Full Length Article

EFFECT OF ORGANIC ACID SUPPLEMENTATION IN DRINKING WATER ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF JAPANESE QUAIL

Farqad Shawqi Ibrahim[1]* and Ali H Al-obaidy[2]

Department of Animal Production
College of Agricultural
University of Diyala
Iraq, MJJ2+R9G

ABSTRACT

This study aimed to evaluate the effects of supplementation of a mixture of acetic acid and citric acid in drinking water on hematological and biochemical parameters in male and female Japanese quails. A total of 540 day-old quails were randomly distributed into four treatments and three replicates with 135 birds for each group as follows: T1 drinking water without acids; T2, T3, and T4 treatments with 1ml+1g, 2ml+2g, and 3ml+3g of acetic acid + citric acid per liter of drinking water, respectively. At 42 days of age, blood samples were collected from six quails per treatments (three repicats per sex)for haematological and biochemical analysis. The results showed that the organic acid mixture significantly($P \leq 0.01$) increased red blood cell count, packed cell volume, haemoglobin concentration, and high-density lipoprotein levels in both males and females. It also significantly decreased cholesterol, triglyceride, low-density lipoprotein, and aspartate aminotransferase levels in both sexes. Significant increase in total protein($P \leq 0.05$) and globulin ($P \leq 0.01$) levels in males, while albumin levels increased significantly ($P \leq 0.01$) in females was also observed. In conclusion, adding acetic and citric acids to drinking water positively influenced haematological and biochemical parameters, indicating enhanced health and productive performance in Japanese quails.

Keywords: Acetic acid, blood biochemistry, citric acid, haematology parameters, Japanese quail

Received : 16.12.2025 Revised :21.03.2026 Accepted : 10.04.2026

*Corresponding author Email Id: farqadibrahim@uodiyala.edu.iq

INTRODUCTION

The increase in demand for animal protein sources and the prosperity of the poultry sector in general (Toor and Goel, 2022) have been accompanied by an increase in the use of nutritional supplements and additives that increase production and improve the health of animals (Sabir *et al*., 2023; Al-Tememy *et al*., 2023; Gayirbegov *et al*., 2018). However, continuous studies for more than 50 years have indicated the danger of using antibiotic growth promoters in poultry feed or drinking water (Scicutella *et al*., 2021; Saragih *et al*., 2024). The use of organic acids in poultry feed or drinking water is a safe and natural alternative to these stimulants due to their antimicrobial nature as well as their role in improving the health and productive performance of poultry (Du *et al*., 2024). Acetic acid and citric acid are weak organic acids that are found naturally in fruits or can be made commercially and show growth-stimulating effects on the growth of poultry and increase feed intake (Jasim and Taha, 2017; Ndelekwute *et al*., 2012). The results of the study conducted by Makofane *et al.*, (2022) showed that the use of citric acid in broiler chicken feed improves the growth of the bird, improves the characteristics of the carcass, increases the performance of the bird's immune system, and works to increase the thickness of the digestive tract, which improves absorption and intake of the feed. Adding citric acid to broiler feed improves production performance and improves the level of albumin and total protein in blood serum (Abd-Elsamee *et al*., 2020). The use of citric acid and acetic acid in broiler chicken feed at a rate of 0.5% to 2% leads to an improvement in blood parameters and blood components of proteins and lipids, and strengthens immunity and antioxidant enzymes (Elnaggar and El-maaty, 2017). The results of the study by Fouladi *et al*. (2018) indicated that the use of acetic acid inJapanese quail feed causes a significant increase in albumin, total protein, and HDL and decreases the content of triglycerides in the blood serum. Using a mixture of acetic and citric acid in broiler chicken feed causes an increase in total protein, albumin, globulin , and ALT liver enzymes, while causing a decrease in the concentration of total lipids and cholesterol (Abou–Ashour *et al.*, 2021). The present study was aimed to find out the effect of adding a mixture of citric acid and acetic acid at different levels onhaemato-biochemical parameters of Japanese quails, which indicates its impact on the physiological status of quails.

