Research Article

Dietary Supplementation of Fresh Betel Leaf (*Piper betle* L.) Improves Growth and Reduces Respiratory Disorders in Ho Chickens Aged 4 to 12 Weeks

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Abstract | The present trial was conducted in Tan Phu Trung commune, Dong Thap province, Vietnam, from May to September 2024, to evaluate whether dietary inclusion of fresh betel leaves (*Piper betle* L.) could enhance the growth performance and health of Ho chickens. A total of 400 four-week-old birds were randomly assigned to four equal groups and fed diets containing 0%, 0.5%, 1.0%, or 1.5% fresh ground leaves. Chickens receiving the 1.5% supplementation showed a significantly higher ($p < 0.01$) average daily weight gain of approximately 30 g/day, compared to 24 g/day in the unsupplemented control group. Feed conversion ratio was also significantly improved (2.74 vs 3.13; $p < 0.05$). The occurrence of respiratory signs, including coughing and nasal discharge, decreased markedly ($p < 0.05$) in the supplemented groups; at 1.0 and 1.5%, the overall incidence declined to only 1% compared with 8% in controls. These findings indicate that fresh betel leaves, a locally available plant in the Mekong Delta, can serve as a natural feed additive to improve productivity and reduce respiratory disorders in indigenous chickens, providing a practical solution for small-scale farmers in tropical areas.

Keywords | *Piper betle* L., Ho chicken, Growth performance, Feed conversion ratio, Respiratory disease, Phytogenic additive, Antibiotic alternative

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INTRODUCTION

Poultry production plays a critical role in food security and rural livelihoods, especially in developing countries. Indigenous and crossbred chicken strains such as the Ho chicken are widely raised in Vietnam due to their adaptability, meat quality, and market preference. Chicken meat is a preferred choice among consumers due to its high protein content and low-fat levels (Ahmad *et al.*, 2018; FAO, 2021), particularly in indigenous chicken breeds. However, respiratory diseases remain a major constraint in semi-intensive and smallholder systems, often leading to poor growth performance, increased mortality, and reduced economic efficiency. Traditional control methods rely heavily on antibiotics, which poses risks of antimicrobial resistance and drug residues in animal products. Due to the emergence of antibiotic resistance, the use of antibiotic growth promoters (AGPs) in animal nutrition has been banned. Furthermore, consumer preferences are increasingly shifting toward animal products that are free from antibiotics (Seidavi *et al.*, 2021).

In poultry production, additives are often introduced into the diet to improve feed quality, promote growth, and safeguard animal health. For many decades, antibiotics were the main growth promoters. Their efficiency was proven, but concerns soon arose about residues in meat and the emergence of resistant bacteria. As a result, the

World Health Organization has recommended banning their routine use in livestock (Charis, 2000; Schwarz *et al*., 2001; Lee *et al*., 2004). With this restriction, consumers have increasingly preferred chicken products free from drug residues (Demir *et al*., 2005). Feed companies and farmers, therefore, began to explore natural options, including herbs, spices, and forage-based ingredients (Singh *et al*., 2015). Research on phytogenic additives such as garlic, ginger, clove, thyme, or black pepper has reported improvements in poultry health and growth, without the drawbacks associated with antibiotics (Ndelekwute *et al*., 2015; Afolabi *et al*., 2017). Most studies, however, examine single plants, and only a few evaluate combinations or blends, leaving gaps in knowledge about their economic and biological effects in starter diets.

Fresh betel leaf (*Piper betle* L.) is one example of a local resource worth attention. The plant is widely grown in Southeast Asia and is traditionally used in folk medicine to treat respiratory problems. As noted by Nursamsiar *et al*. (2023), both *Piper crocatum* Ruiz and Pav and *Piper betle* L. have been recognized in traditional medicine for their therapeutic properties. Its bioactive compounds, including eugenol, chavicol, and flavonoids, have antimicrobial, anti-inflammatory, and immune-supporting properties, making it a potential alternative to antibiotic growth promoters in poultry feeding. Preliminary *in vitro* and *in vivo* studies have suggested its potential to inhibit respiratory pathogens and support mucosal immunity. Betel leaves are characterized by a distinctive aroma and are rich in bioactive compounds with demonstrated antibacterial, anti-inflammatory, and antioxidant properties. These constituents are known to influence various physiological processes within the body (Sarma *et al*., 2018; Das *et al*., 2016). Despite its ethnomedicinal value and availability, limited scientific evidence exists regarding the effects of betel leaf on poultry, particularly in improving respiratory health and growth under tropical production conditions. Furthermore, most studies focus on extracts, whereas fresh leaf forms, which are more practical and accessible to small-scale farmers, remain underexplored.

