Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 1, 113-117

Effect of Piper Betel leaf infusion supplementation on laying quail performance

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ARTICLE INFO

Received: 06 October 2025

Accepted: 22 December 2025

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Keywords:

Laying quail, Piper betel, Infusions, Production

ABSTRACT

Over the years, antibiotic growth promotors (AGP) have been utilized in the agricultural sector to enhance cattle productivity. Nevertheless, the utilization of AGP is prohibited in numerous countries due to its detrimental effects on human health, such as antibiotic resistance. Utilizing piper betel leaf extracts can increase the sustainability and serve as a viable substitute for AGP. The objective of this study was to improve quail performances by piper betel leaf supplementation in drinking water. The experiment was conducted using a completely randomized design comprising 7 treatment groups with 3 replications each. This study consisted of 7 different treatments. Treatment T0 involved the supplementation of vitamins since the Day Old Quail (DOQ); Treatments T1 = 10% betel leaf infusions supplementation starting from DOQ; T2 = 20% betel leaf infusions supplementation starting from DOQ; T3 = 30% betel leaf infusions supplementation starting from DOQ; T4 = 10% betel leaf infusions supplementation starting from laying phase; T5 = 20% betel leaf infusions supplementation starting from laying phase; T6 = 30% betel leaf infusions supplementation starting from laying phase. The results indicated that the inclusion of betel leaf infusions (T1, T2, T3, T4, T5, and T6) especially T6 ($P < 0.05\%$) enhance the productivity, performance, and quality of quail eggs (Coturnix coturnix japonica).

Introduction

According to the Bruinsma (2017), global consumption of animal products has been steadily rising, particularly in developing countries. This is due to the growing public consciousness of the significance of animal proteins. Nevertheless, livestock goods presently have numerous challenges in the realm of marketing. AGP, or antibiotic growth promoters, have been prohibited in several countries for use in animal feed, particularly in poultry (Windisch *et al*., 2007). The restriction was implemented due to the potential of AGP to accumulate in the bodies of livestock and contributing to the antibiotic resistance formation in diseases. Residues present in cattle products provide a risk to consumers as they have the potential to induce allergies and exhibit resistance to specific medications (Gupta *et al*., 2003). Moreover, the existence of antibiotic-resistant bacteria adds complexity to the treatment of livestock that are sick with these germs, particularly when employing the same medication. The purpose of utilizing AGP in animal feed is to eradicate the bacteria residing in the gastrointestinal system in order to enhance the assimilation of nutrients. Moreover, the utilization of AGP might diminish the incidence of diseases and death in animals. These findings indicate that farmers remain dependent on AGPs to enhance livestock output. Consequently, it is imperative to make concerted efforts to substitute the use of these antibiotics (Butaye *et al*., 2003).

Salmonella is an undesirable bacterium that resides in the gastrointestinal tract of poultry. *Salmonella* is a prevalent bacterium that frequently leads to foodborne illness in underdeveloped nations (Dell-Portillo, 2000). *Salmonella* contamination can arise from feces and the environment in which chicken is housed (Dickson and Anderson 1992). Contaminants can come from animal food and liquids that have been polluted (Stearns and Koella, 2008). Salmonellosis, a disease caused by *Salmonella* spp., is particularly prevalent in chicken and can result in significant animal mortality. Consuming livestock products contaminated with *Salmonella* spp. can lead to salmonellosis in humans (Diarrassouba *et al*., 2007). Salmonellosis is a contagious disease that may be transmitted between animals and humans and has become prevalent across the globe. Consequently, certain nations do not allow the export of cattle products from Indonesia that still contain *Salmonella*.

Utilizing natural antibiotics is an endeavor to substitute the use of AGP (Culafic *et al*., 2005). Secondary metabolites derived from plants, known as natural antibiotics, has the ability to hinder or eradicate microorganisms (Lewis and Ausubel, 2006). Utilizing natural antibiotics is a viable alternative to substituting AGP in Indonesia. Indonesia's abundance of potential biological resources, such as medicinal plants, is due to its tropical climate. In addition, the utilization of natural antibiotics can yield many advantages for animals, including the reduction of cholesterol levels. This is due to the fact that natural antibiotics are impure and consist of only a single component. Moreover, it encompasses numerous additional bioactive substances that can enhance the caliber of animal goods. Raw or extracted forms of natural antibiotics can be directly administered for their medicinal properties. Extracts possess superior antibacterial properties compared to raw materials due to their higher concentration of active components.