MATERIALS AND METHODS

Management of Japanese quails and design of the experiment

A total of 540 unsexed day-old Japanese quails were used for the study . The birds were randomly distributed into four treatments equally, each treatment consisting of three replicates, with 45 birds per replicate. Acetic acid and citric acid (99% concentration of each) product by Lamen Techning LTD., were added to the drinking water from one day until the end of the experiment period. The deep litter system of managment was used for the experiment, and manual feeders and drinkers were used. The feeding management included a starter diet during 1-21 days of age with 23% protein and 3000 kcal/kg energy, and a grower diet during 22-42 days of age with 20% protein and 2900 kcal/kg energy, and the conditions of rearing were the similar for all treatments .The treatments as follows:

- T1: Control drinking water without organic acids
- T2: 1 ml acetic acid with 1 g citric acid / L of drinking water .
- T3: 2 ml acetic acid with 2 g citric acid / L of drinking water .
- T4: 3 ml acetic acid with 3 g citric acid / L of drinking water

Evaluation of haematological and biochemical parameters

Blood samples were collected from one male and one female in each replicate at 42 days of age from the jugular vein directly after slaughtering the birds. Blood was collected in tubes containing anticoagulant to calculate the number of red blood cells (RBC), and white blood cells (WBC) were estimated by Natt and Herrick method (1952), packed cell volume (PCV) was estimated by micro-hematocrit method to Archer (1965), and haemoglobin (Hb) was estimated by Varley's method (Varley *et al*.,1980).

The biochemical parameters were measured by using an automatic biochemical analyzer, in a specialized laboratory, which included glucose according to Coles (1986) and uric acid according to Henry *et al*., (1957). Total protein, albumin and globulin were estimated according to Wotton (1964), while, lipid profile (cholesterol, triglyceride, low-density lipoprotein and High-density lipoprotein) according to Franey and Elias (1986). Liver enzymes (Aspartate Transaminase and Alanine Transaminase) according to Reitman and Frankel (1957).

Statistical analysis

The data wereanalyzed using the statistical program SPSS version 26 (SPSS, 2019) and the experiment was designed according to aCompletely Randomized Design (CRD) to evaluatethe effect of organic acids on the studied parameters and Duncan's test (Duncan, 1955) at a significance level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Hematological parameters

The effects of the acetic and citric acid mixture on the haematological parameters of 42-day-old male and female quails are presented in Table 1. The results indicate that while the addition of organic acids did not significantly alter white blood cell (WBC) counts, it led to a significant increase ($P \leq 0.01$) in red blood cell (RBC) counts, packed cell volume (PCV), and haemoglobin (Hb) levels in both sexes. In males, the RBC count was 4.15×106 cells/µl in T1 and increased significantly to 4.31, 4.43, and 4.38×106 cells/µl in T2, T3, and T4, respectively. A similar trend was observed for PCV and Hb, with T2, T3, and T4 significantly exceeding T1 across these parameters. As for females, the results of Table 1 showed a significant increase ($P \leq 0.01$) in RBC count in T2, T3, and T4 compared to T1, which recorded 3.52×106 in T1 cells/µl compared to 3.81, 3.83, and 3.86×106 ×106 cells/µl in T2, T3, and T4, respectively. Haemoglobin concentration reached 10.65 g/100 ml and a significant increase ($P \leq 0.01$) to 11.43 and 11.49 g/100 ml in organic acid-fed treatment groups T2, T3, and T4, respectively. Similarly, PCV was increased ($P \leq 0.01$) in birds fed on organic acid mixtures compared with the control (T1).

The supplementation of organic acids has been associated with significant improvements in haematological parameters, particularly RBC count, PCV, and Hb concentration. These effects are primarily attributed to the beneficial impact of organic acids on intestinal health and nutrient bioavailability, and this leads to enhanced intestinal integrity and more efficient absorption of essential micronutrients, such as iron, copper, and vitamin B12, which are critical cofactors in erythropoiesis and haemoglobin synthesis. Moreover, organic acids have chelating properties that improve the solubility and bioavailability of minerals, particularly iron, thereby facilitating their incorporation into haemoglobin (Saad *et al*., 2024). The increase in PCV and RBC may be due to the fact that organic acids improve oxygen transport and the health status of birds by enhancing the functions of the immune system (Ma *et al*., 2021). The results are largely consistent with those of Orbugh *et al*., (2023), who found that the addition of organic acids to turkey diets increase PCV, RBC, and Hb values, while it did not affect the number of WBC at 12 weeks of age. But the results of the present study do not agree with the findings of Elnagar and EL-Maaty (2017), who concluded that organic acids at a concentration of 2-3% increased in WBC count in ducks. Fikry *et al*., (2021) found that the addition of citric acid to the quail diets leads to an increase in the number of WBC, while there was no significant change in RBC and Hb.

