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Performance and Physicochemical Quality of Quail Eggs Given Bandotan (*Ageratum conyzoides* L.) Leaf Extract

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ABSTRACT

The purpose of this study was to determine the influence of bandotan (*Ageratum conyzoides* L.) leaf extract on the production performance and physicochemical quality of quail eggs raised in tropical settings with ambient temperatures that exceed the comfort zone. A total of 200 female quails aged 6 weeks were reared in colony cages for 30 days. They were separated into four treatments with five replications, each with ten quails. Bandotan leaf extract was administered in dosages of 0%, 0.15%, 0.30%, and 0.45% per quail daily. The parameters measured included feed intake, feed conversion, egg production, egg mass, mortality, egg physical quality, and cholesterol and malondialdehyde (MDA) levels in egg yolks. Adding bandotan leaf extract to drinking water significantly improved quail production performance ($p < 0.05$). A dose of 0.30% resulted in the highest feed consumption, output, and egg mass, as well as significantly lower mortality rates, indicating its effectiveness in boosting the productivity and health of laying quails. Although it did not have a significant influence on the physical quality of eggs, administration of the extract at that dose significantly reduced MDA levels, egg cholesterol levels, and boosted antioxidant activity ($p < 0.05$). This demonstrates that bandotan leaf extract at a dose of 0.30% is beneficial in improving the production performance and chemical quality of heat-stressed quail eggs while preserving their physical quality.

Keywords: bandotan, eggs, malondialdehyde, productive performance, quail.

INTRODUCTION

Quail (*Coturnix coturnix japonica*) is a bird that provides a valuable source of animal protein in the form of eggs and meat. Quail are well-known for their rapid growth and reproduction, low feed requirements, and lack of need for big cages (Widyastuti *et al*. 2014). Despite their numerous advantages, quails are vulnerable to stress from environmental changes such as high temperatures and variable weather (Al-Sagan *et al*. 2020). In 2023, Indonesia's quail population declined by 2.94% (BPS 2024). One of the primary causes of this drop is that the air temperature in 2023 reached 27.2 °C (BMKG 2024), above the optimal temperature range for quail, which is 20 to 24 °C (Ulupi *et al*. 2016). High temperatures can produce heat stress in quail, which can reduce appetite, lower egg production and quality, raise mortality rates, and slow growth and reproduction (Gubali *et al*. 2021). Heat stress in animals can be alleviated by antioxidant treatment, which boosts immunity. Excessive use of antioxidants or synthetic antibiotics might have detrimental consequences, such as disturbing metabolic balance or creating antibiotic resistance, lowering the efficacy of future treatments. (Andiarna *et a*l. 2020; Margaretta *et al*. 2013). As a safer alternative, natural plant compounds with antioxidant and antibacterial capabilities can be employed to improve animal health.

Bandotan (*Ageratum conyzoides* L.) is a wild plant that thrives in tropical locations and has been used in traditional medicine in many countries, including Indonesia. This plant includes active chemicals such flavonoids, tannins, saponins, and triterpenoids, which have antioxidants and antibacterial properties (Agbafor *et al*. 2015). Flavonoids can serve as antioxidants by chelating free radicals by single electron transfer or donating hydrogen atoms, lowering the generation of malondialdehyde (MDA), a byproduct of fat oxidation (Hassanpour *et al*. 2023; Hasbullah *et al*. 2020). According to Almira *et al*. (2022), the leaves contain chemicals with antibacterial activity, such as tannins, that can suppress bacterial growth by interfering with the operation of bacterial DNA gyrase and disturbing bacterial cell function (Khameneh *et al.* 2019). Supriyadi *et al*. (2014) found that administering 5 mL of the leaf extract reduced blood cholesterol levels in broiler chicks. Hussain *et al*. (2021) discovered that active chemicals in bandotan plants, including phenols, flavonoids, and quercetin, had antioxidant properties that can prevent Eimeria infection in broiler chicks. This

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infection can harm the intestines, resulting in symptoms like diarrhea and decreased appetite, which are frequently linked to lipid peroxidation. Although a number of research has demonstrated the benefits of bandotan leaf extract, information on its impact on production performance and physicochemical quality of quail eggs remains scarce. As a result, the purpose of this research is to determine the effect of bandotan extract on quail egg production performance and physicochemical quality.