Therefore, this study was conducted to evaluate the effects of dietary supplementation of fresh betel leaf (*Piper betle* L.) on growth performance and the incidence of respiratory disorders in Ho chickens during the 4–12 week growing phase. The findings aim to provide a scientific basis for integrating herbal strategies in poultry nutrition, contributing to antibiotic-free and sustainable animal production systems.

MATERIALS AND METHODS

Animals

A total of 400 Ho chickens, aged from 5 to 12 weeks, were used in this study. The birds were obtained from a poultry farm located in Tan Phu Trung Commune, Dong Thap Province, Vietnam, from May to September 2024. Prior to the experimental treatments, all chickens were vaccinated against Gumboro disease and Newcastle disease, and were administered anticoccidial medication via drinking water according to standard health management protocols.

To avoid interference with the experimental outcomes, chickens in the treatment groups supplemented with fresh betel (*Piper betle* L.) leaves were not given any antibiotics during the entire experimental period.

Experimental design and diets

In this study, 400 Ho chickens, each 4 weeks of age, were allocated at random to four dietary treatments, with 100 chickens assigned to each group. Separate floor pens located within a single house were used to rear the birds, and bedding was replaced with clean rice husk to ensure hygiene and consistency. All pens were managed under the same husbandry and environmental conditions throughout the 7-week rearing period, corresponding to the growth stage from 4 to 12 weeks of age.

The feeding trial consisted of four treatment groups. Group 1 served as the control and was given the basal di*et al*one. Group 2 was supplemented with 0.5% fresh ground betel (*Piper betle* L.) leaves, whereas Group 3 received 1.0%. The final treatment, Group 4, was offered the basal diet with 1.5% fresh betel leaf inclusion.

Fresh betel leaves were cleaned, ground, and mixed daily with the feed to ensure consistent intake and bioactivity. The inclusion levels were based on fresh weight and calculated as a percentage of total feed offered.

All chickens were fed a pelleted diet containing 18% crude protein (CP) and 3150 kcal/kg metabolizable energy (ME). The diet was formulated using locally available feed ingredients including broken rice, wheat bran, soybean oil, corn, and fish meal. Calcium and phosphorus contents ranged from 0.4%–1.0% and 0.5%–0.8%, respectively. Feed and clean drinking water were provided ad libitum throughout the experiment.

Sampling, measurements and chemical analysis

Growth performance

Each bird was weighed separately at the start of the experiment (week 5) and then at weekly intervals through to the end of the rearing period (week 12). To avoid bias from gut fill, measurements were always taken in the early morning before feed was offered. The weekly body weights were used to estimate weekly gains, while the overall growth rate was expressed as average daily gain (ADG), calculated with the following formula:

$$\text{Average daily gain (g/bird/day)} = \frac{\text{Final average BW} - \text{Initial average BW}}{\text{Number of days}}$$

Feed intake and feed conversion ratio (FCR)

Feed offered and feed refusal were recorded daily to calculate average daily feed intake per bird. Feed conversion ratio (FCR) was calculated for each treatment group over the entire experimental period using the following formula:

$$\text{FCR}\left(\frac{\text{kg feed}}{\text{kg weight gain}}\right) = \frac{Total\ feed\ intake\ (kg)}{Total\ weight\ gain\ (kg)}$$

Survivability

The health status of all chickens was monitored daily in the early morning. Mortality and culling were recorded, and survivability (%) was calculated at the end of the experiment as:

$$\text{Survivability (\%)} = \frac{Number\ of\ surviving\ birds}{Total\ number\ of\ birds} \times 100$$

Respiratory disorder incidence

Chickens were observed daily for clinical signs of respiratory disorders including nasal discharge, sneezing, coughing, or labored breathing. Birds showing such signs were recorded, and the incidence rate was calculated as:

$$\text{Respiratory disorder rate (\%)} = \frac{\text{Number of affected birds}}{\text{Total birds in the treatment group}} \times 100$$

Statistical analysis

Preliminary data processing was performed using Microsoft Excel 2016. Statistical analyses for growth performance parameters, including feed intake, body weight gain, and feed conversion ratio (FCR), were conducted using the General Linear Model (GLM) procedure in Minitab version 16.1.

