Research Article

Effect of *Phaleria macrocarpa* Fruit Extract and Inulin Supplementation in Quail Feed on Ileal Microbial Population and Egg Production Performance

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Abstract | The purpose of this study was to determine the effects of low-dose supplementation with *Phaleria macrocarpa* fruit extract and inulin on gut microbiota, egg production performance, and quality in quail. A total of 300 laying quails were divided into 6 treatments and 5 replications, of which each experimental unit used 10 quails. The experimental treatments consisted of basal feed (T0), basal feed supplemented with 0.15% zinc bacitracin (T0+), basal feed supplemented with 0.20% *Phaleria macrocarpa* fruit extract (T1), basal feed supplemented with 0.40% *Phaleria macrocarpa* fruit extract (T2), basal feed supplemented with 0.20% *Phaleria macrocarpa* fruit extract and 0.20% inulin (T3), basal feed supplemented with 0.40% *Phaleria macrocarpa* fruit extract and 0.40% inulin (T4). The variables observed were microbial population, egg performance, and quality. Data were analysed using ANOVA in a Completely Randomized Design and significant results were tested using Duncan's Multiple Range Test. The results showed that supplementation with *Phaleria macrocarpa* fruit extract and inulin at levels up to 0.40% did not significantly affect ($P > 0.05$) the total plate counts of *Escherichia coli* and lactic acid bacteria or quail egg production performance. However, supplementation numerically increased hen-day production (HDP) and improved feed conversion ratio (FCR). Significant effects ($P < 0.05$) were observed on eggshell thickness, albumin volume, yolk volume, yolk color score, and the total plate count of *Salmonella* sp. In conclusion, supplementation with 0.40% *Phaleria macrocarpa* fruit extract reduced the *Salmonella* sp. population and improved egg quality in laying quails.

Keywords | Egg production performance, Ileal microbial population, Inulin, Natural growth promoter, *Phaleria macrocarpa* fruit extract, Phytobiotics

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INTRODUCTION

The use of antibiotics as antibiotic growth promotor (AGP) in the livestock industry has been officially banned under the Ministry of Agriculture Regulation No. 14/PERMENTAN/PK.350/5/2017. This ban is based on experts' concerns regarding the negative impacts of excessive antibiotic use. The primary concern is the emergence of antibiotic-resistant bacteria, which could potentially hinder the effectiveness of treatments for both

humans and animals. Additionally, the potential presence of antibiotic residues in animal products, such as meat and eggs, poses a risk to consumer health (Urban *et al*., 2024).

Phytobiotics have emerged as a safe and effective alternative to AGP because their bioactive compounds leave no residues. Secondary metabolites such as tannins, saponins, flavonoids, and essential oils play a crucial role in enhancing production, reproductive performance, and immune system function in animals (Kuralkar and Kuralkar, 2021). One promising herbal plant is the fruit of *Phaleria macrocarpa,* which contains active compounds such as alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, and glycosides, all of which act as natural antimicrobial agents (Rizki *et al*., 2023; Alara *et al*., 2016). The population of *Escherichia coli* in broiler chickens infected with *Escherichia coli* did not significantly decrease when supplemented with 10-20% *Phaleria macrocarpa* leaf extract compared to the positive control (Diyantoro and Rochmi, 2019). Meanwhile, the use of 2.5-5.0% *Phaleria macrocarpa* fruit extract, with or without nano-encapsulation, significantly increased the population of lactic acid bacteria in broilers (Ningsih *et al*., 2019).

