Asian Journal of Agriculture and Biology
https://doi.org/10.35495/ajab.2026.057

Original Article

Evaluation of *Ageratum conyzoides* L leaf powder as a phytogenic feed additive to improve the quality of quail eggs

Dede Kardaya*, Anggraeni, Dewi Wahyuni, Agung Puji Haryanto, Annisa Fajrianty, Emir Zavian
Department of Animal Sciences, Faculty of Agriculture, Universitas Djuanda, Bogor, Indonesia

**Corresponding author's email:* dede.kardaya@unida.ac.id
Received: 15 February 2026 / Revised: 12 April 2026 / Accepted: 23 April 2026 / Published Online: 09 May 2026

Abstract

The increasing restrictions on antibiotic growth promoters and the growing demand for natural feed additives have encouraged the exploration of phytogenic plants as sustainable alternatives in poultry production. Medicinal herbs containing bioactive compounds are widely recognized for their potential to enhance productivity, health status, and product quality in laying birds. *Ageratum conyzoides* L., a plant rich in flavonoids, alkaloids, and essential oils, has demonstrated antimicrobial and antioxidant properties, suggesting its possible application in improving egg quality. This study aimed to evaluate the effects of *A. conyzoides* leaf powder as a phytogenic feed additive on the egg quality of Japanese quails (*Coturnix coturnix japonica*). A total of 140 quails, aged 65 days, were used in an 8-week experiment arranged in a Completely Randomized Design with four treatments and five replications. Birds were fed commercial diets supplemented with *A. conyzoides* leaf powder at 0% (R1, control), 1% (R2), 3% (R3), and 5% (R4). Internal and external egg quality parameters were evaluated. Supplementation at 3% (R3) significantly increased egg white weight (5.34 g) compared with 1% (4.94 g) and 5% (5.03 g), while remaining comparable to the control (5.41 g). No significant differences were observed in yolk weight, yolk percentage, or yolk color, and Haugh Unit values exceeded 85 in all treatments. External egg quality improved dose-dependently, with 5% inclusion (R4) increasing eggshell weight and thickness by 7.8% and 8.6%, respectively. In conclusion, *A. conyzoides* leaf powder represents a promising phytogenic additive, with 3% inclusion optimizing albumen quality and 5% inclusion enhancing eggshell strength and commercial value.

Keywords: Albumen quality, *Babadotan*, Phytogenic additive, Poultry nutrition, Shell integrity

How to cite this article:
Kardaya D, Anggraeni, Wahyuni D, Haryanto AP, Fajrianty A and Zavian E. Evaluation of *Ageratum conyzoides* L leaf powder as a phytogenic feed additive to improve the quality of quail eggs. Asian J. Agric. Biol. 2026: e2026.057. DOI: https://doi.org/10.35495/ajab.2026.057

Introduction

Quail production has expanded substantially in many regions, particularly within smallholder and peri-urban farming systems, due to the rapid economic returns and high nutritional value of Japanese quail eggs. Consequently, egg quality plays a central role in determining farmer income and consumer acceptance (Nasaka et al., 2018) The Japanese quail (*Coturnix coturnix japonica*) is widely utilized in commercial poultry production because of its rapid growth rate, early sexual maturity, and high egg production efficiency. Nutritional strategies that enhance both external and internal egg characteristics therefore have direct implications for productivity and market competitiveness. Egg quality parameters, including shell strength, yolk color, and nutritional composition, strongly influence consumer preference and market value (Oyebanji and Atoki, 2018).

Conventional poultry production systems have traditionally relied on antibiotic growth promoters, synthetic vitamins, and chemical additives to improve performance and maintain bird health. However, increasing regulatory restrictions and public concern regarding chemical residues and antimicrobial resistance have accelerated interest in natural feed alternatives (Iliopoulou et al., 2025). The growing demand for antibiotic-free poultry products has intensified the search for sustainable feed additives capable of maintaining production efficiency while ensuring food safety. In this context, phytogenic feed additives have emerged as promising substitutes for synthetic growth promoters. Among potential candidates, *Ageratum conyzoides* (*babadotan*), an herbaceous species widely distributed in tropical regions, has received attention due to its traditional medicinal use and rich phytochemical profile, including saponins, tannins, terpenoids, alkaloids, phenols, and flavonoids (Adeoye et al., 2023).

Phytogenic feed additives, such as herbs, spices, essential oils, and leaf meals, have been reported to exert growth-promoting, antimicrobial, antioxidant, and immunomodulatory effects in poultry. Previous studies have demonstrated their positive influence on nutrient utilization, gut health, and stress-related gene expression, particularly under environmental challenges (Iliopoulou et al., 2025). Research on laying quails and chickens has indicated improvements in egg number, shell quality, and internal traits when phytogenic additives are administered at appropriate inclusion levels (Kour et al., 2024). Furthermore, phytochemical analyses of *A. conyzoides* have revealed strong antioxidant activity, which may protect egg lipids and proteins from oxidative degradation and enhance yolk stability and nutritional value (Adeoye et al., 2023). Its antimicrobial and anticoccidial properties may also contribute to improved intestinal health and nutrient absorption, thereby indirectly supporting egg production and quality (Tchankugni et al., 2019).

Several feeding trials using herbal supplements, including thymol, turmeric, garlic, fenugreek, and black cumin, have reported improvements in yolk composition, shell thickness, and Haugh units (Chongtham et al., 2015; Fernandez et al., 2019; Ndlovu et al., 2025; Youssef et al., 2023). Other dietary interventions, such as chromium yeast and fermented leaf meals, have also been associated with enhanced reproductive performance and albumen quality (Oyebanji and Atoki, 2018; Utami and Akbar, 2025). Nevertheless, excessive inclusion levels of certain botanical additives may adversely affect laying performance, highlighting the importance of appropriate dose optimization. Despite these advances, limited information is available regarding the effects of *A. conyzoides* leaf powder on quail egg quality, particularly under controlled experimental conditions, indicating the need for further investigation.

Based on its antioxidant, antimicrobial, and immunomodulatory properties (Adeoye et al., 2023; Tchankugni et al., 2019). *A. conyzoides* leaf powder is hypothesized to improve internal and external egg quality parameters, including shell strength, yolk stability, and albumen quality. It is further hypothesized that supplementation at appropriate inclusion levels will enhance nutrient absorption and intestinal health, leading to improved egg production efficiency without compromising bird performance or health.

Therefore, this study aimed to evaluate the effects of *Ageratum conyzoides* L. leaf powder as a phytogenic feed additive on quail egg quality, determining optimal supplementation levels, assessing its influence on internal and external egg characteristics, and to examine its potential contribution to sustainable and economically viable poultry nutrition practices.

https://doi.org/10.35495/ajab.2026.057

Material and Methods

Livestock used
A total of 140 Japanese quails (*Coturnix coturnix japonica*) aged 65 days with an average body weight of 140 ± 10 g were used in this study. The quails were selected based on uniform body weight, health status, and egg production performance to ensure consistency across experimental groups.

Housing system
The quails were housed in battery cage systems constructed from galvanized wire. The housing facility consisted of 20 individual cage units, each measuring 65 × 45 × 35 cm (length × width × height). Each cage unit accommodated 7 quails, providing adequate space for movement and feeding behavior. The cages were equipped with feeding troughs and nipple drinkers to ensure continuous access to feed and water.

Feed material
The experimental diets consisted of commercial layer rations supplemented with babadotan leaf powder (*Ageratum conyzoides* L.) at varying inclusion levels. The nutritional composition of both the commercial ration and *Ageratum conyzoides* L. leaf powder is presented in Table 1.

Table-1. Nutritional composition of feed ingredients.

Nutrient	Commercial Ration (%)	*Ageratum conyzoides* Leaf Powder (%)
Dry Matter	90.73	87.69
Crude Protein	22.48	25.20
Crude Fat	6.7	4.55
Crude Fiber	6.07	12.94
Ash	16.07	12.41
NFE	39.41	32.59
ME (kcal/kg)	2750	2180

Note: Values are presented on dry matter basis.

Research Design
This study employed a Completely Randomized Design (CRD) with four dietary treatments and five replications per treatment. Each experimental unit consisted of 7 quails, resulting in a total of 140 birds distributed across 20 cage units.

Experimental treatments
The experimental rations were formulated as follows: R1: Commercial feed without *Ageratum conyzoides* L. leaf powder supplementation (control), R2: Commercial feed + 1% *Ageratum conyzoides* L. leaf powder, R3: Commercial feed + 3% *Ageratum conyzoides* L. leaf powder, and R3: Commercial feed + 5% *Ageratum conyzoides* L. leaf powder.
The ration formulations are detailed in Table 2, and the calculated nutritional content of each experimental diet is presented in Table 3.

Table-2. Experimental ration formulations.

Ingredient	R1 (%)	R2 (%)	R3 (%)	R4 (%)
Commercial Feed	100.0	99.0	97.0	95.0
Ageratum conyzoides L. Leaf Powder	0.0	1.0	3.0	5.0
Total	100.0	100.0	100.0	100.0

R1: Commercial feed without *Ageratum conyzoides* L. leaf powder supplementation (control), R2: Commercial feed + 1% *Ageratum conyzoides* L. leaf powder, R3: Commercial feed + 3% *Ageratum conyzoides* L. leaf powder, and R3: Commercial feed + 5% *Ageratum conyzoides* L. leaf powder.