The extracts from betel leaves possess antibacterial, antioxidative, and antihaemolytic properties (Chakraborty and Shah, 2011). Betel leaves contain numerous bioactive compounds that provide inhibitory effects against a wide range of microorganisms (Caburian and Osi, 2010). Betel leaves contain betlephenol, an active chemical that has the ability to kill certain microorganisms (Sastroamidjojo, 1997). This material has been verified. Multiple researches have demonstrated that betel leaves have the capacity to serve as organic antibiotics for animals in Indonesia. The extraction method is crucial for ensuring the efficacy of betel leaves in suppressing bacterial proliferation. The ethanol extract of green betel leaf exhibited superior efficacy compared to the water-soluble betel leaf extract in suppressing the proliferation of pathogenic bacteria, as reported by Kaveti *et al*. in 2011. Nevertheless, several aqueous extracts have demonstrated superior outcomes compared to ethanol extracts (Pasha *et al*., 2009).

As stated by Sastroamidjojo (1997), Indonesia possesses about 1000 varieties of medicinal herbs, including betel (*Piper betle* L.). Betel, an indigenous tropical plant, originates from Indonesia (Kurniawan, 2010). Throughout history, the people of Indonesia have utilized this plant to alleviate a range of health issues including toothache, cough, nosebleeds,

ISSN: 2090-6277/2090-6269/

haemorrhoids, dizziness, and other diseases (Madhumita *et al*., 2020). The concentration of the active component in the betel leaves was also examined. Betel leaves contain betlephenol, an active chemical that has the ability to prevent the growth of several bacteria (Sastroamidjojo 1997). Utilizing natural antibiotics enables the international market to accept cattle products. Medicinal herbs, such as betel leaves, can provide natural antibiotics. Feeding betel leaves to cattle is anticipated to enhance livestock performance and quality, decrease mortality, and lower *Salmonella* infection in livestock.

Several studies have examined the antibacterial effects of *Piper betle* extract in poultry; however, most have focused on broilers or used ethanol-based extracts. Limited evidence exists on the use of water-based *Piper betle* infusions in laying quails, particularly in relation to the timing (DOQ vs. laying phase) and varying concentrations (Kulnanan *et al*., 2022; Saranya *et al*., 2020) . This study aimed to fill this gap by evaluating both the timing and dosage of *Piper betle* infusion to enhance the productivity of laying quails. This study presents novelty by evaluating the timing of betel leaf infusion administration (from Day-Old Quail vs. the onset of the laying phase) and its concentration on the production performance of quails. Previous studies have not thoroughly examined the interaction effects between administration phase and dosage on productivity and feed efficiency in quails. The addition of betel leaf infusion (*Piper betle* L.) is enhance the productivity, performance, and quality of quail eggs (Coturnix coturnix japonica). A study has been conducted on the concentration of active substances in betel leaves.

Materials and methods

The ‘Belanda’ variety of *Piper betle* leaves used in this study was sourced from Nagrak, Sukabumi. Pictures of betel leaves are shown in Figure 1. The betel leaves were freshly old and young. Old leaves were three leaves from the end of the stalk. Young leaves are one or two leaves from the end of the stalk. The chemical profile of the betel leaves is showed in Table 1.

Table 1. Vitamins composition.

Composition	Dosage in 1 kg
Vitamin A	6.000.000 IU
Vitamin D_3	1.200.000 IU
Vitamin E	2.500 IU
Vitamin K	3.000 mg
Vitamin B_1	2.000 mg
Vitamin B_2	3.000 mg
Vitamin B_6	1.000 mg
Vitamin B_{12}	2 µg
Vitamin C	20.000 mg
Nicotinate acid	15.000 mg
Calcium-D-Pantothenate	5.000 mg
Electrolyte (Na, K, C, Mg)	750.000 mg
Carrier	1 kg

Fig. 1. Betel leaf used in this research.