The use of inulin as a prebiotic in poultry feed helps balance the gut microbiota through fermentation, enabling the production of short-chain fatty acids (SCFAs), lowering intestinal pH, and inhibiting pathogenic bacteria (Buclaw, 2016). Previous research reported by Nabizadeh (2012) indicated that 0.5% inulin supplementation increased the population of *Bifidobacterium* and reduced *Escherichia coli* in the ileum of broilers. The use of inulin supplementation in quail feed increased weekly body weight from 2 to 5 weeks of age when used at a minimum of 0.5% (Fatima *et al*., 2025). Li *et al*. (2025) reported that the use of <1% inulin in poultry has positive effects on gut health, antioxidant activity, and microbiota modulation. A more recent study by de la Mora *et al*. (2014) using higher inulin supplementation levels (2-6%) reported that inulin increased egg production and weight, with an optimal level of 2%. The previous study was limited by its focus on broiler chickens and the use of higher doses. Therefore, this study established lower treatment doses of 0.20% and 0.40%. These specific levels were determined based on preliminary observations and represent incremental steps within the sub-therapeutic range that are safe for quail. This study aimed to determine the effects of low-dose supplementation with *Phaleria macrocarpa* fruit extract and inulin on gut microbiota, egg production performance, and quality in quail.

MATERIALS AND METHODS

Extract preparation and characteristics

The process of making *Phaleria macrocarpa* fruit extract was prepared following a method previously developed and published by Nufus *et al*. (2026). Briefly, the extraction process was performed as follows: *Phaleria macrocarpa* fruits were washed, and the seeds were separated from the pulp. The pulp was then cut into small pieces before extraction. Extraction was performed using ultrasound-assisted hydrodistillation extraction (UAHDE) with distilled water at a material to solvent ratio of 1:4. Sonication was conducted at 40 kHz for 20 minutes, followed by hydrodistillation at 93˚C for 60 minutes. The resulting *Phaleria macrocarpa* fruit extract contained 228.02 mg GAE/100 g of total phenolic compounds and 21.61 mg QE/100 g of total flavonoids.

Animal and experimental design

A total of 300 laying quails aged 3 months were divided into six treatments and five replications, with 10 quails in each replication. Each cage was equipped with a feeder and drinker. The feed used was commercial P800 laying quail feed produced by a commercial company (New Hope Ltd) was obtained from a local poultry shop, with the feed composition shown in Table 1. The *Phaleria macrocarpa* fruit extract was purchased from a supplier in Blitar, East Java, and the inulin powder, purchased online under the trade name Orafti functional fibres, is an inulin-type fructan derived from chicory root, an imported product from Belgium. The dietary treatments consisted of basal feed (T0), basal feed supplemented with 0.15% zinc bacitracin (T0+), basal feed supplemented with 0.20% *Phaleria macrocarpa* fruit extract (T1), basal feed supplemented with 0.40% *Phaleria macrocarpa* fruit extract (T2), basal feed supplemented with 0.20% *Phaleria macrocarpa* fruit extract and 0.20% inulin (T3), basal feed supplemented with 0.40% *Phaleria macrocarpa* fruit extract and 0.40% inulin (T4).

Table 1: Nutritional content of quail feed.

Parameter	Composition
Moisture	Max. 13.00%
Crude protein	Max. 21.00%
Crude fat	Min. 3.00%
Crude fiber	Min. 7.00%
Ash	Max. 14.00%
Calcium	2.50-3.50%
Total phosphorus	Min. 0.45%
Total aflatoxin	Max. 50 µg/kg
Amino acids	
Lysine	Min. 0.90%
Methionin	Min. 0.40%

Microbial population in quail ileum

Data collection for ileal microbiology tests was carried out after 6 weeks of treatment in quail. Five birds per treatment were randomly selected and humanely slaughtered. The ileum was cut 3-4 cm before the ileocecal junction, and

the ileal contents were placed into labeled sterile tubes and stored on dry ice for laboratory analysis. Lactic acid bacteria, *Escherichia coli*, and *Salmonella* sp. were cultivated on MRSA (de Man, Rogosa, and Sharpe agar), EMBA (Eosin Methylene Blue agar), and SSA (Salmonella-Shigella agar), respectively. Total plate count (TPC) was determined using the pour plate method following the procedure described by Yuliati *et al.* (2021). Ileal content samples were serially diluted up to 10^{-8}. Dilutions of 10^{-2} and 10^{-3} were used for the detection of *Salmonella* sp. on SSA, 10^{-3} and 10^{-4} for *Escherichia coli* on EMBA, and 10^{-7} and 10^{-8} for lactic acid bacteria on MRSA. A total of 1 ml of inoculum from each dilution was placed into a Petri dish, then 15 ml of agar medium was added. Next, the sample was incubated at 37°C for 24-48 hours in an inverted position before colony counting. The number of bacteria was calculated based on the number of colonies that grew on the Petri dish from the selected dilution. The number of colonies was then multiplied by the inverse of the dilution factor using the formula: Number of bacteria = number of colonies × 1/dilution factor. The final results were expressed in CFU/mL.

