Research Article

Effect of Addition of African Leaf Extract and Citric Acid on Quail (*Coturnix coturnix japonica*) Performance and Egg Yolk Chemical Quality

Sri Kismiati, Teysar A. Sarjana*, Luthfi D. Mahfudz, Dwi Sunarti, Edjeng Suprijatna, Rina Muryani, Hanna D. Shihah, Binti Ma'rifah, Nur M. Wahyuni

Department of Animal Science, Faculty of Animal Agricultural Sciences, Diponegoro University, Tembalang Campus, Semarang, Central Java, Indonesia.

Abstract | Mixing African leaf extract and citric acid enhanced the effectiveness of phytobiotic compounds in improving quail productivity. This study aimed to evaluate the effect of addition African leaf extract and citric acid separately, and on the performance and quality of quail eggs. This study used 240 female quails, 5 weeks old, with an average body weight of 111.21 ± 3.14 g. Quails were divided into four treatments and five replications, while the treatments tried were: control (T0), the addition of 2% African leaf extract in drinking water (T1), the addition of 0.5% citric acid in drinking water (T2) and a mixture of 2% African leaf extract with 0.5% citric acid in drinking water (T3). This study used a completely randomized design. The measured parameters were drinking water consumption, feed consumption, production performance (egg production, egg weight, egg mass, and feed conversion), and egg quality (protein, cholesterol, and yolk fat content). The results indicated that all treatments significantly improved water and feed consumption, egg weight, egg mass, feed conversion, and yolk composition (cholesterol, fat, and protein) compared to the control (P<0.05), and the best "production performance" result was obtained from T3 without affecting egg production. The study concluded that mixing 2% African leaves extract with 0.5% citric acid in drinking water produced the best results, improving feed conversion and reducing cholesterol and yolk fat content.

Keywords | Quail, Citric acid, African leaves extract, Performance and Egg quality

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***Correspondence** | Teysar Adi Sarjana, Department of Animal Science, Faculty of Animal Agricultural Sciences, Diponegoro University, Tembalang Campus, Semarang, Central Java, Indonesia 50275; **Email:** teysaradisarjana@lecturer.undip.ac.id
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INTRODUCTION

Quail (*Coturnix coturnix japonica*) farming offers promising prospects due to its low capital requirements, rapid returns, and efficient feed utilization compared to other poultry. Quails start producing eggs at 6 weeks, with egg production of 300 eggs/year and an average egg weight of 9-10.5 g (Aryee *et al*., 2020). The weakness of quails is that they are easily stressed, and the eggs produced contain high cholesterol. The optimal temperature for optimal egg production is 17 - 23 °C (Santos *et al*., 2019). Meanwhile, the average air temperature in Indonesia is 22-34.30 °C, which can potentially cause quail stress. The total cholesterol, High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL) contents of quail eggs were higher than those of the chicken eggs. Quail eggs contain 239.30 mg/dl cholesterol, 107.63 mg/dl HDL, and 23.39 mg/dl LDL, while chicken eggs contain 66.97 mg/dl cholesterol, 38.95 mg/dl HDL, and 17.74 mg/dl LDL (Ukachukwu *et al*., 2017). High cholesterol content is an obstacle for consumers because it can interfere with health and affect the income of quail farmers. Therefore, efforts are needed to lower cholesterol levels and reduce the effects of stress to increase egg production and quality. Heat stress affects the physiological and hormonal functions of poultry, reduces health, increases pathogenic bacteria, increases corticosterone hormones, reduces gonadotropin hormones, increases adrenocorticotropic hormones, reduces Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), and reduces egg production (Mangan and Siwek, 2023), intestinal health and immunity (Goel, 2021). Heat stress can reduce quail egg production performance (Batool *et al*., 2021). Vercese *et al*. (2012) proved that feed consumption, daily egg production, egg weight, and egg mass of quails kept at 36 °C were significantly lower than at 21 °C, while at 27 °C, the egg mass and weight decreased. Mangan and Siwek (2024) stated that phytobiotics can overcome heat stress and Melaku *et al*. (2021) stated that citric acid can reduce poultry stress. Mohamed and Hassan (2023) explained that phytobiotics are antimicrobial, antiviral, antioxidant, and can improve intestinal function. The addition of phytobiotics and citric acid significantly increased the height of the villus and the villus/crypt depth ratio in the ileum, jejunum, and duodenum in both immune and healthy poultry products (Gilani *et al*., 2021).