The following statistical model was applied:

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

Where; Y_{ij} is the observed value of the dependent variable for treatment j and replicate i; μ is the overall mean; T_i is the fixed effect of treatment i (i=1-4); ε_{ij} is the random error associated with observation ij.

RESULTS AND DISCUSSION

Effect of fresh *Piper betle* leaf supplementation on growth performance and health of Ho chickens

As shown in Table 1, feed intake increased steadily across all weeks in parallel with higher levels of betel leaf supplementation. In week 4, the control group (Group 1) recorded an average daily feed intake of 36.51 grams per bird, which was significantly lower than the 44.84 grams observed in Group 4, which received 1.5 percent betel leaf ($p < 0.05$). This upward trend continued in week 5, with feed intake values of 42.19, 42.38, 44.27, and 50.75 grams per bird per day for Groups 1 through 4, respectively. The highest feed intake was consistently found in Group 4 throughout the experimental period, reaching 82.17 grams per bird per day in week 12, while the control group remained at 75.21 grams. These findings indicate a positive correlation between betel leaf supplementation and voluntary feed consumption. Betel leaves are known for their nutritional value and have been reported to possess both insecticidal and antitumor activities (Gundala and Aneja, 2014). The improved feed intake observed in this study may be due to the distinctive aroma and palatability of fresh betel leaves, as well as the presence of bioactive compounds that can stimulate digestive enzyme activity and enhance gastrointestinal motility, thereby increasing appetite. Furthermore, phytochemicals found in *Piper betle*, such as eugenol and chavicol, exhibit antimicrobial properties that may contribute to a more balanced gut microbiota, improving nutrient digestion and absorption efficiency.

Table 1: Weekly feed intake of Ho chickens (g/bird/day).

Week	Group 1	Group 2	Group 3	Group 4	SEM	p
4	36.51^{c}	37.12^{bc}	40.23^{b}	44.84^{a}	2.1015	0.041
5	42.19^{c}	42.38^{bc}	44.27^{b}	50.75^{a}	2.0471	0.028
6	52.67^{c}	54.28^{b}	56.89^{ab}	62.14^{a}	1.8633	0.012
7	61.04^{c}	65.82^{b}	66.47^{ab}	69.71^{a}	1.8724	0.005
8	64.22^{c}	67.19^{b}	69.03^{ab}	70.27^{a}	1.6248	0.017
9	66.98^{b}	68.23^{ab}	72.01^{ab}	74.14^{a}	2.6173	0.042
10	70.73^{c}	71.09^{b}	73.18^{ab}	78.84^{a}	1.9659	0.039
11	72.15^{c}	74.04^{b}	76.30^{ab}	79.21^{a}	1.8744	0.018
12	75.21^{c}	75.48^{bc}	76.07^{b}	82.17^{a}	1.3897	0.015

Means with different superscripts in the same row differ significantly ($p < 0.05$).

Improvements in feed intake were closely associated with significant increases in body weight gain, as presented in Table 2. In week 4, chickens in the control group gained 16.21 grams per bird per day, while those receiving 1.5 percent fresh betel leaf gained 20.68 grams per day, representing an increase of more than 27.6 percent ($p < 0.05$). This growth advantage continued throughout the experimental period. For example, in week 6, the control group recorded a gain of 18.34 grams, compared to 19.54 grams in Group 2, 21.05 grams in Group 3, and 22.14 grams in Group 4. By week 12, the control group reached 24.05 grams per day, while Group 4 reached 30.02 grams, indicating a statistically significant improvement (p < 0.05). The consistent enhancement in weight gain among the supplemented groups suggests a combined effect of increased feed intake and improved nutrient utilization. The antioxidant and anti-inflammatory compounds in betel leaf likely contributed to maintaining gut health, reducing oxidative stress, and supporting metabolic efficiency. Furthermore, a lower incidence of subclinical respiratory infections may have allowed more metabolic energy to be directed toward tissue development rather than immune responses. Cross *et al.* (2007) also reported that herbal extract supplementation can enhance immune function and improve carcass and meat quality during storage. These findings support the idea that phytogenic feed additives, such as betel leaf, provide both nutritional and functional benefits in poultry production.