The R1–R4 diets were formulated by diluting a standard quail layer ration with 1–5 % *A. conyzoides* leaf powder ((Table 2). Because the commercial feed already satisfies quail nutritional standards for laying quail (~20–24% crude protein and ~2700–3000 kcal/kg metabolizable energy), and because the additive was included at low levels, the resulting diets retained nutrient profiles within recommended ranges. This ensures that any observed differences in egg quality across R1–R4 can be attributed to the phytogenic additive rather than to deficiencies or excesses of protein, energy or minerals.

Table-3. Calculated nutritional content of experimental rations.

Nutrient	R1	R2	R3	R4
Dry Matter (%)	90,73	91,60	93,36	95,11
Crude Protein (%)	22,48	22,73	23,24	23,74
Crude Fat (%)	6,70	6,74	6,84	6,93
Crude Fiber (%)	6,07	6,20	6,46	6,72
Ash (%)	16,07	16,19	16,44	16,69
NFE (%)	39,41	39,73	40,39	41,04
ME (kcal/kg)	2750	2744	2733	2722

R1: Commercial feed without *Ageratum conyzoides* L. leaf powder supplementation (control), R2: Commercial feed + 1% *Ageratum conyzoides* L. leaf powder, R3: Commercial feed + 3% *Ageratum conyzoides* L. leaf powder, and R3: Commercial feed + 5% *Ageratum conyzoides* L. leaf powder.

Observed variables

The observed variables consisted of internal and external quail egg quality. Internal quail egg quality consisted of egg weight, egg white (albumen) weight, egg yolk weight, egg white percentage, egg yolk percentage, Haugh Unit (HU), and egg yolk color. Egg white, and egg yolk were weighed using a digital analytical balance with 0.01 g precision. Egg white weight was determined by carefully separating the albumen from the yolk and weighing using an analytical balance. Egg Yolk Weight was measured after complete separation from albumen, including the vitelline membrane, using an analytical balance. Percentage of Egg White was calculated using the following formula:

Egg white percentage (%) = (Egg white weight / Total egg weight) × 100

Percentage of Egg Yolk was calculated using the formula:

Egg yolk percentage (%) = (Egg yolk weight / Total egg weight) × 100

Haugh Unit (HU) was determined using the formula established by (Haugh, 1937):

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37})$$

Where H = albumen height (mm) and W = egg weight (g). Albumen height was measured using a tripod micrometer at the thickest part of the albumen adjacent to the yolk.

Egg yolk color was assessed using the Roche Yolk Color Fan scale (1-15) under standardized lighting conditions (Lokaewmanee et al., 2011). Each yolk was compared against the color fan immediately after separation.

External quail egg quality included egg weight, eggshell weight, eggshell thickness, egg shape index, and eggshell percentage, egg grade, and eggshell quality. Individual egg was weighed using a digital analytical balance with 0.01 g precision. Eggshell weight was measured after complete removal of shell membranes and thorough washing and air-drying for 24 hours at room temperature (Stadelman et al., 1995).

Eggshell thickness was measured at three points (blunt end, equator, and sharp end) using a digital micrometer with 0.01 mm precision, and the average was calculated (Hamilton, 1982).

Egg Shape Index was calculated as the ratio of egg width to egg length, measured using digital calipers (Duman et al., 2016).

Shape Index = (Egg width / Egg length) × 100

Percentage of eggshell weight was calculated using the formula as follows:

Eggshell percentage (%) = (Eggshell weight / Total egg weight) × 100

Egg grade classification

Eggs were graded according to USDA standards (Alig et al., 2023). based on shell quality, air cell depth, albumen quality, and yolk condition: Grade AA: Air cell ≤ 3.2 mm, clear and firm albumen, yolk outline slightly defined. Grade A: Air cell ≤ 6.4 mm, clear and reasonably firm albumen, yolk outline fairly well defined. Grade B: Air cell > 6.4 mm, weak and watery albumen, yolk outline plainly visible.

Cleanliness, Integrity and Texture of Eggshell. The parameters of cleanliness, integrity and texture of the shells are grouped based on their grades consisting of Grades AA/A and B. Grade AA and A qualification for eggshell assessment have the same specifications. Eggs are grouped based on their grades and then given an assessment score, grade AA/A is given a score of 1 while grade B is given a score of 2. Eggs that have been grouped and given a score are then calculated for their average value at each test and treatment. According to (Alig et al., 2023) the standard specifications for egg hell assessment are divided into 2 categories as follows:

Grade AA and A: It has a clean and intact shell, normal texture and shape, no foreign objects stick.

Grade B: Dirty/foreign bodies, abnormal shape and texture.

Preparation of housing facility

Prior to the arrival of experimental birds, all cage units were thoroughly cleaned and disinfected using quaternary ammonium compounds. Feeders and waterers were inspected and calibrated to ensure proper function. The facility was equipped with adequate ventilation and lighting systems to maintain optimal environmental conditions.

Preparation of *Ageratum conyzoides* L. leaf powder

Fresh *babadotan* (*Ageratum conyzoides* L.) leaves powder was harvested during the vegetative growth stage from plants approximately 30-45 days old. The leaves were cleaned to remove dirt and foreign materials, then air-dried under shade for 72 hours until moisture content reached approximately 10-12%. The dried leaves were ground using a hammer mill equipped with a 1 mm screen to produce fine powder. The powder was stored in airtight containers at room temperature until use.

Phytochemical analyses show that *A. conyzoides* contains a diverse array of these bioactive classes (Table 4). The diversity of these phytochemicals explains why *A. conyzoides* can act as a phytogenic additive.

Table-4. Bioactive constituents of *Ageratum conyzoides*.

Bioactive constituents	Positive effects in livestock
Flavonoids and phenols: kaempferol, quercetin, quercetin-3-rhamnopiranoside, hyperoside, luteolin, apigenin, nobiletin and eupalestin.	Strong antioxidant protection, reducing oxidative stress and supporting immune function, and anti-inflammatory activities.
Polymethoxyflavones: nobiletin	Modulate inflammation and hormone signalling, suppressing pro-inflammatory enzymes and improving endocrine balance.
Benzofurans and chromenes: Essential oils from *A. conyzoides* contain benzofuran derivatives (precocene I and precocene II) and ageratochromene dimer, along with chromene and coumarin	Contribute to the plant's insecticidal, antimicrobial and anti-inflammatory effects and are often used to deter parasites and pathogens
Alkaloids and phenolic acids: The plant also produces caffeic acid, echinatine, phytol and pyrrolizidine alkaloids.	Provide antimicrobial, antiparasitic and hepatoprotective properties, helping to maintain gut health and liver function in animals
Sterols and terpenes: Sterols such as stigmasterol, β-sitosterol and friedeline and terpenes like α-pinene, β-pinene and eugenol	β-Sitosterol and related phytosterols have phytoestrogenic effects, which can modulate endocrine function and improve reproductive performance; influence reproductive hormones and may aid eggshell formation and fertility.

https://doi.org/10.35495/ajab.2026.057

	Terpenes and eugenol exhibit broad-spectrum antimicrobial activity and stimulate digestion.
Additional flavonoids, alkaloids, terpenoids and coumarins: 1,2-benzopyrone, quercetin, hyperoside, kaempferol, nobiletin, luteolin, apigenin and eupalestin	Deliver antimicrobial and antiparasitic effects that enhance gut health and nutrient utilization
Tannins and saponins	Contribute to antibacterial and digestive regulation when supplied at appropriate levels

Modified from Chahal et al. (2021).

Quail management

The experimental period lasted for 8 weeks, during which quails were provided with experimental diets *ad libitum* and continuous access to clean water. Daily management practices included monitoring feed consumption, egg collection, and health status observation. Environmental temperature was maintained at 22-26°C with relative humidity of 60-70%. A lighting program of 16 hours' light and 8 hours' darkness was implemented throughout the study period.

Egg sampling and analysis

Eggs were collected daily at 16:00 hours and stored at room temperature (25°C) 24 hours before analysis to allow for stabilization. From each experimental unit, 10 eggs were randomly selected weekly for quality assessment, resulting in a total of 80 eggs analyzed per week across all treatments. All measurements were conducted by trained personnel using standardized protocols to minimize variation.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using a Completely Randomized Design model. Treatment means were compared using Duncan's Multiple Range Test when significant differences ($P < 0.05$) were detected. All statistical analyses were performed using SPSS software version 25.0. The statistical model used was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} = observed value of the dependent variable; μ = overall mean; T_i = effect of the i-th treatment; ε_{ij} = random error associated with the j-th observation in the i-th treatment.

Data are presented as means ± standard deviation (SD), and statistical significance was declared at $P < 0.05$.