The preparation of *Piper betle* leaf infusion is illustrated in Figure 2. The betel leaf was steeped in a solution with a concentration of 1:2 (weight/volume), meaning that 1 kilogram of betel leaf was employed in 2 liters of water. Prior to use, betel leaves were thoroughly rinsed with clean water to remove any foreign debris. Subsequently, a quantity of 1 kilogram of betel leaf was combined with 2 liters of water and pulverized using a blender. The solution was thereafter heated to a temperature of 90°C for a duration of 15 minutes. The time was determined based on the temperature at which the pot reached 90°C. A thermometer was used to measure the temperature of each pot. After a duration of 15 minutes, the infusion was removed and pressed while still in a heated state through a flannel fabric. The undiluted infusion (100%) was subsequently mixed with water to achieve concentrations of 10, 20, and 30%. The laying quails were given a daily infusion of betel leaf as their drinking water for a period of 6 weeks.

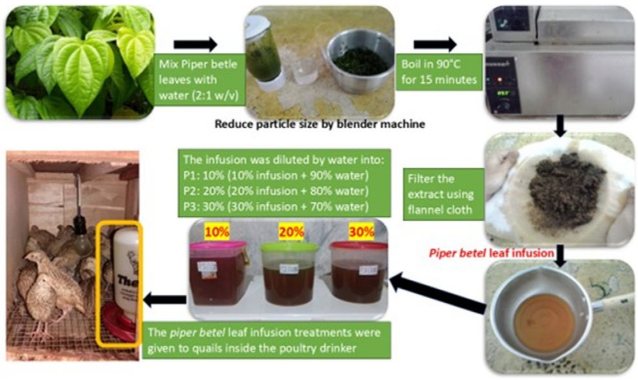

Fig. 2. Preparation and application of *Piper betle* leaf infusion for laying quails.

This study used 210 Quails that were 42 days old with 1 control and 6 treatments which consist of 3 replications for each treatment. The quails were allocated to colony cages for each repetition that consisted of a single enclosure housing a population of 10 quails. The cages utilized were colony cages measuring 20 × 30 × 30 cm and 100 × 30 × 30 cm in dimensions. Performance data of laying quails between the ages of 6 and 12 weeks were collected on daily basis. Feed and water were made available at all time, with the drinking areas were cleansed and replenished with water and piper betel infusion every day. Weekly measurements were taken to determine the quantity of feed and water consumed by weighing both the initial amount of feed supplied and the residual feed. Weekly weighing and egg collecting were conducted to ascertain the mean body weight and egg weight. The data on quail mortality was recorded on a daily basis. The equipment utilized included a digital scale, oven, plastic rations, plastic samples, digital caliper, Roche Yolk color fan, Petri dishes, feed containers, and drinking water containers. Tables 1 and 2 display the specific content of the meals and vitamins used.

Table 2. Nutrients composition of commercial feed product.

Nutrient	Composition (%)
Ash	12.47
Water	10.61
Protein	21.42
Fat	5.14
Crude fiber	4.36
Ca	5.46
P	0.93

Ca: Calcium; P: Phosphorus

Due to constraints in the number of experimental units (quails) and housing facilities, increasing replication was not feasible without compromising animal welfare and management consistency. Hence, a higher number of treatments was prioritized to explore broader treatment options under controlled conditions.

The treatment codes applied in this study are as follows:

T0= basal feed + vitamin;
T1= basal feed + 10% betel leaf infuse (since DOQ);
T2= basal feed + 20% betel leaf infuse (since DOQ);
T3= basal feed + 30% betel leaf infuse (since DOQ);
T4= basal feed + 10% betel leaf infuse (since layer);
T5= basal feed + 20% betel leaf infuse (since layer);
T6= basal feed + 30% betel leaf infuse (since layer)

This study employed a completely randomized design (CRD) and was analyzed using ANOVA. When significant differences were found, Duncan's multiple range test (DMRT) was performed as a post hoc analysis.

Results

The performance of laying quails observed in this study included feed consumption, drinking water consumption, feed conversion, and quail mortality. The ability of quails to produce eggs was also observed by observing the total egg production, total egg mass, and henday from weeks 7 to 12. The results are presented in Tables 3 and 4.

The results showed a statistically significant impact ($P<0.05$) of the therapy on feed consumption, drinking water consumption, and feed conversion. The treatment (T1, T2, T3, T4, T5, and T6) resulted in an overall increase in feed and drinking water consumption in laying quails compared to the control treatment (T0). Laying quails that received treatment from week 5 to week 12 (T4, T5, and T6) showed increased consumption of feed and drinking water compared to quails that were treated since the start of the layer phase.