African (*Vernonia amygdalina*) leaves contain phytobiotics, namely flavonoids, alkaloids, saponins, tannins, and steroids, and the use of 4% African leaves meal rations can cure broilers affected by coccidiosis (Bonjoko *et al*., 2019). The use of 3 g/kg of African leaf flour increased the concentration of amylase and trypsin enzymes, as well as immunoglobulin A and immunoglobulin G, but had no significant effect on the digestive organs, including the pancreas, liver, proventriculus, gizzard, small intestine, and large intestine of broilers (Tokofai *et al*., 2023). Citric acid reduces pathogenic bacteria and intestinal pH, but is influenced by the method of use, age, and sex of poultry (Melaku *et al*., 2021). Mirakzeh *et al*. (2022) stated that the use of citric acid 5 g/kg in the ration had no significant effect on egg production and egg weight, but reduced feed consumption and feed conversion and increased the height, width, and surface area of the jejunal villi and the depth of quail crypts. The use of 6% citric acid increased the width and surface area of the intestinal villi, released the depth of the crypts, released the ratio of the height of the villi and the depth of the crypts of the jejunum, decreased broiler excreta bacteria (Oryza *et al*., 2021), increased quail egg production, and improved feed conversion (Ardianto *et al*., 2020). Citric acid increases the digestibility and absorption of nutrients, and the utilization of phosphorus (Shah and Fatima, 2018). Preciado-Rangel *et al*. (2018) and Dong *et al*. (2023) reported that citric acid can increase phenolic and flavonoid content. Adding flavonoids to feed increases egg production and reduces cholesterol and triglycerides (Nath and Aravindkumar, 2021). Prihambodo *et al*. (2022) stated that adding flavonoids reduces feed conversion; increases egg mass, eggshell compressive strength, and egg color; and reduces egg yolk cholesterol. Mixing citric acid with African leaf extract increased the phenolic and flavonoid contents, improving egg production and quality.

This study aimed to evaluate the effects of African leaf extract and citric acid in drinking water, either separately or in combination, on quail egg production and quality. The study results are expected to be a solution to eliminating the character of quail that is easily stressed, as indicated by improvements in egg production and quality, making it safer to consume.

MATERIALS AND METHODS

Animal ethics

The research was conducted at the Poultry Production Laboratory and Animal Feed Nutrition Laboratory and approved by the Ethical Appropriateness of Research Protocols, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia (60-12/A-24/KEP-FPP), released date June 12th, 2024.

Quail maintenance

Two hundred and forty quails (*Coturnix coturnix japonica*) aged 5 weeks with an average body weight of 111.21±3.14 g and kept in an open cage with an average temperature of 27.64±0.43 ºC and an average humidity of 86.33±2.95% with an additional two circulating 30" fan. Calculated heat stress index (HSI) in our research reaching up to 168,08, which is above the tolerable HSI value 150. The quails were subjected to environmental adaptation for 2 weeks and treatment applied for 7 weeks afterward. The ration used was a product from Charoen Pokphan (code B82P), with nutritional content as presented in Table 1. During maintenance, 50 g/head/day ration was provided for each quail, while drinking water was provided ad libitum.

Table 1: Contents PT. Charoen Pokphand Feed Nutrient Code B82P.

Nutrients	Contents nutrients
Water content (%)	13.0
Crude protein (%)	20.0
Crude fat (%)	7.0
Coarse fiber (%)	5.0
Metabolic energy (kcal /kg feed)	2,800.89
Ash (%)	12.0
Calcium (%)	2.5 – 3.5
Phosphorus (%)	0.6 – 1.0

African leaf extraction process

African leaves were obtained from the Klego District, Boyolali Regency, Central Java, Indonesia. The extract was prepared using the modified decoction method described by Kaneria *et al.* (2012). African leaves were cut into pieces and then oven-dried for 48 h at a temperature of 40 °C (Elshaafi *et al.*, 2020) and made into flour. Furthermore, 50 g of African leaf flour was mixed in 1 liter of water at a temperature of 100 °C in a water bath for 30 min, then cooled at room temperature for 5 min, and filtered using a 220 R micro Hettich centrifuge for 7 min.