Table 2: Weekly body weight gain of Ho chickens (g/bird/day).

Week	Group 1	Group 2	Group 3	Group 4	SEM	p
4	16.21^{c}	16.87^{bc}	16.88^{b}	20.68^{a}	0.4269	0.004
5	16.25^{b}	16.91^{b}	16.93^{b}	21.07^{a}	0.6529	0.003
6	18.34^{c}	19.54^{b}	21.05^{ab}	22.14^{a}	0.5654	0.004
7	20.64^{c}	23.01^{b}	23.78^{ab}	24.49^{a}	0.7032	0.004
8	21.27^{c}	23.48^{bc}	23.75^{b}	24.63^{a}	0.6433	0.006
9	22.76^{c}	24.02^{b}	24.29^{ab}	27.16^{a}	0.8494	0.002
10	23.48^{c}	24.98^{bc}	25.83^{b}	29.05^{a}	0.5951	0.005
11	23.98^{c}	25.06^{bc}	26.32^{b}	29.93^{a}	0.6382	0.006
12	24.05^{c}	25.92^{b}	26.98^{ab}	30.02^{a}	0.6729	0.004

Means with different superscripts in the same row differ significantly (p <0.05).

Feed efficiency, as reflected by the feed conversion ratio (FCR), showed clear improvement in chickens supplemented with fresh *Piper betle* leaves, with notable effects emerging from week 6 onward. As presented in Table 3, the control group had an FCR of 2.872 in week 6, significantly higher than the values recorded in Group 3 (2.703) and Group 4 (2.807) (p = 0.014). This positive trend persisted through subsequent weeks. For instance, in week 7, the FCR in the control group remained elevated at 2.957, while Group 4 achieved a lower ratio of 2.846. By week 12, the FCR in the control group had increased to 3.127, compared to 2.911 in Group 2, 2.820 in Group 3, and 2.737 in Group 4 (p = 0.003), indicating a consistent and statistically significant enhancement in feed efficiency due to betel leaf inclusion. The consistent reduction in FCR among the supplemented groups suggests that birds were able to convert feed into body mass more efficiently. This effect was most prominent in the groups receiving 1.0% and 1.5% supplementation, implying that these inclusion levels may be optimal for growth performance. Notably, the improvement in FCR occurred alongside an increase in feed intake, emphasizing that the gains in body weight were not solely due to greater consumption, but rather to improved nutrient utilization.

Table 3: Feed conversion ratio (FCR) of chickens across weeks.

Week	Group 1	Group 2	Group 3	Group 4	SEM	p
4	2.252	2.200	2.383	2.168	0.3247	0.066
5	2.596	2.506	2.615	2.409	0.5893	0.061
6	2.872^{a}	2.778^{b}	2.703^{c}	2.807^{ab}	0.5831	0.014
7	2.957^{a}	2.860^{ab}	2.795^{c}	2.846^{b}	0.2143	0.008
8	3.019^{a}	2.862^{bc}	2.907^{b}	2.853^{c}	0.0387	0.012
9	2.943	2.841	2.965	2.730	0.1546	0.084
10	3.013^{a}	2.845^{b}	2.833^{bc}	2.714^{c}	0.0491	0.018
11	3.009^{a}	2.954^{ab}	2.900^{b}	2.645^{c}	0.0646	0.024
12	3.127^{a}	2.911^{b}	2.820^{bc}	2.737^{c}	0.0747	0.003

Means with different superscripts in the same row differ significantly (p <0.05).

Similar findings have been reported by Syed *et al.* (2021), who observed enhanced weight gain in starter broiler chickens fed an encapsulated blend of phytogenic feed additives. The beneficial effects observed in the present study may also be linked to the nutritional composition of betel leaves. According to Guha (2006), these leaves are rich in essential vitamins and minerals, which likely contribute to improved digestive function, metabolic efficiency, and overall health. Moreover, reduced stress from respiratory disease in supplemented groups may have minimized energy losses, allowing for more efficient growth.