METHODS

Bandotan leaves were collected from Oelongko Village in Muna Regency, Southeast Sulawesi. The Pharmacology Laboratory of IPB University's School of Veterinary Medicine and Biomedicine extracted the leaves. Phytochemical assays on the leaf extract were performed at the Tropical Biopharmaceutical Study Center Laboratory at IPB University. Quail care was performed at Arkan Quail Farm in Ciampea, Bogor Regency. Egg quality was tested physically at the Poultry Nutrition Science Laboratory, Faculty of Animal Husbandry, IPB University, and chemically at the Biotech Center Laboratory, IPB University.

Bandotan leaves were macerated in 96% ethanol (Suprijatna *et al*. 2022). Khafid *et a*l. (2023) describe their method as qualitative phytochemical analysis. The flavonoids were evaluated by combining 0.2 g of extract with 5 mL of ethanol, boiling for 5 min, and then adding 2N HCl and Mg powder. A dark red color shift suggests the presence of flavonoids. Saponins were measured by combining 0.5 g of extract with 10 mL of distilled water, stirred, and added 2N HCl. The production of steady foam suggests the presence of saponins. Testing tannins with 1% $FeCl_3$ solution (ferric chloride) yields a positive result when a precipitate or dark blue to greenish black tint appears. Triterpenoids and steroids were evaluated using glacial acetic acid and H_2SO_4, resulted brownish colors for triterpenoids and greenish blues for steroids. Alkaloids were examined by adding Wagner's reagent, which produces a reddish-brown precipitate that indicates the presence of alkaloids.

This study included 200 healthy 6-week-old female quails with an average weight of 146 g. The quail went through a two-week adaptation phase prior to receiving the therapy. The quails were kept for a 30-day treatment. Maintenance was performed in a 200 × 75 × 225 cm cage divided into 20 blocks. Each block was filled with ten quails. Bandotan leaf extract was administered at doses of 0%, 0.15%, 0.30%, and 0.45% per 50 mL of water per head each day. The extract was administered every morning at 08.00 WIB. The feed was provided *ad libitum* with a commercial feed P-100. The feed's nutrient composition was as follows: maximum water content of 13.0%, minimum protein of 20.0%, maximum fat of 7.0%, maximum crude fiber of 7.0%, maximum ash of 14.0%, calcium of 2.50−3.50%, and total phosphorus of 0.60−1.00%. A Thermo Hygrometer TC-1 was used to monitor environmental temperature and humidity three times per day, at 07:00, 12:00, and 16:00 West Indonesia Time (humidity measurement range 10−99% RH). The temperature humidity index (THI) was determined using Tao and Xin's (2003) formula. The Animal Ethics Committee of the School of Veterinary Medicine and Biomedicine at IPB University accepted the study's supplies, tools, and procedures (approval number 235/KEH/SKE/VII/2024).

Feed consumption was calculated by subtracting the amount provided from the remaining amount and multiplying it by 100%. Feed conversion was calculated by dividing total feed consumption by egg mass during maintenance. Egg production was estimated by dividing the number of eggs by the number of quails and multiplying by 100%. The egg mass was calculated by multiplying the average egg weight by the egg production given in grams during maintenance. The percentage of quail mortality was determined by dividing the number of quails that died by the number of quails at the start of the observation period.

The egg weight was calculated by dividing the total egg weight by the number of eggs. The weight of the egg white and yolk were determined using a digital scale. The eggshell was weighed on a digital scale with an accuracy of 0.01 g, and the shell thickness was measured with a caliper. An egg yolk color fan (1−15 scale) was used to determine the egg yolk color score. The HU value was determined using the formula HU = 100 log (H+7.57-1.7.W0.37) (Fathi *et al*. 2020).

MDA levels were determined using the method described by Nisa *et al*. (2024). A total of 1.25 g of quail egg yolk was homogenized in 5 mL of phosphate-buffered saline (PBS) before centrifugation at 4000 rpm for 20 min. The resulting supernatant was utilized for MDA analysis, which included reactions with trichloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrochloric acid (HCl) solutions at 80 °C for 1 h. Following that, absorbance was measured at 532 nm. The Liebermann-Burchard method (Aviati *et al*. 2014) was used to measure cholesterol levels. Chloroform extract containing cholesterol reacts with anhydrous acetic acid and concentrated sulfuric acid, resulting in a color that was detected at 420 nm using a UV-visible spectrophotometer. The IC_{50} DPPH (2,2'-diphenyl-1-picrylhydrazyl) technique was used to analyze the antioxidant content of egg yolks (Julizan *et al.* 2019).