Experimental design

This study used a completely randomized design with four treatments and five replicates. The treatments tested were: control (T0), addition of 2% African leaf extract (T1), addition of 0.5% citric acid (T2), and addition of a mixture of 2% African leaf extract and 0.5% citric acid (T3) to drinking water. Considerations level of treatment 2% African leaf combined with 0,5% citric acid determined based on optimum result in our previous research (Kismiati *et al.*, 2023) and Mirakzeh *et al.* (2022). Treatment of 2% African leaf extract was prepared by adding 20ml African leaf extract per L drinking water. T2 prepared by dissolving 5g citric acid powder per L drinking water, while T3 is a mixture of 20ml African leaf extract and 5g citric acid per L drinking water. Treatments was applied starts from 7 to 14 weeks old. The measured parameters included water consumption, feed consumption, production performance, observed and calculated daily then tabulated data provided weekly. The Egg quality is only observed at the end of the research. Egg production represented by quail day production (%); egg weight and egg mass (g).

Data collection

Data on water and feed consumption, egg production, and feed conversion were collected daily during the study, while egg quality (cholesterol, protein, and fat content of yolk) was determined by sampling yolks from three eggs per experimental unit/replication for 3 days at the end of the study. The yolk cholesterol content was analyzed using the Cholesterol Oxidase Para Amino Penazone (CHOD-PAP) method, egg yolk protein was analyzed using the Kjeldahl method, and yolk fat was extracted using the Soxhlet extraction method (AOAC, 2007).

Statistical analysis

Data analysis using ANOVA and Duncan's Multiple Range Test was performed using IBM® SPSS® statistics version 25 64-bit edition at a 95% confidence level.

RESULTS AND DISCUSSION

Water and feed consumption

The effects of addition 2% African leaf extract (T1) and 0.5% citric acid and their mixture on water and feed consumption data are shown in Table 2. Addition African leaf extract and citric acid separately or in combination significantly affected (P<0.01) water and feed consumption

Table 2: Effect of addition African leaf extract, citric acid and their mixtures in drinking water on water and feed consumption.

Weeks	T0	T1	T2	T3	P value
		Water consumption (ml/head/day)			
1	49.66±0.16^{d}	52.66±0.14^{a}	51.57±0.00^{b}	50.52±0.00^{c}	<0.01
2	80.69±0.00^{b}	81.57±0.07^{a}	79.21±0.18^{c}	74.98±0.19^{d}	<0.01
3	97.22±0.23^{c}	103.31±0.33^{a}	99.33±0.35^{b}	88.86±0.41^{d}	<0.01
4	98.09±0.43^{c}	103.90±0.18^{a}	102.59±0.52^{b}	87.19±0.44^{d}	<0.01
5	92.64±0.80^{c}	101.12±0.37^{b}	102.09±0.48^{a}	92.65±0.76^{c}	<0.01
6	99.12±0.28^{c}	104.46±0.14^{a}	100.08±0.30^{b}	100.45±0.90^{b}	<0.01
7	99.88±0.50^{b}	103.07±0.27^{a}	97.11±0.32^{c}	99.64±0.89^{b}	<0.01
Average	88.19±0.17^{c}	92.87±0.15^{a}	90.39^{b}±0.36	84.89±0.21^{d}	<0.01
		Feed consumption (g/head/day)			
1	28.11±0.43	28.14±0.61	28.16±0.51	28.06±1.11	0.99
2	25.89±1.87	25.53±1.83	26.54±1.15	27.56±1.41	0.23
3	28.88±1.85^{a}	25.90±0.10^{b}	28.57±0.53^{a}	29.01±0.85^{a}	<0.01
4	31.03±0.41^{a}	28.16±0.51^{c}	29.35±0.39^{b}	29.43±0.94^{b}	<0.01
5	31.55±0.22^{a}	27.93±0.30^{c}	29.61±1.20^{b}	30.09±0.98^{b}	<0.01
6	30.64±0.27^{a}	28.55±1.24^{b}	27.99±0.69^{b}	28.06±1.24^{b}	<0.01
7	28.35±0.51^{a}	26.84±0.57^{b}	27.41±0.83^{ab}	25.65±1.16^{c}	<0.01
Average	29.24±0.59^{a}	27.29±0.29^{c}	28.23±0.38^{b}	28.27±0.84^{b}	<0.01

^{a,b,c,d} different superscripts on the same row indicate significant differences (P<0.05).