Levels of Lipid profile

The data in Table 2 showed a significant decrease ($P \leq 0.01$) in cholesterol, triglyceride (Tri), and low-density lipoprotein (LDL) in T2, T3 and T4 compared with control treatment T1 for male and female quails in both sexes of quails. While High-density lipoprotein (HDL) recorded in T1 was 45.00 mg/dl and significantly ($P \leq 0.01$) increased to 51.00, 50.67 and 52.33 mg/dl in organic acid treatments viz., T2, T3, and T4, respectively. The results also showed a significant increase ($P \leq 0.01$) in HDL level in females of which recorded 50.66, 53.00 and 53.67 mg/dl in T2, T3, and T4 treatments, respectively compared with 43.67 mg/dl in the control treatment T1.

The studies inclusion of organic acids in the diet of poultry has been shown to beneficially modulate the lipid profile by reducing serum cholesterol and triglyceride levels while simultaneously HDL concentrations. This effect can be attributed to several interconnected physiological and biochemical mechanisms. Firstly, organic acids lower the pH of the gastrointestinal tract, creating an unfavorable environment for pathogenic microorganisms while promoting the growth of beneficial microbiota. This improved microbial balance enhances gut health and nutrient absorption, particularly lipids and fat-soluble vitamins, which play a role in regulating lipid metabolism (Ghazvinian *et al*., 2018). Also, organic acids may suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis in the liver. Inhibiting this enzyme leads to a decrease in endogenous cholesterol production. Moreover, organic acids can enhance β-oxidation of fatty acids, thereby reducing the accumulation of triglycerides in the blood (Deng *et al*., 2020). Additionally, organic acids improve bile acid secretion and emulsification of dietary fats, which facilitates more efficient lipid digestion and absorption. Enhanced bile flow also promotes the excretion of cholesterol in the form of bile salts (Waghmare *et al*., 2025). These results are consistent with the findings of Al-Fadhli *et al.,* (2018), who show that adding organic acids causes a decrease in the level of triglycerides. According to the results of a study by Elnagar and EL-Maaty (2017), organic acids in duck diets cause a decrease in cholesterol and triglycerides, as well as the level of LDL. While Saleem *et al*., (2020) indicated that the addition of organic acids did not cause a significant change in the levels of HDL and LDL in the blood plasma of broiler chickens. As well as the data of Pourreza *et al*., (2023), adding organic acids did not lead to a change in the levels of cholesterol, triglycerides, HDL and LDL. These results are not consistent with the results of Kaya *et al*., (2015) when using a mixture of organic acids for laying hens did not affect the level of cholesterol and HDL.

Levels of Protein profile

The effect of the organic acid mixture on the level of protein profiles in the blood serum of male and female Japanese quails is shown in Table 3. In males, total protein increased significantly (P ≤0.05) to 2.40 and 2.53 mg/dl in T3 and T4 treatments compared with 2.30 and 2.33 mg/dl in T1 and T2 treatments, respectively. Also, globulin in males had a significant (P ≤ 0.01) increase in T4 (1.46 mg/dl) compared with T1 (1.23 mg/dl). In females, it was found that T3 and T4 had recorded significantly (P≤0.01) higher levels ofin albumin, which recorded 1.20 and 1.16 mg/dl albumin, respectively, compared to 0.96 mg/dl in T1.

The rise in serum total protein, albumin and globulin levels observed with organic acid supplementation is primarily due to enhanced nutrient absorption, improved liver protein synthesis, and stimulation of the immune system. These changes collectively contribute to better health, growth performance, and disease resistance in poultry, an increased rate of protein degradation and the improvement in protein digestion resulting from the increased activity of protein-digesting enzymes due to the increased acidity of the intestine. (Khan and Iqbal, 2016 ; Waghmare *et al*., 2025). The present results are consistent with the findings of Salem and El-Garhy (2021); they indicated a significant increase in total protein, albumin, and globulin when organic acids were added to the feed of laying hens. It also agrees with the results of Fikry *et al*. (2021), who found that adding citric acid to Japanese quail diets increased the levels of protein in the blood. However, these results of the present trail were not in agree with those of Pourreza *et al*., (2023), who reported that the addition of a mixture of organic acids, including acetic acid, did not cause a significant change in the levels of albumin in the blood serum of broiler chickens.