The data were analyzed using variance with the SPSS® 21.0 software. If there was a significant difference, the analysis was carried out using an honest

significant difference test with a 5% significance threshold.

RESULTS AND DISCUSSION

Environmental Conditions of the Cage

Based on Temperature Humidity Index (THI) data collected at three distinct times, the quail's heat stress levels fluctuated throughout the day. At 07.00 WIB, the THI was recorded at 27.77°C, which is within the normal category (<27.8°C) according to Hahn *et al*. (2021), indicating no thermal abnormalities. At 12.00 WIB, the THI reached 34.46°C, above the criterion of ≥30.0°C and placing the quail in the emergency category. This indicates extreme heat stress that could harm their health and productivity. At 16.00 WIB, the THI dropped to 30.65°C, but it remained in the emergency category and posed a risk of heat stress. The quails experiencing heat stress exhibit panting symptoms, which include breathing with an open mouth and an increase in respiratory rate. Under extended heat stress, this system becomes inefficient, resulting in electrolyte imbalance and decreased productivity (Ulupi *et al.* 2016). One way to mitigate the effects of heat stress is to administer natural antioxidants, such as bandotan leaf extract, which contains bioactive components that can boost quail tolerance to oxidative stress caused by high temperatures. Table 1 shows the temperature, humidity, and THI values.

Phytochemical Content

The qualitative phytochemical examination of bandotan leaf extract revealed that it contains flavonoids, saponins, tannins, triterpenoids, and steroids. The categories of active chemicals found in the leaf extract are listed in Table 2. Flavonoids have antioxidant action via binding reactive oxygen species, either by donating hydrogen atoms or transferring single electrons (Hassanpour *et al*. 2023). Saponins are antimicrobials that impair the stability of bacterial cell membranes (Chaudhary *et al*. 2018). Tannins can reduce blood cholesterol by inhibiting the activity of the HMG-CoA reductase enzyme involved in the synthesis of cholesterol in the liver. (Alagawany *et al.* 2021). Triterpenoids exhibit antiviral and antibacterial properties, preventing viral replication and destroying bacterial cell membranes (Hidayah *et al.* 2023). Steroids like sitosterol and stigmasterol reduce blood cholesterol by decreasing cholesterol absorption in the intestine and enhancing lipase enzyme activity (Cheng *et al*. 2020).

Production Performance

Bandotan leaf extract exhibited a substantial influence (p <0.05) on quail feed consumption, egg production, egg mass, and mortality rates. The 0.30% dose treatment produced the most feed, eggs, and egg mass, while the 0.30% and 0.45% dose treatments had the lowest death rates. Table 3 shows the findings of the quail performance observations made during the investigation.

Bandotan extract, which contains flavonoids, saponins, tannins, triterpenoids, and steroids, boosts poultry productivity through unique mechanisms in each constituent. Flavonoids operate as antioxidants by lowering oxidative stress in the digestive tract, activating Nrf2, and inducing the production of

Table 1 Temperature and humidity of the maintenance cage environment

Measurement time (West Indonesia Time)	Temperature (°C)	Humidity (%)	THI (°C)
07:00	24–28	70–89	27.77
12:00	31–35	64–77	34.46
16:00	25–31	69–84	30.65

Table 2 Active compounds of bandotan leaf extract

Active ccmpounds	Result
Flavonoids	(+)
Saponins	(+)
Tannins	(+)
Triterpenoids	(+)
Steroids	(+)