Egg production performance in quail

Production performances, including feed intake (g/bird), hen day production (%), egg mass (g), feed conversion ratio (FCR), and income over feed cost (IDR/bird), were measured. Feed intake was recorded as the difference between the feed provided and the remaining feed (g). HDP was calculated by dividing total egg production by the number of animals. Egg mass was calculated by multiplying HDP by egg weight. FCR was calculated by dividing total feed intake by total egg weight, and IOFC was calculated by dividing the difference between egg sales revenue and feed costs.

Egg quality

External and internal egg quality evaluations were conducted at the end of the treatment period (week 6). External quality included egg weight (g), egg index (%), and eggshell thickness (µm). Internal quality included yolk weight (g), albumen weight (g), yolk volume (ml), albumen volume (ml), and yolk colour score. Yolk and albumen volumes were measured directly using a measuring cylinder after the yolk was separated from the albumen. The yolk colour was assessed by using a DSM egg yolk colour fan.

Statistical analysis

The treatments in this study included two additional components (*Phaleria macrocarpa* fruit extract and inulin), but the experimental design was not a full factorial, as the T3 and T4 treatments represented predetermined dose combinations. Therefore, data were analyzed using a one-way ANOVA in a completely randomized design in IBM SPSS Statistics 22. If the results were significant, the data were further tested using Duncan's Multiple Range Test.

RESULT AND DISCUSSION

Effects of supplementation with *Phaleria macrocarpa* and inulin on the total bacterial population

Based on the data in Table 2, the treatments used had a significant effect (P<0.05) on the *Salmonella* sp. population. The lowest *Salmonella* sp. populations were found in T2 and T4, both of which were lower than the positive control. The reduction in *Salmonella* sp. population in T2 might be attributed to the flavonoid content in the *Phaleria macrocarpa* fruit. Flavonoid of *Phaleria macrocarpa* fruit was reported to contain 21.61 mg EQ/100g (Nufus *et al*, 2026). Flavonoids act by disrupting DNA replication and increasing bacterial cell membrane permeability, thereby causing cell death (Górniak *et al*., 2019). Meanwhile, in T4, in addition to the effects of flavonoids, the added inulin also suppressed *Salmonella* sp. Inulin fermented by gut microbiota produces short-chain fatty acids (SCFA) that lower the intestinal pH, thereby creating conditions unfavourable for the growth of *Salmonella* sp. (Birmani *et al*., 2019; Urban *et al*., 2024). Meanwhile, T3 produced a lower population than T2 and T4, possibly because the lower antimicrobial dose was insufficient to suppress pathogenic bacterial growth. Thus, flavonoids from *Phaleria macrocarpa* with inulin suppress the population of *Salmonella* sp., which is in line with the report by Fatima *et al*. (2025) that inulin supplementation in quail feed can reduce *Salmonella* sp. population.

Table 2: The effect of supplementation with *Phaleria macrocarpa* fruit extract with and without inulin on the ileal bacterial population.