Table 3: Effect of addition african leaf extract. Citric acid and their mixtures in drinking water on egg production, egg weight, egg mass and feed conversion.

Weeks	T0	T1	T2	T3	P-Value
Quail day production (%)					
1	65.00 ± 5.92	63.09±5.71	59.76 ± 2.84	70.24±12.11	0.20
2	80.24±15.26	78.57±8.79	80.71 ± 5.91	84.53 ± 5.05	0.79
3	87.86±10.08	83.81±6.66	87.14 ± 6.04	93.81 ± 6.65	0.24
4	88.57 ± 7.46	85.24±8.97	88.10 ± 6.30	93.10 ± 7.21	0.45
5	89.76 ± 8.85	86.90±8.42	86.43 ± 5.02	92.62 ± 4.93	0.49
6	83.33±11.54	91.43±6.09	85.95±10.22	90.48 ± 5.95	0.44
7	80.19± 5.90	84.81±7.08	82.59 ± 4.13	84.55 ± 5.92	0.58
Average	82.05± 6.87	81.98±5.34	81.52 ± 2.85	87.05 ± 4.75	0.31
Egg weight (g)					
1	11.55±0.28	11.36±0.21	11.46±0.21	11.24±0.17	0.20
2	10.55±0.14	10.55±0.20	10.51±0.25	10.70±0.11	0.55
3	10.73±0.13[bc]	10.63±0.21[c]	10.89±0.16[b]	11.18±0.12[a]	0.00
4	10.99±0.16[c]	11.36±0.19[b]	10.87±0.25[c]	11.83±0.30[a]	0.00
5	11.32±0.09[ab]	11.47±0.31[a]	11.10±0.06[b]	11.59±0.30[a]	0.00
6	10.89±0.15[bc]	10.94±0.20[b]	10.69±0.11[c]	11.62±0.14[a]	0.00
7	10.98±0.10[c]	11.26±0.09[b]	11.21±0.17[b]	11.51±0.08[a]	0.00
Average	10.95±0.08[c]	11.11±0.10[b]	11.02±0.08[bc]	11.44±0.06[a]	0.00
Egg mass (g)					
1	6.92±0.68	6.71±0.46	6.48±0.28	7.51±1.25	0.22
2	8.52±1.74	8.39±1.03	8.71±0.58	9.30±0.54	0.57
3	9.39±1.09[b]	9.02±0.72[b]	9.56±0.67[b]	10.67±0.74[a]	0.03
4	9.70±0.81	10.01±0.82	9.70±0.66	11.01±0.82	0.06
5	9.84±0.97	10.06±0.97	9.84±0.58	11.01±0.58	0.11
6	9.11±1.26	10.02±0.67	9.21±1.10	10.64±0.68	0.07
7	8.80±0.75	9.65±0.77	9.24±0.56	10.03±1.14	0.16
Average	8.90±0.80[b]	9.13±0.54[b]	8.96±0.33[b]	10.02±0.61[a]	0.03
Feed conversion					
1	4.15±0.36	4.38±0.32	4.63±0.21	3.88±0.63	0.06
2	3.18±0.67	3.11±0.47	3.10±0.20	2.99±0.12	0.91
3	3.12±0.23	2.95±0.23	3.02±0.21	2.73±0.14	0.06
4	3.23±0.25[a]	2.85±0.23[bc]	3.06±0.23[ab]	2.71±0.22[c]	0.01
5	3.25±0.32[a]	2.85±0.32[b]	3.03±0.09[ab]	2.75±0.12[b]	0.02
6	3.47±0.49[a]	2.87±0.11[b]	3.06±0.36[ab]	2.66±0.08[b]	0.01
7	3.27±0.28[a]	2.80±0.19[bc]	3.00±0.10[ab]	2.63±0.27[c]	<0.01
Average	3.38±0.26[a]	3.12±0.18[ab]	3.27±0.10[a]	2.91±0.14[b]	<0.01

[a,b,c] different superscripts on the same row indicate significant differences (P<0.05).