Table 3 Quail production performance given bandotan leaf extract

Parameter	Treatment			
	T0	T1	T2	T3
Feed consumption (g/bird)	19.62±0.52^{a}	22.04±0.49^{b}	25.93±0.74^{c}	21.75±1.30^{b}
Feed conversion ratio	2.85±0.09	2.81±0.07	2.70±0.08	2.80±0.15
Egg production (%)	73.32±2.26^{a}	75.57±5.41^{ab}	80.07±2.83^{b}	74.63±2.83^{ab}
Egg mass (g/bird/day)	206.34±2.00^{a}	235.16±1.03^{b}	287.21±1.89^{c}	232.68±2.09^{b}
Mortality (%)	14.00±5.48^{a}	6.00±5.48a^{b}	4.00±5.48^{b}	4.00±5.48^{b}

Remarks: Values are followed by superscripts $^{a, b, c}$ in the same row indication a significant difference (p < 0.05). T0 = 0% (Control); T1 = 0.15% extract; T2 = 0.30% extract; T3 = 0.45% extract.

antioxidant genes including HO-1, which improves nutrient absorption and feed consumption (Pratiwisitha and Margiana 2023). Furthermore, flavonoids have been shown to soothe the central nervous system, reduce anxiety, and enhance the release of digestive fluids, resulting in increased hunger (Aliwu *et al.* 2020). Saponins balance intestinal microbiota by lowering infections and activating TLR4 (Toll-like receptor 4), which boosts the immune system and improves digestion (Ruysschaert and Lonez 2015). Tannins attach to bacterial proteins, killing them, and boost calcium absorption via Cav1 (calcium channel), promoting the development of stronger eggshells (Ningsih and Sanjaya 2022). Triterpenoids improve energy metabolism by activating AMPK (AMP-activated protein kinase), reduce inflammation by reducing NF-κB expression, and enhance reproductive health by enhancing ovulation (Khafid *et al*. 2023). Steroids activate estrogen receptors (ERα and ERβ) to promote folliculogenesis and ovulation, leading to increased egg production (Wicaksono *et al*. 2013). In general, bandotan extract can boost feed consumption and egg quality while decreasing quail mortality rates by enhancing digestive health, increasing energy metabolism, and strengthening the immune system.

According to Agbafor *et al.* (2015), bandotan leaves contain a variety of minerals, including calcium and phosphorus, as well as microminerals including Fe, Cu, Mn, and Zn. These minerals' content is critical in supporting biological activities, such as quail egg formation. Minerals such as calcium and phosphorus are required for eggshell production, whereas microminerals such as Fe, Cu, Mn, and Zn help with body metabolism, ovarian health, and hormonal balance, all of which promote the reproductive process.

Physical Quality of Eggs

The administration of bandotan leaf extract did not show any significant differences between the treatments given. Guo *et al.* (2020) stated that bioactive compounds have more influence on biological parameters such as liver health and the immune system compared to the physical quality of eggs directly (Table 4). The weight of quail eggs given the extract ranged from 11.53 to 11.98 g, in line with the report of Suci *et al.* (2019). The weight of egg white ranged from 6.80 to 7.00 g, and the weight of egg yolk was 3.40 to 3.60 g, higher than the findings of Nastiti *et al.* (2014). The administration of leaf extract revealed no significant changes between the treatments administered. According to Guo *et al*. (2020), bioactive chemicals have a greater influence on biological indicators such as liver health and the immune system than they do on egg quality.

The weight of quail eggshells varied from 1.23 to 1.29 g, which is higher than Najian *et al*. (2021), and the shell thickness was 0.19−0.20 mm, which is thicker than previous finding by Najian *et al*. (2021). The color score of egg yolk ranged between 3.5 to 3.8, which was greater than Kurnia *et al.* (2022). The Haugh unit value ranged from 89.12 to 92.73, placing it in the Quality I category according to BSNI 01-3926-2008.

Chemical Quality of Quail Eggs

Table 5 shows the results of evaluating the physical quality of quail eggs. Bandotan leaf extract significantly decreased the levels of MDA, cholesterol, and antioxidant activity (IC_{50}) in quail egg yolks. The leaf extract used at a 0.45% treatment dose can reduce MDA, cholesterol, and IC_{50}. Ridwan *et al*. (2020) showed that animals fed high-tannin feed may experience stress because tannin reduces nutrient absorption and digestive function, as well as interfering with body metabolism by suppressing digestive enzymes such as amylase and protease (Akbar *et al*. 2023). The administration of extract at doses of 0.15% and 0.30% produced the greatest results with the lowest MDA levels, 0.75 and 0.73 mg/kg. The leaf extract's significant antioxidant activity may explain the decrease in MDA levels in egg yolks. This conclusion is consistent with the findings of Nisa *et al*. (2024), who showed that administering moringa flour containing antioxidant chemicals in the form of flavonoids in quail diet reduced MDA levels in quail egg yolks.