The control treatment (T0) and quail that received therapy from DOQ (T1, T2, and T3) had the highest conversion rates. Quails that were treated from the beginning of the layer phase (T4, T5, and T6) achieved a lower feed conversion rate. A lower feed conversion value signifies that the treatment enhances the efficacy of laying quails in turning feed into products.

Total egg production and total egg mass were presented in Table 4. The highest total egg production and total egg mass were obtained at T6 of 30.63 eggs^{-1} and the lowest was in the control (T0) of 26.20 eggs^{-1}. The results showed that the total egg production and total egg mass was higher when the infusion level was increased. The treatment administered since the beginning of the layer phase also resulted in a higher total production and total egg mass when compared to the treatment administered since DOQ.

Table 5 shows the quail-day production results from weeks 7 to 12. Egg production at week 7 showed that the treatment administered at the beginning of the layer phase produced a higher value when compared to the treatment administered at DOQ. Egg production in the following

Table 3. layering quail performances week 7-12.

Treatment	Feed consumption (g head^{-1})	Water consumption (mL head^{-1})	Feed Conversion	Mortality (head)
T0	675.0±8.60b	1675.00±35.00b	2.19±0.05d	0
T1	682.7±14.30ab	1685.00±5.00b	2.21±0.07d	0
T2	686.0±2.73ab	1743.33±11.55a	2.14±0.06cd	0
T3	674.0±8.32b	1733.33±36.17a	2.08±0.06bc	0
T4	684.8±7.17ab	1733.33±10.41a	2.00±0.03ab	0
T5	686.9±5.02ab	1746.67±20.21a	1.93±0.03a	0
T6	691.3±1.84a	1758.33±24.66a	1.92±0.03a	0

T0= basal feed + vitamin; T1= basal feed + 10% betel leaf infuse (since DOQ); T2= basal feed + 20% betel leaf infuse (since DOQ); T3= basal feed + 30% betel leaf infuse (since DOQ); T4= basal feed + 10% betel leaf infuse (since layer); T5= basal feed + 20% betel leaf infuse (since layer); T6= basal feed + 30% betel leaf infuse (since layer)

Table 4. Average total egg mass and total egg production week 7-12.

Treatment	Total egg production (egg head^{-1})	Total egg mass (g head^{-1})
T0	26±0.400d	307.67±5.10d
T1	26±0.252d	309.81±3.49d
T2	27±0.611c	320.31±6.93c
T3	27±0.551c	323.98±4.37c
T4	29±0.379b	342.83±7.27b
T5	30±0.346a	356.00±2.77a
T6	30±0.231a	359.55±4.83a

T0= basal feed + vitamin; T1= basal feed + 10% betel leaf infuse (since DOQ); T2= basal feed + 20% betel leaf infuse (since DOQ); T3= basal feed + 30% betel leaf infuse (since DOQ); T4= basal feed + 10% betel leaf infuse (since layer); T5= basal feed + 20% betel leaf infuse (since layer); T6= basal feed + 30% betel leaf infuse (since layer)

Table 5. average quailday production week 7-12.

	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
T0	41±5.15a	64±0.83a	76±0.83a	67±2.47a	67±1.43a	80±3.46
T1	37±4.59a	65±1.65ab	75±3.78a	71±1.43a	68±1.43a	80±2.31
T2	38±3.60a	69±2.18b	76±3.30a	79±4.59b	68±1.43a	80±4.62
T3	36±2.18a	66±2.97ab	76±2.18a	81±2.97b	70±1.65ab	79±4.16
T4	57±5.15b	76±2.18c	76±0.83a	81±1.43b	72±0.00bc	73±1.16
T5	62±0.00b	78±2.86cd	81±0.83b	83±1.65b	72±2.18bc	72±8.08
T6	62±1.43b	82±3.30d	79±2.18ab	83±2.97b	74±3.78c	77±3.06

T0= basal feed + vitamin; T1= basal feed + 10% betel leaf infuse (since DOQ); T2= basal feed + 20% betel leaf infuse (since DOQ); T3= basal feed + 30% betel leaf infuse (since DOQ); T4= basal feed + 10% betel leaf infuse (since layer); T5= basal feed + 20% betel leaf infuse (since layer); T6= basal feed + 30% betel leaf infuse (since layer) week showed decreased quail-day production in the treatment administered at the beginning of the layer phase, and egg production in the treatment administered at DOQ was stable in accordance with the control treatment. Quail-days at week 10 showed a decrease in all treatments.