in weeks 1-7. Water consumption at T1, T2, was higher than that at T0, and T3 was the lowest of all; whereas feed consumption decreased with increasing water consumption (P < 0.01). According to Yoshida *et al.* (2022), the oral cavity of poultry has taste receptors, thus affecting water consumption behavior. Poultry prefer sweet, savory, sour, and fragrant odors at specific concentrations (Dey *et al.*, 2023). The addition of citric acid to drinking water increases water consumption (Ali *et al.*, 2020) and decreases feed consumption (Abou-Ashour *et al.*, 2021; Mirakzehi *et al.*, 2022). In other research, the inclusions of high-level CABP (Citric acid by product) increase quail water intake and reduces feed intake (Tanpong *et al.*, 2021). In our research, the citric acid level is lower, but the interaction effect with African leaf phytobiotics properties suggests possibilities in contributing to a decrease in feed intake. However, there have been no studies on the effects of African leaves on drinking water consumption.

Theoretically, the active compounds in African leaves can increase nutrient absorption, improve intestinal health, and modulate metabolic processes, which cause changes in feed and water consumption patterns. In a study conducted by Hosny *et al.* (2024), supplementation with flavonoid-rich additives resulted in increased water consumption and increased growth performance in quail, showing a positive impact on nutrient metabolism, which can indirectly have a positive impact on feed consumption (Xiyun *et al.*, 2009). African leaves have antioxidant activity, calculated based on the flavonoid content, with a capacity of 14,846 mg QE/g (Sukmawati *et al.*, 2017). In addition to flavonoid content, the active ingredient tannin plays a role in modifying lipid metabolism, which can decrease cholesterol levels in quail eggs. This can affect feed intake by changing energy balance and nutrient utilization (Erwan *et al.*, 2023). This can affect the feed consumption pattern. Quail has a normal feed consumption ranging from 20 to 35 g/head/day (Kurniawan *et al.*, 2015; Ashour *et al.*, 2021); changes in feed consumption in this study are still within the standard consumption pattern of laying quails. Therefore, the decrease in consumption in the combination of citric acid and African leaf additives is still positive because was accompanied by improved egg mass, which is a representation of improvements in the utilization of feed nutrients.

Egg production performance

The effects of addition 2% African leaf extract (*Vernonia amygdalina*), 0.5% citric acid, and their mixtures to drinking water on the production performance of quail are shown in Table 3. We found no significant difference in egg production and egg mass between T1 and T2 compared with the control, except for T3, which is the best among all. We speculate that citric acid has a synergetic effect with African leaf extract phytobiotics in enhancing their activities and improving feed digestibility and performance. Citric acid has been found to have a synergistic effect with antioxidant compound activity, such as alkaloids, polyphenols (Borda *et al.*, 2021), flavonoids activation (Dong *et al.*, 2023) and providing chelating properties which in turn facilitate better utilization of tannins (Shah *et al.*, 2018). Feed consumption decreased, whereas water consumption increased significantly (Table 2). The study showed that phytobiotic compounds from African leaf extracts "when combined with acidifier" could improve health and intestinal morphology and increase nutrient absorption, which is indicated by a decrease of FCR and an increase of feed efficiency up to 13,9% compared to the control. The increase in water consumption increases bioactive and citric acid intake, thus improving feed efficiency. The results of a study by Adetoro-Awopetu *et al.* (2021) showed that using 2% African leaf flour in the feed had no significant effect on egg weight. However, they also improved the quality of laying hen eggs. Using 3 g/kg of African leaf flour in feed increased the concentrations of amylase and trypsin enzymes, immunoglobulin A, and broiler immunoglobulin B (Tokofai *et al.*, 2023). African leaves contain phytobiotic/bioactive compounds such as terpenoids, tannins, alkaloids, flavonoids, and saponins (Edo *et al.*, 2023). Phytobiotics increase crypt depth and the ratio of villus height to crypt depth in the duodenum, jejunum, and ileum (Musa *et al.*, 2023), increasing lactic acid bacteria in the ileum, thereby increasing feed digestibility (Rabelo-Ruiz *et al.*, 2021; Khasanah *et al.*, 2024). Flavonoids have potent antioxidant effects, improve intestinal health and nutrient absorption, increase egg production increases (Negasa, 2024), and increase immunity and egg weight (Yang *et al.*, 2023). The data in Table 3 show that the addition of 0.5% citric acid had no significant effect on egg production, egg weight, or feed conversion, but increased egg mass only in the third week. Citric acid is antimicrobial, healthy, lowers intestinal pH (Melaku *et al.*, 2021), increases immunity (Fikry *et al.*, 2021), and improves intestinal morphology, microbiota, and nutrient absorption (Mirakzehi *et al.*, 2022; Xue *et al.*, 2023; Sedghi *et al.*, 2024). The study's results by Ardianto *et al.* (2020) show that adding 0.6% citric acid to feed increases egg production and improves quail feed conversion. According to Dhiab (2020), supplementation with 0.4% citric acid increased egg production and mass, increased egg weight, and decreased or improved feed conversion of laying hens, whereas Mirakzehi *et al.* (2022) stated that citric acid supplementation had no significant effect on egg production.