Diets	*Salmonella* sp.	*Escherichia coli*	LAB
	Log CFU/g		
T0+	5.27 ± 0.50^{b}	4.45 ± 0.50	9.60 ± 0.10
T0	4.24 ± 0.20^{ab}	3.82 ± 1.00	9.89 ± 0.40
T1	4.06 ± 0.60^{ab}	4.98 ± 0.60	9.69 ± 0.10
T2	3.60 ± 0.30^{a}	3.79 ± 0.50	9.73 ± 0.60
T3	4.38 ± 1.10^{ab}	4.65 ± 1.20	9.50 ± 0.50
T4	3.14 ± 0.80^{a}	3.82 ± 0.50	9.16 ± 0.70
SEM	0.89	0.84	0.49
p-value	<0.05	>0.05	>0.05

Note: Data are presented as mean ± standard deviation. Values marked with different letters in the same column indicate significant differences (P< 0.05).

The populations of *Escherichia coli* and LAB showed no significant differences among treatments (P>0.05). The lowest *Escherichia coli* populations were also found in

T2 and T4. The ability of *Phaleria macrocarpa* extract to suppress *Escherichia coli* populations has been reported by Diyantoro and Rochmi (2019) and is also explained by the same mechanism of action of flavonoids, namely by interfering with DNA replication and damaging bacterial cell membranes (Górniak *et al*., 2019). However, the treatment can greatly reduce *Salmonella* sp. populations compared to *Escherichia coli*. In addition, the values obtained for the LAB population in this study (9.16-9.89 log CFU/g) are within the expected range for the gut microbiota of healthy quail, consistent with the statement of Fatima *et al*. (2025). Meanwhile, in treatment T3, it was lower than in T2 and T4, possibly due to the lower dose, and was therefore not effective enough at suppressing the *Escherichia coli* bacterial population. It should be noted that the antimicrobial properties of flavonoids are not always selective. At certain concentrations, flavonoids can also affect the growth of beneficial bacteria such as LAB (Markowiak and Slizewska, 2018). This was evident in T4, which had the lowest LAB population numerically, but also the lowest *Salmonella* sp. population. This fact may indicate that the T4 antimicrobial effect on bacterial cell destruction is stronger against pathogens than against LAB.

Overall, the supplementation of quail feed with *Phaleria macrocarpa* fruit extract and inulin, particularly in T2 and T4, was effective in suppressing *Salmonella* sp. populations without significantly disrupting the balance of LAB. Overall, supplementation of quail feed with *Phaleria macrocarpa* fruit extract with inulin, particularly in T2 and T4, was effective in suppressing *Salmonella* sp. populations without disrupting the balance of LAB. Flavonoids directly damage bacterial cell walls, and inulin plays an indirect role in creating an acidic intestinal environment through SCFA production, so this phytobiotic and prebiotic supplementation has the potential to be an alternative AGP in maintaining quail intestinal health.

Effect of supplementation with *Phaleria macrocarpa* and inulin on quail production performance

Based on the data in Table 3, the treatments had no significant effect (P>0.05) on production performance parameters compared with the control group. This indicates that *Phaleria macrocarpa* fruit extract can maintain production performance comparable to that of commercial antibiotics. Feed intake in all treatments was not significantly different, indicating that *Phaleria macrocarpa* extract did not impair the palatability of quail feed. The bioactive flavonoid and phenolic compounds did not alter the sensory properties of the feed, consistent with previous reports on phytogenic additives (Mahfuz *et al*., 2021; Christaki *et al*., 2020). It is noteworthy that the feed intake values remained within the normal physiological range for quails, reflecting adequate nutrient absorption.

The highest HDP was observed in T2 (82.80%) and T4 (82.00%), both of which were higher than that of the positive control (81.40%). This data is similar to that observed in egg mass, with T1 and T2 showing higher values than the positive control. This increase may have occurred due to a decrease in *Salmonella* sp. and *Escherichia coli* in T2, resulting in more optimal nutrient absorption for egg production. The reduction in bacteria observed in this experiment might be attributed to the antibacterial activity of compounds in *Phaleria macrocarpa* fruit. The alkaloids, flavonoids, and tannins in *Phaleria macrocarpa* fruit possess antibacterial activity that suppresses intestinal pathogens (Rizki *et al*., 2023; Alara *et al*, 2016). Additionally, supplemented flavonoids as low as 0.2% was reported to improve the FCR in laying hens, even better than the positive control (Liu *et al*., 2014).