Discussion

In a previously published study (Widjaya *et al*., 2016), *Salmonella* sp. population in the small intestine of quails was reduced by approximately 98.9% when treated with 30% *Piper betle* leaf infusion starting from the laying phase. This treatment (P6) also resulted in the best production performance parameters. These findings support the current study, which focused on evaluating the production performance of laying quails rather than re-measuring microbial counts. Therefore, the reduction effect is not represented numerically but serves as the biological basis for performance improvements observed here.

Betel leaves contain several compounds such as d-germaken, lepidosen, cariopilene, murolen, selinol, kadine, cineol, hydroxychavicol, stearic acid, and palmitic acid. According to Poudel *et al*. (2024), betel leaf infusions were found to contain alkaloids, flavonoids, phenols, steroids, triterpenoids, tannins, and saponins. Row and Ho (2009) found that phenolic compounds exhibit antibacterial properties. Chakraborty and Shah (2011) state that betel nuts contain sterols, which have antibacterial properties. Betel leaves are rich in phenols and sterols. Friedman *et al*. (2002) identified carvacrol, eugenol, cavibetol, and eugenol isomers as the active antibacterial components found in betel leaves. The volatile, non-volatile, and volatile extracts have demonstrated efficacy in preventing the growth of the bacteria S. aureus, E. Coli, S. thypurium, L. Monocytogenes, P. aeruginosa (Abu-Niaaj *et al*., 2024). The portions of betel nut exhibited significant inhibition against *Salmonella* sp., with an inhibitory diameter ranging from 10 to 26 mm (Arambewela *et al*., 2005). According to Boripun *et al*. (2022), the growth of *Salmonella* can be effectively inhibited by a 15% (v/v) concentration of betel leaf extract. Furthermore, a concentration of 20% (v/v) with a ratio of 1:1 or 1:2 (w/v) can completely kill all *Salmonella* bacteria.

The primary determinants influencing the efficacy of herbal extracts are the specific plant components utilized, their physical characteristics, origin, time of harvest, and compatibility with other constituents in the feed (Wang *et al*., 1998). The heated extracts were ineffective in suppressing *Salmonella* spp.. Nevertheless, the volatile extracts and essential oils have a significant inhibitory impact on *Salmonella* bacteria (Meenu *et al*., 2022). Prior to being fed to animals, it is necessary to do a study to ensure that the substance can be utilized properly without any adverse effects (Fallah *et al*., 2013). A study conducted by Widjaya *et al*. (2017) found that the infusion of betel leaf is efficient in preventing the growth of *Salmonella* spp. in the small intestine of quails. The study conducted by Widjaya *et al*. (2017) found that betel leaf infusion can achieve different levels of inhibition, namely low, medium, and strong. These levels correspond to concentrations of 10% (2.5 mm), 20% (3.5 mm), and 30% (7 mm), respectively. Pan *et al*. (2009) reported that the suppression of bacteria can be categorized as robust (more than 6mm), moderate (between 3-6mm), and feeble (less than 3mm). Elevated concentrations can diminish the palatability of quails (El-Hack, 2016). The palatability of food declines when the fiber and nutritional content increases (Ghazalah and Ali, 2008).

The performance of laying quails observed in this study included feed consumption, drinking water consumption, feed conversion, and quail mortality. The ability of quails to produce eggs was also observed by observing the total egg production, total egg mass, and henday from to 7-12 weeks. The results are presented in Tables 3 and 4.