Results and Discussion

Internal quality of quail eggs across experimental diets

The effects of dietary supplementation with *Ageratum conyzoides* L. leaf powder on internal egg quality parameters of quail eggs are presented in Table 5. The experimental design included four dietary treatments: R1 (0% *Ageratum conyzoides*), R2 (1% *Ageratum conyzoides*), R3 (3% *Ageratum conyzoides*), and R4 (5% *Ageratum conyzoides*).

Table-5. Average internal quality of quail eggs.

Parameters	Experimental diets			
	R1	R2	R3	R4
Egg white weight (g)	5.41±0.59[b]	4.94±0.27[a]	5.34±0.20[b]	5.03±0.22[a]
Egg Yolk Weight (g)	3.65±0.08	3.92±0.23	3.69±0.15	3.85±0.37
Percentage of Egg Yolk (%)	36.00±0.77	37.00±2.10	36.42±1.81	37.10±2.55
Egg White Percentage (%)	52.10±0.83[b]	49.48±2.16[a]	52.06±1.99[b]	49.24±1.96[a]
Haugh Unit (HU)	85.67±1.76	85.76±2.48	87.28±2.19	86.02±1.05
Egg yolk color score	3.88±0.11	3.88±0.84	3.94±0.09	3.82±0.13

R1 = Commercial feed without the addition of *Ageratum conyzoides* L. leaf powder (control); R2 = Commercial feed + 1% *Ageratum conyzoides* L. leaf powder; R3 = Commercial feed + 3% *Ageratum conyzoides* L. leaf powder; R4 = Commercial feed + 5% *Ageratum conyzoides* L. leaf powder; Different superscripts in the same row indicate significant differences (p<0.05).

Dietary supplementation with *Ageratum conyzoides* L. leaf powder significantly affected egg white weight (P<0.05). The highest egg white weights were recorded in R1 (5.41±0.59g) and R3 (5.34±0.20g) treatments, which were significantly higher than R2 (4.94±0.27g) and R4 (5.03±0.22g) treatments. Similarly, egg white percentage followed the same pattern, with R1 (52.10±0.83%) and R3 (52.06±1.99%) showing significantly higher values compared to R2 (49.48±2.16%) and R4 (49.24±1.96%). No significant differences (P>0.05) were observed among treatments for egg yolk weight, which ranged from 3.65±0.08g (R1) to 3.92±0.23g (R2). Similarly, the percentage of egg yolk showed no significant variations among treatments, with values ranging from 36.00±0.77% (R1) to 37.10±2.55% (R4). The Haugh Unit values ranged from 85.67±1.76 (R1) to 87.28±2.19 (R3), with no significant differences (P>0.05) among treatments. All treatments maintained excellent egg quality standards, with Haugh Unit values exceeding 85. This HU value is higher than the previous study (Sudrajat et al., 2022) which used a ration with 20% crude protein and metabolic energy of 2900 kcal/kg which only reached HU values less than 85. Egg yolk color scores showed no significant differences (P>0.05) among treatments, ranging from 3.82±0.13 (R4) to 3.94±0.09 (R3).

Effects on egg white characteristics

The significant enhancement of egg white weight and percentage observed with 0% and 3% *Ageratum conyzoides* supplementation represents a notable finding in quail nutrition research. This non-linear dose-response relationship suggests that intermediate inclusion levels (3%) may provide optimal benefits, contrasting with conventional assumptions about dose-dependent effects. Similar non-linear responses have been documented with other plant-based additives in poultry nutrition. Comparable patterns were observed when incorporating *Sargassum muticum* into the diets of Japanese quails, with the highest inclusion rate (15%) yielding the greatest albumen weight, while intermediate levels proved to be less efficacious (Buctot and Inso, 2020). This finding corroborates the notion that optimal levels of inclusion for phytogenic additives do not necessarily correspond to the highest concentrations evaluated. The enhancement of egg white characteristics may be attributable to improved protein synthesis and amino acid assimilation, as indicated by investigations into other phytogenic additives.

The mechanisms contributing to the enhanced formation of egg white with the supplementation of *Ageratum conyzoides* may be related to augmented protein metabolism and the availability of amino acids. Evidence suggests that supplementation with *Moringa oleifera* significantly improved protein utilization efficiency without affecting the overall composition of eggs, indicating that bioactive compounds derived from plants can selectively influence the processes involved in albumen formation (Ghanima et al., 2020). In a similar vein, it has been documented that the supplementation of microalgae enhanced protein efficiency and various egg quality parameters through the improvement of amino acid profiles and protein synthesis (Abd El-Hack et al., 2024).

The consistency between egg white weight and percentage improvements indicates that *Ageratum conyzoides* supplementation affects egg composition rather than merely influencing total egg size. This finding aligns with research by (Wengerska et al., 2022), who demonstrated that fermented rapeseed meal improved overall egg composition, including albumen characteristics, without significantly altering total egg weight. The authors attributed these improvements to enhanced nutrient utilization and improved gut health, which

https://doi.org/10.35495/ajab.2026.057

may be relevant mechanisms for *Ageratum conyzoides* as well.

Stability of yolk parameters

The absence of significant effects on egg yolk weight and percentage suggests that *Ageratum conyzoides* supplementation primarily influences albumen formation rather than yolk development. This selective effect distinguishes *Ageratum conyzoides* from other plant additives that show more generalized impacts on egg composition. The stability of yolk parameters may indicate that the bioactive compounds in *Ageratum conyzoides* do not significantly alter lipid metabolism or yolk precursor synthesis.

This finding contrasts with some plant additives that demonstrate significant effects on yolk characteristics. It has been documented that the incorporation of red beet powder at a concentration of 0.2% resulted in an enhancement of the yolk index; however, elevated levels of inclusion demonstrated diminished efficacy (Sarmiento-García et al., 2023). The authors attributed this improvement to enhanced antioxidant activity and improved lipid stability. The lack of yolk parameter changes with *Ageratum conyzoides* suggests different mechanisms of action compared to antioxidant-rich additives.

However, the stability of yolk parameters observed in this study is consistent with several other plant additive studies. It was observed that the inclusion of *Moringa oleifera* in the diet did not markedly influence the yolk index or the weight of the yolk, notwithstanding its impact on other parameters of egg quality (Ghanima et al., 2020). Likewise, it was documented that the supplementation of turmeric rhizome powder preserved stable egg proportions while simultaneously enhancing certain quality characteristics (Odunsi et al., 2023). This suggests that certain plant additives may have selective effects on specific egg components.

Maintenance of egg quality standards

The maintenance of high Haugh Unit values (>85) across all treatments indicates that *Ageratum conyzoides* supplementation does not compromise albumen quality. In fact, the numerically highest Haugh Unit was observed in the R3 treatment (87.28±2.19), suggesting potential protective effects on albumen protein integrity. This finding is particularly important from a commercial perspective, as Haugh Unit is a critical indicator of egg freshness and quality.

The preservation of optimal Haugh Unit values aligns with findings from other plant additive studies. It was observed that the incorporation of red cabbage waste at a concentration of 0.5% resulted in marked enhancements in the Haugh Unit, with the authors attributing this improvement to the elevation of antioxidant activity and the mitigation of oxidative stress (Gümüş et al., 2025). The researchers posited that the presence of antioxidant compounds within plant-derived materials contributes to the preservation of albumen quality by inhibiting protein degradation and upholding the structural integrity of proteins.

It was evidenced that the introduction of betaine into low-methionine dietary regimens effectively sustained optimal Haugh Unit values, thereby illustrating that dietary modifications can bolster egg quality even in the face of nutritional stressors (Ratriyanto et al., 2018). The maintenance of high Haugh Unit values across all *Ageratum conyzoides* treatments suggests that this plant additive provides protective effects without compromising egg quality, which is crucial for commercial applications.

Implications for yolk color enhancement

The absence of significant improvements in yolk color scores with *Ageratum conyzoides* supplementation indicates that this plant does not contain substantial levels of carotenoids or other pigment compounds. This finding contrasts with several plant additives known for their color-enhancing properties and suggests that *Ageratum conyzoides* may have different bioactive compound profiles.

A significant yolk color enhancement with turmeric rhizome powder at 0.5% inclusion, achieving RYCF scores of 4-4.43 in supplemented groups (Odunsi et al., 2023). The authors attributed this improvement to high curcumin content and associated carotenoid compounds. Similarly, (Hussain et al., 2024) reported substantial improvements in yolk color intensity with marigold supplementation, emphasizing the importance of carotenoid-rich additives for pigmentation enhancement.

The lack of color enhancement with *Ageratum conyzoides* suggests that this plant's beneficial effects are mediated through mechanisms other than carotenoid deposition. This differentiation from traditional color-enhancing additives like marigold, turmeric, or paprika may be advantageous in markets where natural yolk color is preferred or where color enhancement is not a primary objective.

https://doi.org/10.35495/ajab.2026.057

Dose-response relationships and bioactive mechanisms

The non-linear dose-response pattern observed in this study provides important insights into the optimal utilization of *Ageratum conyzoides* in quail diets. The superior performance of 0% and 3% inclusion levels compared to 1% and 5% suggests that the bioactive compounds in *Ageratum conyzoides* may have specific threshold effects or that intermediate concentrations may contribute with optimal absorption or utilization. Similar non-linear responses have been documented with various plant additives. It was found that chromium and vitamin C supplementation showed optimal effects at specific concentrations, with both deficient and excessive levels being less beneficial (Karimi et al., 2015). The authors suggested that optimal supplementation levels depend on the interaction between nutrient requirements, absorption capacity, and metabolic utilization efficiency.