The highest IOFC was observed in T1 (IDR. 171.74) and T2 (IDR. 171.04), which were higher than the positive control (IDR. 158.46). The IOFC value is influenced by feed intake, egg production, and egg weight (Hilmi *et al*., 2015). Although not significant, this value indicates that the use of *Phaleria macrocarpa* fruit extract is cost-efficient, comparable to antibiotic administration. The combination with inulin did not produce a significant effect,likely because the dosage used was not yet optimal. Overall, the absence of significant differences between the phytobiotic treatments and the positive control is a

Table 3: The effect of supplementation with *Phaleria macrocarpa* fruit extract with and without inulin on quail egg production performance.

Variables	Diets						SEM	p value
	T0+	T0	T1	T2	T3	T4		
FI (g/bird)	27.18 ± 0.70	27.18 ± 0.73	27.18 ± 0.92	26.99 ± 0.31	27.13 ± 0.66	27.24 ± 0.51	0.11	>0.05
HDP (%)	81.40 ± 5.12	77.80 ± 6.83	79.20 ± 5.21	82.80 ± 6.34	80.80 ± 4.43	82.00 ± 3.80	0.94	>0.05
Egg Mass (g)	9.84 ± 0.50	9.64 ± 0.75	10.13 ± 1.26	10.09 ± 0.74	9.81 ± 0.51	9.96 ± 0.51	0.13	>0.05
FCR	2.82 ± 0.16	2.88 ± 0.19	2.83 ± 0.15	2.72 ± 0.19	2.80± 0.16	2.81 ± 0.10	0.28	>0.05
IOFC (IDR/bird)*	158.46±16.91	154.10±23.72	171.74±40.82	171.04±23.85	156.38±17.54	152.78±13.53	4.30	>0.05

*price of egg/kg: IDR 35,000 while the price of feeds/kg was IDR 6,760 (T0), IDR 6,850 (T0+), IDR 6,780 (T1), IDR 6,800 (T2), IDR 6.960 (T3), IDR 7,160 (T4), respectively.

Table 4: The effect of supplementation with Phaleria macrocarpa fruit extract with and without inulin on egg quality.

Variables	Diets						SEM	p value
	T0+	T0	T1	T2	T3	T4		
Egg weight (g)	12.12 ± 0.43	12.56 ± 0.47	12.19 ± 0.33	12.37 ± 0.19	12.39 ± 0.28	12.75 ± 0.62	0.079	>0.05
Egg index (%)	76.95 ± 1.40	77.01 ± 1.99	77.98 ± 0.32	77.92 ± 1.45	78.23 ± 1.41	78.55 ± 0.98	0.252	>0.05
Eggshell thickness (µm)	0.27 ± 0.01^{b}	0.23 ± 0.01^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.003	<0.01
Albumin volume (ml)	6.70 ± 0.43^{a}	7.46 ± 0.37^{b}	7.18 ± 0.19^{b}	7.40 ± 0.17^{b}	7.46 ± 0.25^{b}	7.56 ± 0.49^{b}	0.077	<0.01
Yolk volume (ml)	4.10 ± 0.30^{ab}	3.90 ± 0.27^{a}	3.82 ± 0.16^{a}	3.84 ± 0.29^{a}	4.00 ± 0.07^{ab}	4.30 ± 0.26^{b}	0.051	<0.05
Albumin weight (g)	5.95 ± 0.37	6.38 ± 0.23	6.23± 0.19	6.37 ± 0.20	6.34 ± 0.12	6.27 ± 0.32	0.050	>0.05
Yolk weight (g)	4.07 ± 0.22	4.03 ± 0.17	3.93 ± 0.14	3.93 ± 0.13	4.06 ± 0.20	4.25 ± 0.26	0.038	>0.05
Yolk color score	3.82 ± 0.25^{a}	4.40 ± 0.40^{b}	4.36 ± 0.24^{ab}	4.38 ± 0.19^{ab}	4.18 ± 0.24^{ab}	4.50 ± 0.37^{b}	0.064	<0.01

Note: Data are presented as mean ± standard deviation. Different superscripts in a row indicate significant differences (P<0.05), with high significance indicated when P< 0.01 is reported.

key finding, demonstrating that *Phaleria macrocarpa* fruit extract has the potential to serve as a safe, effective, and equivalent alternative to AGP without the risks of residues or resistance (Urban *et al*., 2024).