The mixture of 2% African leaf extract and 0.5% citric acid (T3) did not significantly affect egg production, but increased egg weight and mass, and improved feed conversion. We speculate that the results of this study may demonstrate synergy between the bioactive compounds of African leaves and the effects of citric acid on digestion. It is known that citric acid can enhance the bioavailability and stability of certain bioactive compounds (Sivaraj *et al.*, 2024). This could potentially amplify their effects on lipid metabolism. Citric acid itself has been shown to influence lipid metabolism by acting as an intermediate in the citric acid cycle, which is crucial for energy production and lipid synthesis. When combined with bioactive compounds, there could be a synergistic effect that enhances lipid metabolism modulation. According to Salas-Pérez *et al.* (2018), citric acid significantly increased the phytochemical biosynthesis of phenolic and flavonoid compounds. This resulted in no significant differences in egg production, egg weight, and feed conversion at T3 (Table 3), even though feed consumption was the lowest (T2).

Table 4: Effect of addition African leaf extract. Citric acid and their mixtures in drinking water on cholesterol. protein and fat content of egg yolk.

Parameter	T0	T1	T2	T3	P-value
Cholesterol (mg/100g)	8.58±0.61[a]	8.38±0.44[a]	7.82 ±0.62[a]	6.54±1.11[b]	0.02
Fat (%)	14.13±0.42[a]	13.52±0.37[ab]	13.04±0.18[b]	11.91±0.67[c]	<0.01
Protein (%)	14.80±0.49[a]	14.82±0.26[a]	14.40±0.13[a]	13.79±0.29[b]	<0.01

[a,b,c] different superscripts on the same row indicate significant differences (P<0.05).

Chemical quality of egg yolk

The cholesterol, fat, and protein content of the egg yolk are shown in Table 4. The addition of 2% African leaf extract (*Vernonia amygdalina*) (T1) did not significantly affect the cholesterol, fat, and protein contents of the yolk, whereas the addition of 0.5% citric acid (T2) significantly reduced yolk fat, but cholesterol and protein levels were not significantly different. These different patterns might be caused by different pathways of general fat, cholesterol, and protein absorption. While both fats and cholesterol are absorbed in the intestine (Sun *et al*., 2015; Mu *et al*., 2019), fats are primarily absorbed in the jejunum and ileum Rodriguez-Sanchez (2019 a, b), whereas cholesterol absorption is facilitated by specific transporters in the enterocytes. Fatty acids are transported as chylomicrons via the lymphatic system (Sun *et al*., 2015), whereas cholesterol is transported in micelles and incorporated into chylomicrons within enterocytes (Sun *et al*., 2015; Mu *et al*., 2019). In fat metabolism, metabolic pathways involve beta-oxidation and storage as triglycerides, whereas cholesterol metabolism includes conversion to bile acids and steroid hormones (Arshad *et al*., 2021; Bontempo *et al*., 2018; Chen *et al*., 2024). While there is no explicit connection between cholesterol pathways and protein absorption, both processes share some common transport mechanisms and cellular pathways thus efficiency of protein absorption can be influenced by the type and quality of dietary proteins, as well as the presence of other dietary components through metabolic interactions (He and Giussepin, 2014; Ten Have *et al*., 2007). The results of the study are in accordance with the research of Suci *et al*. (2023), that the administration of 3 ml/day of African leaf extract had no significant effect on egg yolk cholesterol, and Mirakzehi *et al*. (2022), that the addition of 0.5% citric acid had no significant effect on egg yolk cholesterol but increased egg weight. Yolk cholesterol was not significantly different, indicating that the phytochemical/bioactive compounds from African leaf extract could not lower cholesterol, as was citric acid. Darmawan *et al*. (2022) stated that the addition of bioactive compounds can reduce yolk cholesterol, and the effective dose to reduce cholesterol was 300 mg/kg. Different things in the mixture of African leaf extract (*Vernonia amygdalina*) 2% and citric acid 0.5% (T3) resulted in a decrease in cholesterol, fat, and protein contents in the yolk (P < 0.01).