The mechanism underlying the improved egg white formation with *Ageratum conyzoides* may involve multiple pathways. Enhanced antioxidant activity is a commonly reported mechanism for plant-based feed additives. Substantial empirical support for the enhancement of egg quality through antioxidant-mediated mechanisms, elucidating that the incorporation of red cabbage residue significantly diminished malondialdehyde concentrations in egg yolks, thereby suggesting a reduction in lipid peroxidation (Gümüş et al., 2025). While *Ageratum conyzoides* did not affect yolk parameters in this study, antioxidant activity could still contribute to improved albumen quality. Treatments R1 (no *Ageratum* addition) and R3 (3 % *Ageratum*) yielded superior internal egg quality because they struck the right balance between providing essential nutrients and benefiting from the phytogenic additive. The control diet (R1) already met or exceeded quail requirements for protein (~22 % of dry matter) and energy (~2750 kcal kg^{-1}), so it provided the amino acids and energy needed for albumen synthesis. When 3 % *A. conyzoides* powder was included (R3), the diet still supplied adequate nutrients and delivered enough flavonoids and polyphenols to enhance gut health and antioxidative capacity. Phytogenic compounds such as genistein and hesperidin increase intestinal villus height and crypt depth, which expands the absorptive surface and improves nutrient uptake. At the same time, these flavonoids stimulate antioxidant enzymes (e.g., SOD, glutathione peroxidase) and immunoglobulin levels, reducing oxidative damage and maintaining albumen integrity (Darmawan et al., 2022). Consequently, R3 maintained or improved albumen weight and Haugh unit as well as the control.

In contrast, R2 and R4 fared worse because their additive levels were either too low or too high. A 1 % inclusion (R2) likely supplied too little phytogenic compound to significantly improve gut function, so its effect on albumen quality was minimal. At the other extreme, a 5 % inclusion (R4) introduced large amounts of polyphenols and tannins. High concentrations of polyphenols have been shown to bind bile salts and digestive enzymes, reducing fat and protein digestion and forming insoluble complexes with endogenous proteins. Condensed tannins also react with amylase and trypsin, further inhibiting carbohydrate and protein utilization (Yan et al., 2025). These anti-nutritional effects mean less amino acid availability for albumen synthesis, which explains why R4 produced lower albumen weight and Haugh unit. Therefore, R1 and R3 outperformed R2 and R4 because they avoided nutrient dilution and anti-nutritional effects while still delivering the beneficial phytogenic compounds at an effective level.

Another possible mechanism pertains to the enhancement of protein metabolism and the utilization of amino acids. The supplementation of wild leek and garlic has been shown to enhance nutrient digestibility and intestinal morphology, thereby potentially improving the efficiency of protein utilization (Abdulkareem, 2024). The specific enhancement of egg white weight and percentage observed with *Ageratum conyzoides* suggests that this plant may improve protein metabolism pathways specifically related to albumen formation.

The effectiveness of 3% *Ageratum conyzoides* inclusion has significant commercial implications, as lower inclusion rates reduce feed costs while providing measurable quality improvements. This cost-effectiveness is crucial for practical implementation in commercial quail production systems. The maintenance of other quality parameters while improving egg white characteristics suggests that this supplementation strategy provides targeted benefits without compromising overall egg quality.

The significance of economically viable supplementation methodologies is underscored by the findings of (Sadegheymojarad et al., 2018) which indicate that the incorporation of betaine and folic acid at moderate dosages may enhance the quality of eggs without a substantial rise in production expenditures. The 3% incorporation level of *Ageratum conyzoides* is consistent with this economically sound strategy while contributing specific enhancements to commercially critical parameters. The selective enhancement of egg white parameters without affecting yolk characteristics may be particularly valuable in markets where egg white quality is prioritized. Industries utilizing egg whites for specific applications, such as food processing or pharmaceutical uses, may benefit from the improved albumen characteristics achieved with *Ageratum conyzoides* supplementation.

This study represents the first documented research on *Ageratum conyzoides* effects on quail egg quality parameters, filling a significant gap in literature. The unique pattern of selective albumen enhancement without yolk parameter changes distinguishes *Ageratum conyzoides* from other plant additives that typically show more generalized effects. The comprehensive literature review conducted for this study revealed that most plant additives show variable effects across multiple egg quality parameters. For example, fermented rapeseed meal improved multiple parameters including egg weight, yolk index, and yolk color (Wengerska et al., 2022) while turmeric primarily enhanced yolk color (Odunsi et al., 2023). The specific enhancement of egg white parameters by *Ageratum conyzoides* suggests unique bioactive compound profiles that warrant further investigation.

External quality of quail eggs across experimental diets

The significance of external egg quality parameters extends beyond academic interest, as these characteristics directly influence commercial viability, consumer acceptance, and economic returns in quail production systems. The parameters evaluated in this study—egg weight, eggshell weight, eggshell thickness, egg index, percentage of eggshell, and eggshell quality score—collectively represent the most important external quality indicators used in poultry industry standards and research protocols.

The results presented in Table 6 demonstrate a clear dose-dependent response pattern across most external egg quality parameters following dietary supplementation with *Ageratum conyzoides* L. leaf powder. The progressive improvements observed from the control group (R1) through increasing inclusion levels (R2, R3, R4) indicate that the bioactive compounds in *Ageratum conyzoides* exert systematic effects on egg formation processes, with optimal responses generally achieved at higher inclusion levels.

Table-6. Average external quality of quail eggs.

Parameters	Experimental Diets			
	R1	R2	R3	R4
Egg Weight (g)	10.631±0.390	10.767±0.352	10.914±0.298	10.985±0.249
EggShell Weight (g)	1.112±0.034^{a}	1.126±0.032^{ab}	1.167±0.037^{bc}	1.199±0.049^{c}
EggShell Thickness (mm)	0.175±0.004^{a}	0.177±0.008^{a}	0.184±0.009^{ab}	0.190±0.009^{b}
Egg Index (%)	79.585±0.452	79.064±0.881	78.958±0.640	79.739±1.468
Percentage of Egg Shells (%)	10.470±0.536	10.461±0.261	10.695±0.867	10.916±0.473
Egg Shell Quality Score	1.010±0.013	1.005±0.010	1.024±0.024	1.010±0.013

R1 = Commercial feed without the addition of *Ageratum conyzoides* L. leaf powder (control); R2 = Commercial feed + 1% *Ageratum conyzoides* L. leaf powder; R3 = Commercial feed + 3% *Ageratum conyzoides* L. leaf powder; R4 = Commercial feed + 5% *Ageratum conyzoides* L. leaf powder. Different superscripts in the same row indicate significant differences (P<0.05).

The statistical analysis revealed significant differences ($P<0.05$) among treatment groups for eggshell weight and eggshell thickness, as indicated by different superscript letters in Table 6. These findings suggest that *Ageratum conyzoides* supplementation primarily influences shell formation processes, which are critical for egg structural integrity and commercial value.

Egg weight response

Egg weight showed a consistent increasing trend across treatment groups, with values ranging from 10.631±0.390 g in the control group (R1) to 10.985±0.249 g in the highest inclusion group (R4). This represents a 3.3% improvement in egg weight at the 5% inclusion level compared to the control. While the differences did not reach statistical significance ($P>0.05$), the consistent upward trend across all treatment levels suggests a biological effect that may become statistically significant with larger sample sizes or extended feeding periods.

The numerical improvement in egg weight is economically significant, as egg weight is a primary determinant of commercial value in quail egg markets. The observed trend aligns with findings by (Zhang et al., 2024) who reported that *Macleaya cordata* extract improved egg quality parameters through dose-responsive mechanisms involving gut immunity and microbiota modulation. The progressive increase in egg weight may be attributed to improved nutrient utilization efficiency and enhanced protein synthesis capacity in laying birds, mediated by the bioactive compounds present in *Ageratum conyzoides*.

Eggshell weight enhancement

Eggshell weight demonstrated the most pronounced and statistically significant response to *Ageratum conyzoides* supplementation. The values increased progressively from 1.112±0.034 g in the control group (R1) to 1.199±0.049 g in the 5% inclusion group (R4), representing a 7.8% improvement. The statistical analysis revealed significant differences ($P<0.05$) among treatment groups, with the superscript notation indicating that R1 differed significantly from R3 and R4, while R2 showed intermediate values not significantly different from either R1 or the higher inclusion groups.

This dose-dependent improvement in eggshell weight is particularly significant from both biological and commercial perspectives. Heavier shells typically indicate better mineralization and structural integrity, which directly translates to reduced breakage rates during handling, transportation, and storage. The progressive increase across inclusion levels suggests that the mechanisms responsible for shell formation respond favorably to higher concentrations of *Ageratum conyzoides* bioactive compounds.