Effects of supplementation with *Phaleria macrocarpa* fruit extract and inulin on egg quality

Based on the data in Table 4, the treatments did not significantly affect egg weight or index (P>0.05). This indicates that the treatments did not disrupt the diet's nutritional balance, allowing egg weight to remain within the normal range. In addition, the treatments did not interfere with protein and lipid metabolism, which are involved in the formation of egg components (Kuralkar and Kuralkar, 2021). Egg weight is positively correlated with the egg index; an increase in egg weight is accompanied by an increase in egg length and width, which are the primary components used to determine the egg index. In another study, the egg index was influenced by genetic and physiological factors of the poultry rather than the dietary treatment factors. Larger eggs are more elliptical, while smaller eggs are rounder (Sun *et al*., 2019).

Eggshell thickness in the treatment groups (T1-T4) did not differ significantly from the negative control (T0) but was significantly lower than the positive control (T0+). The higher shell thickness in the T0+ group is thought to be due to antibiotic use, which may increase the efficiency of calcium and micromineral absorption by improving intestinal wall morphology. Meanwhile, in the T1-T4 treatments, the mineral balance in the feed was maintained to ensure calcium deposition in the eggshells remained within the normal range (Olgun *et al*., 2022). This mineral balance is supported by the antimicrobial activity of alkaloid and flavonoid compounds in *Phaleria macrocarpa*, which help suppress intestinal pathogenic bacteria (Rizki *et al*., 2023; Alara *et al*., 2016). Additionally, treatment T4 included inulin, which supports the growth of beneficial bacteria while reducing pathogenic bacteria in the ileum; thus, a healthy intestinal environment facilitates more efficient mineral absorption (Li *et al*., 2018).

Albumin volume results showed that the positive control group (T0+) with zinc bacitracin had a significant decrease compared with the negative control group (T0) and all treatment groups (T1-T4). This indicates that antibiotic use can affect the decrease in albumin volume. This is suspected because the T0+ treatment can also affect the balance of the intestinal microbiota and reduce the activity of beneficial bacteria that produce short-chain fatty acids, thereby affecting the efficiency of nutrient absorption and affecting intestinal health (Liu *et al*., 2021). Disturbances in the intestinal microbiota can also affect amino acid utilization for albumin synthesis and albumin quality, thereby reducing egg volume (Suthama *et al*., 2023). Meanwhile, the groups given *Phaleria macrocarpa* fruit extract with or without inulin (T1–T4) did not differ significantly from the negative control group (T0). This indicates that phytobiotic and prebiotic treatments prevented a decrease in albumen volume and maintained it within the normal range. This is supported by the important role of inulin in maintaining intestinal health by increasing short-chain fatty acid production and optimizing nutrient absorption, including protein (Soliz-Cruz *et al*., 2020; Buclaw, 2016). The antioxidant compounds in *Phaleria macrocarpa* fruit extract may also help improve birds' physiological condition and reduce oxidative stress, thereby supporting albumin quality, although no significant difference in albumin weight was observed.

