 a	0.03±1.57 a
Sig.	*	*	*	*

Based on the results shown in Table (4), the seventh treatment, which used a mixture of organic acids, was superior in enhancing the immune response. This significant improvement may be attributed to the fact that organic acids lower the pH in the birds' digestive tract, creating an environment unfavorable to pathogenic bacteria and stimulating antibody production (Haque *et al.*, 2010). These results are consistent with those of Abdel-Fattah *et al.* (2008), who found that combining more than one organic acid has a stronger effect on improving health and immunity compared to using a single acid. This is due to their combined ability to improve protein metabolism and increase the response of lymphoid tissues. This positive effect on the immune response (cellular and humoral) and the weight of the bursa of Fabricius upon the addition of citric and acetic acids is consistent with the findings of Adil *et al.* (2010). They confirmed that adding organic acids to broiler diets led to a significant increase in the weight of lymphoid organs (bursa of Fabricius and spleen). Organic acids act as natural growth promoters, reducing oxidative stress and allowing lymphoid organs to grow more effectively.

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