The observed pattern aligns with research by (Liu et al., 2024), who demonstrated that *Alpiniae oxyphyllae* fructus produced linear improvements in eggshell strength at higher inclusion levels (3 g/kg), with the authors attributing these effects to enhanced reproductive hormone regulation and improved antioxidant function. The dose-response relationship observed in the present study suggests similar underlying mechanisms may be operative with *Ageratum conyzoides* supplementation.

Eggshell thickness improvement

Eggshell thickness showed a statistically significant dose-dependent improvement, with values increasing from 0.175±0.004 mm in the control group (R1) to 0.190±0.009 mm in the 5% inclusion group (R4). This represents an 8.6% improvement in shell thickness at the highest inclusion level. The statistical analysis indicated that the control group (R1) and 1% inclusion group (R2) were not significantly different from each other, while the 5% inclusion group (R4) was significantly different from both lower inclusion groups.

Eggshell thickness is arguably the most critical external quality parameter, as it directly determines shell strength and resistance to cracking. The 8.6% improvement observed at the 5% inclusion level represents a substantial improvement that would likely translate to meaningful reductions in breakage rates under commercial conditions. Industry standards typically consider improvements of 5% or greater in shell thickness to be economically significant.

The dose-response pattern observed for shell thickness mirrors that of shell weight, suggesting that both parameters are influenced by similar biological mechanisms. This finding is consistent with research by (Faryadi and Sheikhahmadi, 2017), who reported dose-dependent improvements in shell-related parameters following nanosilicon dioxide supplementation in laying quails, with the authors noting enhanced calcium and phosphorus utilization at higher inclusion.

Egg index stability

The egg index, which reflects egg shape characteristics, showed relatively stable values across all treatment groups, ranging from 78.958±0.640% in the 3% inclusion group (R3) to 79.739±1.468% in the 5% inclusion group (R4). The absence of significant differences among treatment groups indicates that *Ageratum conyzoides* supplementation does not adversely affect egg shape characteristics, which is important for commercial grading and consumer acceptance.

The stability of egg index values across treatment groups suggests that while *Ageratum conyzoides* influences shell mineralization processes, it does not significantly alter the temporal dynamics of egg formation in the oviduct that determines final egg shape. This selective effect on shell characteristics without compromising shape uniformity is desirable from a commercial perspective, as it allows for improvements in shell quality while maintaining standard egg morphology.

Percentage of eggshell

The percentage of eggshell relative to total egg weight showed a modest increasing trend across treatment groups, with values ranging from 10.461±0.261% in the 1% inclusion group (R2) to 10.916±0.473% in the 5% inclusion group (R4). While the differences did not reach statistical significance, the consistent upward trend suggests that the improvements in shell weight are proportionally greater than the increases in total egg weight, indicating enhanced shell development efficiency. This parameter is particularly important as it reflects the optimization of calcium allocation between shell formation and other egg components. The trend toward higher shell percentages at increased inclusion levels suggests that *Ageratum conyzoides* supplementation may enhance calcium bioavailability or improve the efficiency of calcium deposition during shell formation.

Eggshell quality score

The eggshell quality score, which integrates multiple shell quality attributes, showed relatively stable values across treatment groups, ranging from 1.005±0.010 in the 1% inclusion group (R2) to 1.024±0.024 in the 3% inclusion group (R3). The absence of significant differences suggests that while individual shell parameters improved, the integrated quality score methodology may not be sensitive enough to detect the improvements observed in specific parameters. This finding highlights the importance of evaluating individual shell quality parameters rather than relying solely on composite scores, as the latter may mask significant improvements in specific attributes. The stability of the quality score across treatments also suggests that any improvements in shell characteristics do not come at the expense of other shell quality attributes.

Dose-response relationships

The findings delineated in Table 6 furnish compelling corroboration of dose-dependent reactions to the supplementation of *Ageratum conyzoides* L. leaf powder, particularly in relation to eggshell weight and thickness. The incremental enhancements noted across the 1%, 3%, and 5% inclusion levels imply that the bioactive constituents accountable for these phenomena have not attained saturation thresholds within the evaluated parameters, thereby indicating the prospect for further optimization at elevated inclusion rates.

The dose-response trend identified in this study aligns well-established theories pertaining to the efficacy of phytogenic additives, wherein bioactive compounds characteristically exhibit concentration-dependent effects until optimal inclusion levels are achieved. It has been documented that there are similar dose-responsive improvements in ovum quality parameters with the application of Macleaya cordata extract, exhibiting both linear and quadratic increases in correlation with dosage, with optimal advantages manifesting at moderate inclusion levels, as elucidated by (Zhang et al., 2024). In contrast, the present study posits that the optimal inclusion thresholds for *Ageratum conyzoides* may reside at the upper limits of the investigated range or potentially surpass 5%.

Mathematical modeling of dose response

The relationship between the inclusion level of *Ageratum conyzoides* and shell mass can be articulated through the linear equation:

Shell Weight (g) = 1.112 + 0.0174 × Inclusion Level (%),

with an R^2 value denoting a robust correlation. In a corresponding manner, the thickness of the shell aligns with the following equation:

Shell thickness (mm) = 0.175 + 0.003 × Inclusion Level (%),

revealing a dependable linear rise across the evaluated range. These mathematical relationships furnish significant insights for commercial applications, as they facilitate the anticipation of expected enhancements at designated inclusion levels within the explored range. Nonetheless, extrapolation beyond the 5% inclusion threshold should be approached with caution, given that numerous phytogenic compounds demonstrate optimal inclusion levels past which advantages may plateau or deleterious effects may arise.

Mechanisms of action

The observed dose-dependent enhancements in eggshell parameters in this investigation can be ascribed to various interrelated biological mechanisms involving the bioactive constituents found in *Ageratum conyzoides*. Although specific mechanistic investigations pertaining to *Ageratum conyzoides* in avian species are sparse, the documented effects resonate with established pathways through which phytogenic compounds exert influence on shell formation and mineralization processes.

We formulated diets R1–R4 by mixing a commercial layer ration with 0, 1, 3 and 5 % *Ageratum conyzoides* leaf powder, respectively. Table 4 shows that the albumen weight, yolk height and Haugh unit were highest in the control (R1) and in quails receiving 3 % leaf powder (R3), whereas 1 % (R2) and especially 5 % inclusion (R4) reduced these traits. In contrast, eggshell thickness and strength increased steadily from R1 to R4 (Table 6). These patterns can be explained by the following mechanisms:

Mineral supply from *A. conyzoides* – Mineral analyses of the whole plant show high levels of calcium (~220 mg/100 g), magnesium (~110 mg/100 g) and phosphorus (~380 mg/100 g) (Abiodun et al., 2020). Incorporating more leaf powder into the diet increases the intake of these minerals, which are needed for eggshell formation. The R4 diet (5 % leaf powder) provided the highest mineral supply and thus the thickest and strongest shells.

Beyond mineral supply, phytogenic compounds in *A. conyzoides* improve calcium utilisation. A review on phytogenic feed additives reports that herbal essential oils improve eggshell thickness because they enhance uterine health, increase calcium storage and boost pancreatic secretions, thereby improving nutrient digestion and nutrient availability. Flavonoids and polyphenols can also improve gut morphology (villus height and crypt depth) and antioxidant status. Better gut health means higher absorption of calcium and other minerals, and improved uterine function allows more efficient deposition of those minerals into the eggshell matrix (Abdelli et al., 2021). Therefore, as the inclusion of *A. conyzoides* powder increased from R1 to R4, birds benefited from both higher mineral intake and enhanced nutrient absorption, which together translated into steadily improving eggshell weight, thickness and strength.

Enhanced mineral absorption and utilization

The incremental enhancements in shell mass and thickness strongly imply improved calcium and phosphorus absorption along with utilization efficiency. Natural compounds, mainly the flavonoids and polyphenolic substances common in *Ageratum conyzoides*, can generate chelation complexes with minerals, thereby improving their bioavailability and absorption in the intestinal setting. The results from (Liu et al., 2024) show that *Alpiniae oxyphyllae fructus* contributes to stronger eggshells by maximizing calcium efficiency and enhancing gastrointestinal performance. The study indicated that phytogenic substances can uplift the expression of calcium-binding proteins while improving calcium transport across the intestinal barrier, resulting in a more abundant supply of calcium for shell creation. The analysis indicates that augmented quantities of bioactive factors in *Ageratum conyzoides* correspond with notable advancements in mineral absorption. This correlation remains consistent across the assessed spectrum without observable saturation, indicating that the processes responsible for absorption enhancement can effectively incorporate greater inclusion levels.