The administration of 0.15% and 0.30% leaf extract resulted in the lowest cholesterol levels (742.69 mg/dL and 666.09 mg/dL, respectively). Supriyadi *et al*. (2014) found that bandotan leaf extract can lower blood cholesterol in broiler chicks. Choi and Kim (2020) discovered that tannins can reduce HMG-CoA activity in poultry livers, lowering cholesterol levels. Liu *et al*. (2020) have shown that tannins can suppress cholesterol metabolism. According to Chaudhary *et a*l. (2018), saponins can reduce blood cholesterol by binding to and enhancing the excretion of bile acids. Cheng *et al*. (2020) found that steroid chemicals like stigmasterol can lower cholesterol by inhibiting absorption in the intestine and activating the lipase enzyme. Sentosa *et al.* (2017) discovered that reduced blood cholesterol levels cause cholesterol to be transported to eggs to decrease, resulting in lower cholesterol levels in egg yolks.

The IC_{50} values of antioxidants in egg yolks treated with bandotan leaf extract (0.15%, 0.30%, and 0.45%) were lower than the control, indicating that the extract has a greater ability to neutralize free radicals. According to Sulistyani *et al*. (2024), the lower the IC_{50} value, the more effective the molecule is at combating free radicals. Liu *et al*. (2020) have discovered that active substances such flavonoids, tannins, and saponins can boost antioxidant capacity by enhancing the activity of endogenous antioxidant enzymes.

Table 4 Physical quality of quail eggs given bandotan leaf extract

Parameter	Treatment			
	T0	T1	T2	T3
Egg weight (g)	11.34±0.89	11.98±0.42	11.86±0.44	11.53±0.23
Albumin weight (g)	6.65±0.46	6.65±0.15	7.00±0.43	6.80±0.07
Egg yolk weight (g)	3.40±0.33	3,40±0,25	3.60±0.02	3.50±0.17
Egg shell weight (g)	1.29±0.12	1.29±0.04	1.25±0.04	1.23±0.03
Egg shell thickness (mm)	0.20±0.01	0.20±0.01	0.19±0.01	0.19±0.00
Egg yolk color value	3.55±0.61	3.55±0.77	3.75±0.60	3.85±0.80
Haugh unit	92.73±1.46	92.73±1.93	89.12±1.46	89.77±1.79

Remarks: T0 = 0% (Control); T1 = 0.15% extract; T2 = 0.30% extract; T3 = 0.45% extract.

Table 5 Chemical quality of quail eggs given bandotan leaf extract

Parameter	Treatment			
	T0	T1	T2	T3
MDA (mg/kg)	$1.73±0.04^{a}$	$0.75±0.01^{b}$	$0.73±0.01^{b}$	$1.53±0.58^{a}$
Cholesterol (mg/dL)	$829.39±7.78^{a}$	$742.69±4.18^{b}$	$666.09±16.41^{c}$	$788.01±23.69^{d}$
Antioxidants IC_{50} (µg/mL)	$873.00±30.25^{a}$	$758.06±35.01^{bc}$	$739.48±25.81^{c}$	$824.92±36.86^{ab}$

Remarks: Values are followed by superscripts $^{a, b, c}$ in the same row indicating a significant difference ($p < 0.05$). T0 = 0% (Control); T1 = 0.15% extract; T2 = 0.30% extract; T3 =0.45% extract.

Endogenous antioxidant enzymes limit the number of free radicals in the body and strengthen the antioxidant defense system, so protecting the lipids in egg yolks from oxidative damage.

CONCLUSION

Bandotan (*Ageratum conyzoides* L.) leaf extract administered at a concentration of 0.30% through drinking water has been shown to improve quail production performance. This treatment can enhance feed consumption, egg production, and mass while decreasing mortality rates. Furthermore, the leaf extract reduces malondialdehyde (MDA) and cholesterol levels in quail eggs that have been exposed to high temperatures.

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