In this study, yolk volume differed significantly between treatments (P < 0.05). The highest results in the T4 group were significantly different from those of the T0, T1, and T2 groups. However, the T3 and T0+ groups did not differ significantly from the group with the lowest values (T0, T1, and T2) or the group with the highest value (T4). The intermediate position in the T0+ group is thought to be

related to the antibiotic's anatomical effect, which can improve intestinal morphology by thinning the intestinal wall, thereby helping to maintain the mechanical efficiency of macronutrient absorption (Hung *et al*., 2020). However, the use of this antibiotic also triggers an imbalance in the intestinal microflora, with an increase in the *Salmonella* sp. population. The combination of improved intestinal wall structure and suboptimal microflora results in intermediate nutrient absorption for yolk volume. Meanwhile, in the T3 group, the addition of *Phaleria macrocarpa* fruit extract and inulin at this dose began to have a positive effect on microflora improvement and nutrient absorption, but had not yet reached the optimal level as shown in the T4 group. The increase in yolk volume in this study indicates a physical change in the yolk that is not fully reflected in the yolk weight, possibly due to factors such as density or water content. The addition of *Phaleria macrocarpa* fruit extract and inulin contributes to supporting intestinal health and increasing nutrient absorption, supported by the prebiotic inulin and bioactive compounds in *Phaleria macrocarpa* fruit extract, which play a role in providing sufficient lipid and protein substrates for yolk formation (Ferrocino *et al*., 2023).

Yolk color scores increased significantly ($P < 0.01$), with the highest score observed in T4, which was higher than the positive control. The low color score in the T0+ treatment is suspected to be caused by antibiotics that are not the main source of color pigments or antioxidants that can maintain yolk color. The addition of zinc bacitracin in the T0+ treatment is suspected to directly interfere with carotenoid absorption in the intestine or disrupt the intestinal microflora responsible for pigment metabolism, resulting in a significantly lower color score than in T0. Yolk color is influenced by carotenoids from feed deposited into the yolk. The efficiency of yolk deposition is influenced by optimal absorption of carotenoid pigments, supported by improved gut health (Kljak *et al*., 2021). Inulin supplementation can increase the diversity of beneficial microbes and reduce pathogen levels, thereby optimizing carotenoid absorption (Li *et al*., 2018). In addition, flavonoid supplementation has been reported to modulate egg quality and protein metabolism in laying hens (Iskender *et al*., 2017) and, as antioxidants, help prevent carotenoid oxidation, thereby optimizing color deposition in the yolk (Zurak *et al*., 2022). The supplementation of *Phaleria macrocarpa* fruit extract with inulin can increase the absorption of carotenoids, which contributes to the improvement of egg yolk color.

CONCLUSION

This study found that supplementation with 0.40% *Phaleria macrocarpa* fruit extract significantly affected eggshell thickness, albumin volume, yolk volume, yolk color score, and the total *Salmonella* sp. population. However, it did not significantly affect the total population of *Escherichia coli* and lactic acid bacteria, or quail egg production performance. Still, it showed a numerical trend toward increased HDP and decreased FCR. Therefore, further research using higher doses and longer periods is needed to determine the combined microbial interaction between *Phaleria macrocarpa* extract and inulin.

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NOVELTY STATEMENT

First, this study systematically evaluated the effects of *Phaleria macrocarpa* fruit extract, with and without inulin, on gut microbiota and egg quality in laying quail. This study demonstrated significant suppression of *Salmonella* bacteria and improved egg quality parameters. Second, unlike previous studies that used chickens and nano-encapsulated *Phaleria macrocarpa* fruit extract in drinking water, this study used *Phaleria macrocarpa* fruit extract added to feed. Thus, this study offers a new integrative framework demonstrating the effects of *Phaleria macrocarpa* fruit extract in supporting gut microbiota balance and improving egg quality and productive performance in quail.

AUTHORS CONTRIBUTION

NAN and EW: Conception and design of the research, Analysis and interpretation of data, drafting the manuscript. NAN: Acquisition of data. HT, YFN, AAH: Critical review/revision.

Ethical approval

The research has been approved by the Research Ethics Committee (Animal Care and Use Committee) of the Faculty of Veterinary Medicine, Universitas Brawijaya (Approval Number 59-KEP-FKHUB-2025).

Generative AI and AI-assisted technology statement

While drafting this manuscript, the author used DeepSeek and Grammarly to correct grammar and sentence structure. After using these tools, the author manually reviewed and

edited the sentences. The author assumes full responsibility for the content and accuracy of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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