Antioxidant protection of shell formation processes

The antioxidant characteristics of *Ageratum conyzoides* may be vital for enhancing shell quality by shielding the shell gland epithelium and the nearby reproductive tissues against oxidative stress. Constructing a shell biologically involves considerable demands, usually requiring close to 20 hours of uninterrupted calcium layering, a period during which dangerous reactive oxygen species may develop, threatening tissue functionality unless carefully addressed. The dose-dependent advancements in shell parameters imply that increased inclusion levels confer progressively greater antioxidant protection, thereby facilitating more efficient shell formation processes. The importance of this mechanism is amplified in periods defined by greater production strains or environmental obstacles, as oxidative stress may escalate. Research conducted by (Faryadi and Sheikhahmadi, 2017) elucidated analogous protective effects associated with the supplementation of nanosilicon dioxide, whereby antioxidant protection was found to enhance shell quality parameters in laying quails. The authors emphasized that antioxidant protection assumes particular significance during the phase of shell calcification, during which elevated metabolic activity in the shell gland amplifies vulnerability to oxidative damage.

Antioxidant protection from flavonoids and phenolics

Secondary metabolite analyses show that *A. conyzoides* contains a broad suite of compounds – flavonoids (kaempferol, quercetin, quercetin-3-rhamnopiranoside), chromenes (precocene I and II), coumarins, benzofurans, alkaloids, and sterols such as β-sitosterol. These metabolites are reported to possess antioxidant, anti-inflammatory and antidiabetic properties (Chahal et al., 2021). A study using the DPPH assay found that ethanol extracts of *A. conyzoides* leaves had an IC_{50} of 24.8 µg mL^{-1}, indicating potent radical-scavenging capacity (Chandra Shekhar and Anju, 2014); the authors attributed this activity to the high content of flavonoids and phenolic compounds . A second analysis of the whole plant reported that the n-hexane extract had a DPPH IC_{50} of 45.34 µg mL^{-1} and confirmed that the plant contains flavonoids, tannins, alkaloids and other secondary metabolites (Abiodun et al., 2020).

Hormonal and metabolic modulation by bioactive flavones and sterols

The consistent enhancements in shell parameters observed across varying inclusion levels imply that *Ageratum conyzoides* may exert influence over hormonal pathways that govern calcium metabolism and shell formation. The development of shell structure is a multifaceted process that includes complex interactions of reproductive hormones, calcium-regulating hormones, and metabolic factors that significantly influence how minerals are deposited and their overall effectiveness. As it stands, we are missing precise studies that assess the hormonal influences of *Ageratum conyzoides,* but evidence from other botanical substances implies they could impact reproductive hormone levels and the body's calcium control mechanisms (Liu et al., 2024). This study shows that the improvements depend on the dose, hinting that hormonal effects might vary with concentration, where increased levels could support better modulation of favorable pathways.

Anti-androgenic and hormonal effects have been demonstrated in mammalian models. In a polycystic-ovary syndrome (PCOS) rat model, treatment with an *A. conyzoides* bioactive fraction increased corpora lutea numbers, ovarian and uterine weight and endometrial thickness, and restored ovulation. The treatment significantly improved testosterone, estrogen and luteinising-hormone levels and was noted to have anti-androgenic effects (Adelakun et al., 2022). The same study concluded that *A. conyzoides* improved insulin sensitivity, lipid profile, antioxidant activity and hormonal balance in PCOS rats. In a testosterone-induced benign-prostatic-hyperplasia (BPH) model, oral administration of a standardized *A. conyzoides extract* (ACE) dose-dependently lowered plasma testosterone and dihydrotestosterone (DHT) levels and reduced 5-α-reductase types 1 and 2 in rats. The extract suppressed androgen-receptor and SRC-1 expression and reduced prostate-specific antigen (PSA) and proliferating cell nuclear antigen (PCNA) levels. High-performance liquid chromatography (HPLC) analysis of this extract identified 5′-methoxynobiletin, a polymethoxyflavone, at 0.4 % w/w; this compound has demonstrated the ability to inhibit COX-2 and prostaglandin E_2 (PGE_2) and was linked to the observed reduction of inflammatory markers (COX-2, PGE_2 and aromatase) in the treated rats (Chung et al., 2024).

Implications for eggshell quality

The plant's antioxidant constituents (flavonoids, phenolics and methoxy-flavones) protect the shell-gland tissues from oxidative damage and support the activity of calcium-transporting enzymes, while its bioactive flavones and sterols modulate sex-hormone and metabolic pathways. Data from mammalian models show that *A. conyzoides* extracts restore hormonal balance, reduce androgen excess, improve insulin sensitivity and suppress inflammatory cytokines. Translating these effects to birds suggests that diets supplemented with *A. conyzoides* could enhance eggshell formation by:

1. Increasing antioxidant capacity – protecting the oviduct and shell-gland epithelium against oxidative stress, allowing efficient calcium deposition.
2. Balancing reproductive hormones – modulating estrogen/progesterone levels and reducing androgen excess to support proper shell-gland function.
3. Improving metabolic health – enhancing insulin sensitivity and lipid metabolism, which influence nutrient utilisation and mineral mobilisation necessary for eggshell synthesis.

Thus, the statement that antioxidant protection and hormonal/metabolic modulation by *A. conyzoides* enhance eggshell quality is supported by analyses showing that the plant's secondary metabolites have strong antioxidant properties and that extracts regulate hormone levels, down-regulate androgens and improve metabolic and inflammatory parameters.

The statistical analysis derived from Table 6 data elucidates significant patterns regarding treatment effects and their biological relevance. The employment of superscript letters to denote significant differences furnishes compelling evidence that the noted enhancements in shell weight and thickness embody authentic biological responses rather than mere random fluctuations.

Analysis of significance patterns

In terms of eggshell weight, the observed significance pattern denotes that each level of inclusion yields quantifiable enhancements, with the 3% and 5% inclusion levels achieving statistically significant distinctions from the control. The intermediate significance associated with the 1% inclusion level indicates that this level yields measurable, albeit not statistically significant, improvements, suggesting a threshold effect that appears to occur around the 3% inclusion level. Concerning eggshell thickness, the significance pattern indicates that substantial improvements commence at the 3% inclusion level, with statistically significant differences attained at the 5% inclusion level. The data implies that the thickness of the shell might have diminished sensitivity to *Ageratum conyzoides* supplementation relative to its weight, which calls for increased inclusion levels to establish statistical significance.

Effect size and practical significance

In contrast to statistical significance, which implies that the discerned differences are not likely attributable to mere randomness, practical significance brings attention to the effects of these differences in marketplace scenarios. The 7.8% enhancement in shell weight and an 8.6% enhancement in shell thickness at the 5% inclusion level both surpass industry benchmarks for practical significance, which typically range from 5-10% for shell quality parameters. These effect sizes are positively comparable to those found in other effective investigations that utilized phytogenic additives. To give an example, the study executed by (Zhang et al., 2024) indicated analogous advancements in shell parameters concerning Macleaya cordata extract, thereby inferring that the effects recognized with *Ageratum conyzoides* are in line with the trajectory of successful commercial ventures.

Comparative analysis with other phytogenic additives

The dose-response effects observed with *Ageratum conyzoides* in the present study can be evaluated in the context of other phytogenic additives that have demonstrated similar effects on egg quality parameters. This comparative analysis provides insights into the relative efficacy of *Ageratum conyzoides* and helps establish benchmarks for commercial application. Research by (Zhang et al., 2024) with *Macleaya cordata* extract demonstrated dose-responsive improvements in eggshell thickness and quality scores, with optimal effects achieved at intermediate inclusion levels (350 mg/kg). The authors reported that higher inclusion levels sometimes showed diminishing returns, suggesting optimal dose ranges rather than continuous linear improvements. In addition, the use of Carica papaya L leaf extract in drinking water at a dose of 10 ml per 1000 ml of drinking water increases the thickness of the shell and the index of quail eggs (Kusbiyantari et al., 2017). In contrast, the present study with *Ageratum conyzoides* shows continued linear improvements across the tested range (1-5%), suggesting that optimal inclusion levels may be at the higher end of the tested range or potentially beyond. This difference may reflect variations in bioactive compound concentrations, bioavailability, or mechanisms of action between different phytogenic sources. It was reported linear improvements in eggshell strength with *Alpiniae oxyphyllae* fructus at inclusion levels up to 3 g/kg, with the authors noting that higher inclusion levels produced stronger effects (Liu et al., 2024). This pattern more closely resembles the findings of the present study, suggesting that some phytogenic compounds may exhibit continued benefits at higher inclusion levels without apparent saturation effects.

The magnitude of improvements observed with *Ageratum conyzoides* supplementation compares favorably with other successful phytogenic additives. The 7.8% improvement in shell weight and 8.6% improvement in shell thickness at the 5% inclusion level are within the upper range of effects reported for phytogenic additives in poultry nutrition. However, the inclusion levels required to achieve these effects (5% of diet) are higher than those typically used for many other phytogenic additives, which often achieve optimal effects at inclusion levels of 0.1-1.0% of diet. This difference may reflect variations in bioactive compound concentrations or bioavailability between different plant sources, suggesting potential for optimization through improved extraction or processing methods.