Biochemical parameters

Table 4 shows the effect of the mixture of citric acid and acetic acid on glucose level and certain indicators of liver and kidney functions in Japanese quails. The results indicate that in males showed a significant increase (P≤0.01) in glucose level across T2, T3 and T4, which recorded 251.42, 275.33

and 243.30 mg/dl respectively, compared to the control (235.33 mg/dl). In contrast, AST significantly decreased ($P \leq 0.01$) to 178.00, 168.67 and 167.66 U/L in organic acid treatments T2, T3, and T4, respectively when compared to control group (191.33 U/L). But, organic acid treatments had no significant effect on creatinine, uric acid, or ALT levels. The result also indicated that that female quails in the organic acid treatment groups (T2, T3, and T4) had a significant decrease ($P \leq 0.01$) in uric acid and AST levels compared with the control birds

The results indicate an increase in glucose levels in T2 and T4 males, as well as a reduction in uric acid and AST levels across most organic acid treatments for both sexes of Japanese quails. These results are in consistent with the findings of Elnagar and EL-Maaty (2017), who reported that adding citric acid to duck feed decreased the levels of urea, AST, ALT and creatine. The results of the study conducted by Dehghani-Tafti and Jahanian (2016) also witnessed that organic acids in broiler feed reduce uric acid. These findings are consistent with a study by Salem and El-Garhy (2021), who observed that using organic acids either alone or as a mixture including citric acid led to a slight decrease in AST levels in broilers. However, contrary results were obtained by Tomar *et al*., (2020) in broilers fed with organic acids had higher uric acid and AST . The addition of organic acids may increase the metabolic rate, as well as enhance energy mobilization, leading to the observed rise in serum glucose levels. In addition, organic acids may influence energy metabolism by modulating the expression of genes involved in gluconeogenesis and glycolysis, supporting a more efficient energy supply (Fouladi *et al*., 2018). Also, organic acids may improve the health and function of the gastrointestinal tract by lowering intestinal pH, which limits the proliferation of pathogenic bacteria and promotes beneficial microbiota. This inturn results in reduced endotoxin production and translocation into the blood, which decreases liver stress and liver cell integrity is preserved, leading to lower leakage of hepatic enzymes (Ma *et al*., 2021). Moreover, in birds, uric acid is the primary nitrogenous waste product resulting from amino acid catabolism. Improved digestive efficiency and amino acid absorption due to organic acid supplementation reduce the need for protein catabolism, thereby decreasing uric acid synthesis and excretion (Alaeldein *et al*., 2017).

CONCLUSION

Based on the findings from this study, we can conclude that supplementation of acetic acid and citric acid mixture in drinking water to japanese quail improves the hematological and certain biochemical parameters studied, which positively affects the health and productivity of the bird.

Table.1. The effect of an organic acid mixture on haematological parameters of Japanese quails at 42 days of age (Mean ± SE).

Male					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
WBC(×10³/µl)	1.36±0.01	1.33±0.00	1.35±0.00	1.35±0.02	NS
RBC (×10⁶/µl)	4.15±0.02[c]	4.31±0.00[b]	4.43±0.02[a]	4.38±0.01[a]	**
Hb (g/100 ml)	12.45±0.06[c]	12.93±0.01[b]	13.29±0.07[a]	13.14±0.00[a]	**
PCV (%)	39.35±0.21[c]	40.79±0.05[b]	41.87±0.22[a]	41.42±0.10[a]	**
Female					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
WBC(×10³/µl)	1.31±0.33	1.32±0.33	1.39±0.05	1.36±0.02	NS
RBC (×10⁶/µl)	3.52±0.05[b]	3.81±0.01[a]	3.83±0.02[a]	3.86±0.04[a]	**
Hb (g/100 ml)	10.65±0.17[b]	11.43±0.05[a]	11.49±0.07[a]	11.59±0.11[a]	**
PCV (%)	31.68±0.51[b]	34.29±0.15[a]	34.47±0.22[a]	34.77±0.33[a]	**

Means with different letters in the same horizontal row differ significantly (P ≤ 0.05); N.S= Non-Significant; (P≥0.05); = significant (P≤0.01).**

Table.2. Effect of an organic acid mixture on the lipid profile of quails at 42 days of age (Mean ± SE).