Mixing African leaf extract with citric acid increases the effectiveness of phytochemicals in reducing yolk cholesterol. Salas-Pérez *et al*. (2018) reported that citric acid could significantly increase phenolic and flavonoid biosynthesis. Polyphenols reduce yolk cholesterol (Li *et al*., 2022; Tan *et al*., 2022) and inhibit adipogenesis, and increase lipolysis and apoptosis in adipose tissue cells, thereby reducing fat and cholesterol in poultry products (Tan *et al*., 2022) but bind lysozyme and change the structure of egg yolk protein (Gil *et al*., 2024). Flavonoids activate the lipase enzyme, which breaks down fat into fatty acids and glycerol, so that fat is not deposited in the product (Widyamanda *et al*., 2013). A mixture of herbs and organic acids lowers intestinal pH, reduces the activity of bile salt hormone (BSH) in converting conjugated bile salts into unconjugated and free primary bile acids (Dibamehr *et al*., 2021) and increases fat catabolism, and inhibits fat synthesis, thereby reducing fat deposition in the product (Arshad *et al*., 2021). The addition of flavonoids to feed reduces egg fat and cholesterol (Nath and Aravindkuma, 2021). Singh *et al*. (2020) reported that flavonoids lower cholesterol levels and increase protein synthesis. However, our results showed that a mixture of 2% African leaf extract and 0.5% citric acid reduced the protein content of the egg yolk. Even though there were % of yolk protein reduction, this condition is compensated by the significant increase in egg weight and egg mass, which represent higher total protein deposition, thus this change in yolk protein is insignificant.

CONCLUSIONS AND RECOMMENDATIONS

The study demonstrated that the addition of African leaf extract (2%) and citric acid (0.5%) in drinking water, either separately or in combination, significantly improved the performance and egg quality of quails (*Coturnix coturnix japonica*). While all treatments showed positive effects compared to the control, the combination of African leaf extract and citric acid (T3) yielded the most pronounced benefits. Specifically, T3 improved feed conversion efficiency, reduced yolk cholesterol and fat content, and increased egg weight and mass, without adversely affecting egg production. These findings highlight the synergistic effects of African leaf extract and citric acid. The phytobiotic compounds in African leaves, such as flavonoids, tannins, and alkaloids, likely enhanced nutrient absorption and intestinal health, while citric acid improved gut microbiota balance and nutrient utilization. Together, they optimized metabolic processes, leading to better feed efficiency and healthier egg composition. This study provides a practical and cost-effective solution for quail farmers aiming to enhance productivity and egg quality. Future research could explore the long-term effects of these additives, optimal dosage variations, and their impact on other poultry species. Additionally, investigating the mechanisms behind the observed synergy could further validate these findings and expand their applicability in poultry nutrition.

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NOVELTY STATEMENT

The addition of African leaf extract or citric acid separately has been proven effective in prior studies has been proven effective in improving laying hens performance and quality of egg yolks. We found that the combined addition of both was significantly more effective in improving the performance and nutritional quality of quail egg yolks.

AUTHOR'S CONTRIBUTION

Sri Kismiati and Teysar Adi Sarjana planned and designed the study, recorded and analyzed the data, and drafted and revised the manuscript. Luthfi Djauhari Mahfudz, Dwi Sunarti, Edjeng Suprijatna: Prepared the materials, conducted the study, and drafted the manuscript. Rina Muryani, Hanna Dzawish Shihah, Binti Ma'rifah, and Nur Maulida Wahyuni: writing-review and editing, formal analysis, and data curation.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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