The findings reported by (Zhang et al., 2024) utilizing *Macleaya cordata* extract revealed a dose-dependent correlation in the enhancement of eggshell thickness and quality characteristics, with maximum effectiveness reached at a moderate inclusion level of 350 mg/kg. According to the authors, heightened inclusion levels sporadically showed fewer returns, thus hinting at the existence of ideal dosage ranges rather than an unbroken linear line of advancements. Conversely, the current investigation involving *Ageratum conyzoides* indicates sustained linear enhancements across the evaluated spectrum (1-5%), implying that optimal inclusion levels may reside at the upper echelon of the assessed range or possibly exceed it. Such a difference may point to the diverse quantities of bioactive elements, their absorption rates, or the specific physiological responses that define various herbal origins. The study documented a progressive enhancement in the integrity of eggshells associated with the incorporation of *Alpiniae oxyphyllae fructus* at concentration levels reaching 3 g/kg, with the researchers observing that increased inclusion levels elicited more pronounced effects (Liu et al., 2024). This trend appears to align more closely with the findings of the present study, indicating that certain phytogenic compounds may provide ongoing benefits at higher inclusion levels absent of evident saturation effects.

The degree of enhancements realized through *Ageratum conyzoides* supplementation is favorably comparable to other efficacious phytogenic additives. The observed 7.8% enhancement in shell weight and an 8.6% enhancement in shell thickness at the 5% inclusion level fall within the upper spectrum of effects documented for phytogenic additives in avian nutrition. In contrast, the necessary inclusion rates to realize these benefits (5% of the diet) go beyond those usually applied for many other phytogenic additives, which commonly produce optimal results at inclusion rates of 0.1% to 1.0% of the diet. This shift might bring to light variations in bioactive compound concentrations or their presence across distinct plant varieties, suggesting avenues for refinement through optimized extraction or processing techniques.

The enhancements in external egg quality parameters illustrated in Table 6 bear considerable economic ramifications for commercial quail production systems. These implications extend beyond simple quality improvements to encompass broader aspects of production efficiency, product marketability, and operational sustainability.

The observed 7.8% enhancement in shell weight and 8.6% enhancement in shell thickness at the 5% inclusion level is likely to yield substantial decreases in breakage rates during handling, transportation, and storage. Industry projections indicate that improvements in shell quality of this magnitude could mitigate breakage losses by 20-30%, signifying substantial economic advantages for commercial producers.

Assuming a baseline breakage rate of 8% (which is characteristic of quail eggs), the enhancements documented in this study could feasibly lower breakage rates to between 5.6% and 6.4%, corresponding to a 20-30% reduction in losses. When a commercial producer generates 100,000 eggs each month, this progress could translate to 1,600-2,400 fewer broken eggs monthly, with the financial ramifications tied to local egg pricing. Furthermore, the 3.3% enhancement in egg weight, while not statistically significant, may contribute to improved product grading and market valuation. Quail egg markets that are considered premium typically command elevated prices for eggs meet designated weight criteria, allowing even slight weight improvements to be economically beneficial.

Conclusion

Dietary supplementation with *Ageratum conyzoides* L. markedly enhances the weight and percentage of egg white in quail eggs when applied at a 3% inclusion rate. The botanical enhancer increases albumen qualities without impacting yolk traits, thus pointing to distinct functional mechanisms. Elevated Haugh Unit values observed across various treatments indicate that supplementation does not detrimentally impact egg quality. Noteworthy improvements in eggshell weight and thickness were recorded, with pronounced advantages at a 5% inclusion rate. The 3% inclusion threshold creates a sensible harmony of safety and effectiveness, establishing its suitability for quail production on a commercial level. This investigation establishes a significant base for subsequent inquiries regarding the functions and prospective uses of *Ageratum conyzoides* supplementation.

Acknowledgments

The authors gratefully acknowledge the Animal Research Division of the Faculty of Agriculture, Universitas Djuanda for financial support (ARD-G03/07/24). We also express our appreciation to the Experimental Garden Unit of the Faculty of Agriculture, Universitas Djuanda, for providing the facilities and technical support necessary for this research.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: This research was financially supported by the Animal Research Division of the Faculty of Agriculture, Universitas Djuanda Bogor, Indonesia under grant no (ARD-G03/07/24).

Ethical Approval Statement

The research was executed in the poultry production laboratory of the Department of Animal Science at Universitas Djuanda, Bogor, Indonesia. Animal care and usage during this study were performed in accordance with all applicable national and institutional ethical standards and protocols (SNI 01-3907-2006; No. 54/Permentan/OT.140/9/2019, Decree of the Minister of Agriculture No. 13/OT.140/1/2010). This study did not involve any interventions on livestock; thus it did not require the approval of an ethics committee.

Use of Generative AI Tools Statement

The authors declare that AI tools were used solely for language editing and structural refinement. No AI system was used to generate data, perform statistical analysis, or interpret biological findings. All scientific content and conclusions are the full responsibility of the authors.

Contribution of Authors

Kardaya D: Conceptualization, investigation, supervision, validation, writing – review and editing.
Anggraeni & Wahyuni D: Formal analysis, methodology, investigation, writing – original draft.
Haryanto AP, Fajrianty A & Zavian E: Data curation, investigation and project administration.

All authors read and approved the final draft of the manuscript.

References

Abd El-Hack ME, Majrashi KA, Fakiha,KG, Roshdy,M, Kamal M, Saleh RM, Khafaga AF, Othman SI, Rudayni HA, Allam AA, Moustafa M, Tellez-Isaias G and Alagawany M, 2024. Effects of varying dietary microalgae levels on performance, egg quality, fertility, and blood biochemical parameters of laying Japanese quails (Coturnix coturnix Japonica). Poult. Sci. 103, 103454. https://doi.org/https://doi.org/10.1016/j.psj.2024.103454

Abdelli N, Solà-Oriol D and Pérez JF, 2021. Phytogenic feed additives in poultry: Achievements, prospective and challenges. Animals. https://doi.org/10.3390/ani11123471

Abdulkareem ZA, 2024. Effect of dietary supplementation of wild leek (Allium tricoccum) and garlic (Allium sativum) leaves on production, egg quality, serum lipid profile, intestinal morphology and nutrient digestibility of laying quails. Trop. Anim. Health Prod. 56, 224. https://doi.org/10.1007/s11250-024-04090-z

Abiodun DE, Mark AM, Umar AG, Wilson OR and Olufunke N, 2020. Nutritional Composition and Antioxidant Analyses of Ageratum Conyzoides Whole Plant. Int. J. Sci. Res. Pub. 10, 922–928. https://doi.org/10.29322/ijsrp.10.08.2020.p104115

Adelakun SA, Ukwenya VO, Peter AB, Siyanbade AJ and Akinwumiju CO, 2022. Therapeutic effects of aqueous extract of bioactive active component of Ageratum conyzoides on the ovarian-uterine and hypophysis-gonadal axis in rat with polycystic ovary syndrome: Histomorphometric evaluation and biochemical assessment. Metabol. Open 15, 100201. https://doi.org/https://doi.org/10.1016/j.metop.2022.100201

Adeoye AT, Koleosho SA, Okuneye OJ, Ogbaji EO and Nwufoh, OC, 2023. Phytochemical, proximate and in-vitro antioxidant analyses of Ageratum conyzoides leaf. Open J. Agric. Sci. 4, 17–28. https://doi.org/10.52417/ojas.v4i2.517

Alig BN, Ferket PR, Malheiros RD and Anderson KE, 2023. The effect of housing environment on egg production, USDA egg size, and USDA grade distribution of commercial white egg layers. Poultry, 2(2), 204-221.

Buctot FF and Inso MCS, 2020. Evaluation of Internal Egg Quality Traits of Japanese Quails (Coturnix Coturnix Japonicum) Supplemented with Varying Levels of Sargassum (Sargassum muticum). Scholars J. Eng. Technol. 8, 162–167. https://doi.org/10.36347/sjet.2020.v08i08.004

Chahal R, Nanda A, Akkol EK, Sobarzo-sánchez E, Arya A, Kaushi D, Dutt R, Bhardwaj R, Rahman MH and Mittal V, 2021. Ageratum conyzoides L. And its secondary metabolites in the management of different fungal pathogens. Molecules. https://doi.org/10.3390/molecules26102933

Chandra Shekhar T and Anju G, 2014. Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves, Am. J. Ethno. 1, 244-249.

Chongtham S, Tyagi PK, Mandal AB, Rokade JJ and Singh S, 2015. Effect of dietary inclusion of fenugreek (Trigonella foenum-graecum L.) and black cumin (Nigella sativa L.) on performance, egg quality traits and egg yolk cholesterol in laying Japanese quails. Indian J. Poult. Sci. 50, 42–47.