The results showed a statistically significant impact ($P<0.05$) of the therapy on feed consumption, drinking water consumption, and feed conversion. The treatment (T1, T2, T3, T4, T5, and T6) resulted in an overall increase in feed and drinking water consumption in laying quails compared to the control treatment (T0). Laying quails that received treatment from week 5 to week 12 (T4, T5, and T6) showed increased consumption of feed and drinking water compared to quails that were treated since the start of the layer phase. Leeson and Summers (2005) suggest that water consumption is influenced indirectly by factors such as feed intake and the quantity of feed consumed. The antibacterial activity of betel leaf infusion might lead to increased feed digestibility, resulting in higher feed consumption. According to Windisch*et al*. (2009), plant extracts can enhance feed consumption and feed conversion, but they do not have a substantial impact on body weight. According to Mountzouris *et al*. (2009), plant-derived extracts can contribute to better feed palatability, improved growth performance, and greater nutrient utilization in animals, and overall function of intestinal microbiota, the digestive tract, immune system, and carcass quality. The control treatment (T0) and quail that received therapy from DOQ (T1, T2, and T3) had the highest conversion rates. Quails that were treated from the beginning of the layer phase (T4, T5, and T6) achieved a lower feed conversion rate. A lower feed conversion value signifies that the treatment enhances the efficacy of laying quails in turning feed into products (Hafsah *et al*., 2025). The research conducted on broiler chickens demonstrates that the feed conversion rate is notably distinct from the control group. Moreover, it is seen that the feed conversion rate is superior during the finisher phase compared to the grower phase when herbal extracts are administered (Rahimi *et al*., 2011). Leeson and Summers (2005) identified several parameters that affect ration conversion, including egg output, nutritional composition of diets, egg weight, and temperature. The therapy administered during the layer phase has been superior to the treatment given because to the positive impact of DOQ on feed efficiency, feed consumption, and drinking water. The rise in feed consumption can be attributed to the increasing intake of drinking water. The consumption of drinking water can increase due to the infusion, which can enhance the physiological condition of quails. The infusion of betel leaf contains compounds that have anti-stress properties, capable of reducing stress levels in quails. This can be demonstrated by the reduction in feed conversion, which suggests that the infusion of betel leaf can enhance the egg production performance of quails.

Total egg production and total egg mass are shown in Table 4. The highest total egg production and total egg mass were obtained at T6 of 30.63 eggs-1 and the lowest was in the control (T0) of 26.20 eggs-1. The results showed that the total egg production and total egg mass increased when the infusion level was increased. The treatment administered since the beginning of the layer phase also resulted in a higher total production and total egg mass when compared to the treatment administered since DOQ. Mariot *et al*. (2024) explained that ration consumption and low energy consumption in the production phase of quail results in decreased egg production and egg mass production. Livestock performance showed that feed consumption was increased in the treated group. This is thought to have caused the total production and mass of research quail eggs to increase.

Table 5. shows the quailday production results from weeks 7 to 12. Egg production at week 7 showed that the treatment administered at the beginning of the layer phase produced a higher value when compared to the treatment administered at DOQ. Egg production in the following week showed decreased quailday production in the treatment administered at the beginning of the layer phase, and egg production in the treatment administered at DOQ was stable in accordance with the control treatment. This shows that the betel leaf infusion had a negative effect. Quaildays at week 10 showed a decrease in all treatments. This may have been influenced by noise that occurred at that time.

Additives of plant origin can improve the performance of livestock (Windisch *et al*. 2007). One of these mechanisms is to increase the digestibility and secretion of digestive enzymes (Fallah *et al*., 2013). It also exhibits antiviral, antibacterial, and antioxidant effects (Cross *et al*. 2007). Antimicrobial activity is the main mechanism by which livestock performance and health are improved (Fallah *et al*., 2013).

The superior results observed in the T6 group (30% infusion from the laying phase) suggest that administering betel leaf infusion at a time of high metabolic demand enhances its bioavailability and physiological impact. This aligns with findings by Widjaya *et al* (2017), which showed higher inhibition of *Salmonella* with increased infusion concentration. In contrast, administration since DOQ might lead to adaptation of gut microbiota, reducing the long-term effectiveness of the extract. Therefore, timed administration during the laying period may provide more targeted antibacterial and performance-enhancing effects.

Conclusion

The infusion of betel leaf can enhance the total productivity of laying quail. The most effective concentration for enhancing livestock performance with betel leaf infusion is 30%, administered from the onset of the egg-laying phase. The betel leaf infusion has been applied at a concentration of 10% starting from the onset of the egg-laying phase. Additional research is required to assess the impact of betel leaf infusion on the maximum egg production of laying quails. Administering infusions at specific intervals is necessary to avoid escalating production expenses associated with daily administration. Further investigation into the administration of infusions to other cattle species should be carried out.

Acknowledgements

This research was conducted independently without external funding. The authors gratefully acknowledge the personal support and encouragement from colleagues, friends, and family during the research and writing process.

Conflict of interest

The authors confirm that there are no competing of interest related to this work, including personal, institutional or financial connection that could have influenced the submitted manuscript within the past three years.

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