Male					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
Cholesterol(mg/dl)	250.33±2.60[a]	239.67±2.9[b]	233.31±0.8[b]	231.67±2.0[b]	**
Tri (mg/dl)	172.33±1.45[a]	163.67±1.4[b]	163.00±1.5[b]	152.00±1.7[c]	**
HDL (mg/dl)	45.00±1.15[b]	51.00±0.57[a]	50.67±0.67[a]	52.33±1.20[a]	**
LDL (mg/dl)	78.00±0.57[a]	67.33±0.33[b]	67.31±0.32[b]	66.57±0.29[b]	**
Female					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
Cholesterol(mg/dl)	246.00±1.15[a]	236.32±1.20[b]	234.67±1.85[b]	235.00±1.52[b]	**
Tri (mg/dl)	183.33±1.33[a]	170.00±0.57[b]	172.00±1.00[b]	152.33±1.20[c]	**
HDL (mg/dl)	43.67±1.20[b]	50.66±0.88[a]	53.00±1.15[a]	53.67±1.21[a]	**
LDL (mg/dl)	77.00±0.53[a]	68.00±0.57[b]	65.01±0.57[c]	67.66±0.88[b]	**

Means with different letters in the same horizontal row differ significantly ** = significant (P≤0.01).

Table.3. Effect of an organic acid mixture on the protein profile of quails at 42 days of age (Mean ± SE).

Male					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
Total protein(g/dl)	2.30±0.05[c]	2.33±0.03[c]	2.40±0.06[b]	2.53±0.03[a]	*
Albumin (mg/dl)	1.06±0.03	1.10±0.05	1.23±0.03	1.07±0.03	NS
Globulin (mg/dl)	1.23±0.08[b]	1.20±0.08[b]	1.16±0.03[c]	1.46±0.06[a]	**
Female					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
Total protein (g/dl)	2.46±0.17	2.67±0.08	2.67±0.13	2.80±0.10	NS
Albumin (mg/dl)	0.96±0.03[b]	1.03±0.03[b]	1.20±0.05[a]	1.16±0.03[a]	**
Globulin (mg/dl)	1.50±0.20	1.63±0.12	1.46±0.08	1.63±0.12	NS

Means with different letters in the same horizontal row differ significantlyNS Non-Significant (P≥0.05);* = significant (P≤0.05), ** = significant (P≤0.01).

Table.4. Effect of an organic acid mixture on select serum indicators of renal and hepatic function in quails at 42 days of age (Mean ± SE).

Male					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
Glucose (mg/dl)	235.33±5.84[c]	251.42±1.20[b]	275.33±3.10[a]	243.30±4.90[b]	**
Creatinine(mg/dl)	0.43±0.08	0.37±0.06	0.33±0.06	0.33±0.03	NS
Uric acid (mg/dl)	3.33±0.03	3.33±0.00	3.67±0.01	3.71±0.00	NS
AST (U/L)	191.33±4.06[a]	178.00±1.00[ab]	168.67±1.20[b]	167.66±1.45[b]	**
ALT(U/L)	9.67±0.88	7.17±0.72	9.16±0.60	7.83±0.44	NS
Female					
Parameters	**T1**	**T2**	**T3**	**T4**	**Significance**
Glucose (mg/dl)	224.67±1.76	225.31±1.85	225.29±0.67	224.33±1.66	NS
Creatinine(mg/dl)	0.43±0.03	0.27±0.03	0.33±0.03	0.30±0.05	NS
Uric acid (mg/dl)	4.67±0.33[a]	3.66±0. 31[ab]	2.67±0.30[b]	2.67±0.29[b]	**
AST (U/L)	182.33±2.07[a]	167.30±0.88[b]	165.27±2.81[b]	166.67±1.45[b]	**
ALT(U/L)	8.00±0.57	5.53±0.29	6.67±0.31	6.66±1.45	NS

Means with different letters in the same horizontal row differ significantly NS Non-Significant (P≥0.05) ; = significant (P≤0.01).**

ACKNOWLEDGEMENT

We would like to thank and appreciate the College of Agriculture - University of Diyala and animal production department for completing this study.

CONFLICT OF INTEREST

All authors of this study declare no conflict of interest.

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