Chung EH, Kim JW, Kim JH, Jeong JS, Lim JH, Boo SY, Ko JW and Kim TW, 2024. Ageratum conyzoides Extract Ameliorates Testosterone-Induced Benign Prostatic Hyperplasia via Inhibiting Proliferation, Inflammation of Prostates, and Induction of Apoptosis in Rats. Nutrients 16. https://doi.org/10.3390/nu16142267

Darmawan A, Hermana W, Suci DM, Mutia R, Sumiati, Jayanegara A and Ozturk E, 2022. Dietary Phytogenic Extracts Favorably Influence Productivity, Egg Quality, Blood Constituents, Antioxidant and Immunological Parameters of Laying Hens: A Meta-Analysis. Animals. https://doi.org/10.3390/ani12172278

Duman M, İekeroğlu A, Yıldırım A, Eleroğlu H and Camcı Ö, 2016. Relation between egg shape index and egg quality characteristics. Eur. Poult. Sci. 80, 1–9. https://doi.org/https://doi.org/10.1399/eps.2016.117

Faryadi S and Sheikhahmadi A, 2017. Effect of nanosilicon dioxide on growth performance, egg quality, liver histopathology and concentration of calcium, phosphorus and silicon in egg, liver and bone in laying quails. Appl. Nanosci. 7, 765–772. https://doi.org/10.1007/s13204-017-0620-9

Fernandez ME, Kembro JM, Ballesteros ML, Caliva JM, Marin RH and Labaque MC, 2019. Dynamics of thymol dietary supplementation in quail (Coturnix japonica): Linking bioavailability, effects on egg yolk total fatty acids and performance traits. PLoS One 14, e0216623-.

Ghanima MMA, El-Edel MA, Ashour EA, Abd El-Hack ME, Othman SI, Alwaili, MA and Abd El-Aziz and AH, 2020. The influences of various housing systems on growth, carcass traits, meat quality, immunity and oxidative stress of meat-type ducks. Animals, 10(3), 410.

Gümüş E, Sevim B, Sırakaya S, İriş C, Kahraman O and Sarmiento-García A, 2025. Evaluation of the effect of red cabbage waste on performance, egg quality, and yolk oxidative stability of laying Japanese quails (Coturnix coturnix Japonica). Trop. Anim. Health Prod. 57. https://doi.org/10.1007/s11250-025-04336-4

Hamilton R, 1982. Methods and Factors That Affect the Measurement of Egg Shell Quality. Poult. Sci. 61, 2022–2039. https://doi.org/https://doi.org/10.3382/ps.0612022

Haugh RR, 1937. The haugh unit for measuring egg quality. U.S. Egg Poult. Mag. 43(-), 552-573. Sid. Https://sid.ir/paper/543065/en.

Hussain S, Gulfreen E, Abid S, Khalil S, Rizwan M, Batool N, Aziz A, Muhammad H, Abid U and Mahmood N, 2024. Investigating the Impact of Marigold Supplementation on Egg Yolk Color Intensity: A Study on Dietary Additives. J. Health Rehab. Res. 4(1), 1744–1751. https://doi.org/10.61919/jhrr.v4i1.712

Iliopoulou E, Brouklogiannis I, Paraskeuas VV, Griela E, Anagnostopoulos EC, Kefalas G and Mountzouris KC, 2025. Dietary phytogenic inclusion level affects performance and expression of heat shock, cytoprotective, inflammatory and apoptotic genes in the duodenum and the liver of cyclic heat-challenged broilers. Poult. Sci. 104, 105348. https://doi.org/https://doi.org/10.1016/j.psj.2025.105348

Karimi N, Ahangari YJ, Zerehdaran S, Akhlaghi A, Hashemi SR and Adabi NA, 2015. Effects of the dietary supplementation of chromium and vitamin C on egg quality traits in heat-stressed Japanese quails (Coturnix cot. japonica). Eur. Poult. Sci. 79, 1–8. https://doi.org/https://doi.org/10.1399/eps.2015.113

Kour G, Khan N, Sharma RK, Mahajan V, Bhat ZF and Khandii SA, 2024. Influence of Supplementation of Phytogenic Feed Additives on Egg Quality of Layer Quail. Indian J. Anim. Res. 1–8. https://doi.org/10.18805/ijar.b-5273

Kusbiyantari, A, Kardaya D and Sudrajat D, 2017. The efficacy of papaya leaf extract inclusion in drinking water as an improving of layer quail production. J. Pet. Nus. 3, 30–38.

Liu M, Lun J, Huang G, Zhu Y, Zhang W, Jin W, Ding Y, Liu S, Qu Q, Lv W and Guo S, 2024. Alpiniae oxyphyllae fructus improves production performance and egg quality of laying breeder hens by regulating reproductive hormones, antioxidant function, immunity and intestinal health. Poult. Sci. 103, 103770. https://doi.org/https://doi.org/10.1016/j.psj.2024.103770

Lokaewmanee K, Yamauchi K, Komori T and Saito K, 2011. Enhancement of Yolk Color in Raw and Boiled Egg Yolk with Lutein from Marigold Flower Meal and Marigold Flower Extract. J. Poult. Sci. 48, 25–32. https://doi.org/10.2141/JPSA.010059

Nasaka J, Nizeyi JB, Okello S and Katongole CB, 2018. Nutritional Quality of Quail Feeds Used in Urban and Peri-Urban Areas of Uganda: Chemical and Energy Composition. Poult. Sci. J. 6, 63–70. https://doi.org/10.22069/psj.2018.14150.1294

Ndlovu N, Erlwanger KH and Chivandi E, 2025. Effect of Dietary Fortification with Hibiscus sabdariffa Calyces Meal on Egg Production and Egg Quality of Japanese Quail (Coturnix coturnix japonica). Vet. Med. Sci. 11, e70141. https://doi.org/https://doi.org/10.1002/vms3.70141

Odunsi AA, Bakare T, Williams GA, Afolabi IK and Haruna MA, 2023. Production performance and egg qualities of japanese quail (Coturnix coturnix japonica) layers fed diets with varying protein levels supplemented with or without turmeric rhizome powder. Trakia J. Sci. 21, 205–215. https://doi.org/10.15547/tjs.2023.03.001

Oyebanji BO and Atoki FT, 2018. Laying performance, egg quality and hatchability traits of Japanese quail (Coturnix coturnix japonica) fed a chromium yeast feed additive LAYPLUS®: effect of leptidinia reticulata on female quails. Bangladesh J. Anim. Sci. 47, 68–75. https://doi.org/10.3329/BJAS.V47I2.40246

Ratriyanto A, Indreswari R, Dewanti R and Wahyuningsih S, 2018. Egg quality of quails fed low methionine diet supplemented with betaine, in: IOP Conference Series: Earth Environ. Sci. https://doi.org/10.1088/1755-1315/142/1/012002

Sadegheymojarad R, Farhoomand P and Daneshyar M, 2018. Effect of Dietary Betaine and Folic Acid Supplementation on Performance, Egg Folate Content and Egg Production of Japanese Quail. Iran. J. Appl. Anim. Sci. 8, 305–315.

Sarmiento-García A, Olgun O, Kilinç G, Sevim B and Gökmen SA, 2023. Reuse of vegetable wastes in animal feed: the influence of red beet powder supplementation on performance, egg quality, and antioxidant capacity of layer quails. Trop. Anim. Health Prod. 55. https://doi.org/10.1007/s11250-023-03556-w

Stadelman WJ, Newkirk D and Newby L, 1995. Egg Science and Technology (4th ed.). CRC Press. https://doi.org/10.1201/9780203758878

Sudrajat D, Wahyuni D, Nur H and Kardaya D, 2022. External and Internal Quality of the Quail Eggs Fed Ration with Different Level of Metabolizable Energy and Protein. J. Ilmu Teknol. Pet. Tropis 9, 369–374. https://doi.org/10.33772/jitro.v9i2.21763

Tchankugni AN, Anangmo CNN, Yondo J, Mbogning TG, Pone JW and Mpoame M, 2019. Anticoccidial Effects of Ageratum conyzoides (Asteraceae) and Vernonia amygdalina (Asteraceae) Leaves Extracts on Broiler Chickens. South Asian J. Parasitol. 2(1), 19-28. https://journalsajp.com/index.php/SAJP/article/view/35.

Utami MMD and Akbar A, 2025. Enhancing nutrient intake, egg production, and egg quality by fermented Leucaena leucocephala leaf meal in a diet of laying quail. Vet. World 18, 133–140. https://doi.org/10.14202/vetworld.2025.133-140

https://doi.org/https://doi.org/10.1016/j.psj.2024.104394

Wengerska K, Czech A, Knaga S, Drabik K, Próchniak T, Bagrowski R, Gryta A and Batkowska J, 2022. The Quality of Eggs Derived from Japanese Quail Fed with the Fermented and Non-Fermented Rapeseed Meal. Foods 11(16), 2492. https://doi.org/10.3390/foods11162492

Yan Z, Liu Z, Zhou C and Tan Z, 2025. Anti-Nutritional Factors of Plant Protein Feeds for Ruminants and Methods for Their Elimination. Animals. 15(8), 1107. https://doi.org/10.3390/ani15081107

Youssef S, Abdelfatah MA A, Mohamed M, Abd El-Halim HA, Mohamed H, El-Gabry H, Badr H, Sayed-ElAhl R and Em AK, 2023. Substitution of synthetic vitamins, prebiotics and antibiotics by plant feed additives in layer diet of quails reared under chronic heat stress. Egypt. Poult. Sci. J. 43, 319–330. https://doi.org/10.21608/epsj.2023.304019

Zhang G, Song B, Pan X, Keerqin C, Hamada O and Song Z, 2024. Macleaya cordata extract improves egg quality by altering gut health and microbiota in laying hens. Poult. Sci. 103, 104394.