Effect of fresh *Piper betle* leaf supplementation on the incidence of respiratory diseases in Ho chickens

The addition of fresh *Piper betle* leaves not only supported growth but also contributed to better respiratory health. As presented in Table 4, clinical signs including nasal discharge, coughing, sneezing, and difficult breathing were concentrated in the control group. For instance, in week 5, seven birds in Group 1 showed symptoms, compared with only one case in Group 4. A similar pattern was seen in week 6, where the control group registered seven new cases, while no infections occurred in the supplemented groups. By week 7, five birds in the control and three in Group 2 were affected, whereas Groups 3 and 4 showed none. From week 8 to 12, respiratory illness appeared sporadically only in the control group, while the treated groups remained almost entirely free from infection.

Across the entire experimental period, infection was recorded in 6.0% of the control birds, while the groups supplemented with 0.5%, 1.0%, and 1.5% betel leaves showed lower incidences of 0.75%, 0.25%, and 0.25%,

Table 4: Summarizes the occurrence of respiratory signs in each treatment group across the experimental period.

Week	Total chickens	No. of Sick/infected chickens			
		Group 1	Group 2	Group 3	Group 4
4	400	0	0	0	0
5	400	7	0	0	1
6	400	7	0	0	0
7	400	5	3	0	0
8	400	3	0	0	0
9	400	1	0	1	0
10	400	0	0	0	0
11	400	0	0	0	0
12	400	1	0	0	0
Total	400	24	3	1	1
Infection rate (%)		6.0%[a]	0.75%[b]	0.25%[b]	0.25%[c]

respectively. These findings are consistent with the range of respiratory disease incidence reported for broilers, which can vary between 2.6% and 14.8% depending on housing and management conditions (Roussan *et al*., 2008). Earlier studies also emphasized that poultry, including crossbred Ho chickens during the 5–12 week stage, are particularly vulnerable to respiratory problems such as chronic infections and asthma-like disorders (Crawley and Fahey, 1955; Gross, 1990). The sharp decline in respiratory cases among supplemented groups indicates that fresh betel leaves may provide antimicrobial and immune-modulating benefits. Glevitzky *et al*. (2019) reported that betel leaves contain a high level of polyphenols (68.01 mg/g) and flavonoids (6.17 mg/g), indicating their potential as antioxidant, antifungal, and antibacterial agents. In traditional medicine, betel leaves have been employed as cough remedies, general tonics, and astringent agents (Joesoef *et al*., 1996; Arambewela *et al*., 2010; Chowdhury *et al*., 2020). This effect has been attributed to several bioactive constituents. For instance, chavinol, a phenolic compound in betel leaf oil, possesses antiseptic properties and contributes to the leaf's strong aroma (Dwivedi and Tripathi, 2014). Antibacterial effects have also been linked to sterols and polyphenols, which can damage bacterial membranes (Tan and Chan, 2014). The eugenol present in betel leaf essential oil has been demonstrated to disrupt bacterial and fungal cell wall enzymes, leading to enhanced membrane permeability and subsequent leakage of intracellular materials (Madhumita *et al*., 2019; Gulçin, 2011). In addition, the plant contains antioxidants such as vitamin C, which plays roles in collagen synthesis, neurotransmitter production, and energy metabolism (Molnar *et al*., 2014). Vitamin C is a potent free-radical scavenger (Shah *et al*., 2016), and its concentration in different betel varieties has been reported at around 5.2 mg per 100 g (Guha, 2006). These levels are sufficient to neutralize reactive oxygen species and enhance antioxidant status (Aguirre and May, 2008). Feeding trials further showed that dietary betel leaves can elevate serum antioxidant levels, including both vitamins C and E (Saravanan *et al*., 2003). Taken together, the reduced infection rate observed in this study, along with known antimicrobial and antioxidant properties of *Piper betle*, supports its role as a promising supplement to improve respiratory health in poultry raised under tropical conditions.

CONCLUSION

Supplementing the diet of Ho chickens with 0.5–1.5% fresh betel leaves from 4 to 12 weeks of age improved feed intake, growth rate, feed efficiency, and respiratory health. The 1.5% level gave the clearest benefits across traits. These results indicate that fresh betel leaves, which are locally available and inexpensive, may be used as a natural additive to support both growth and disease resistance in smallholder poultry systems in tropical regions. Beyond reducing reliance on antibiotics, their use may also contribute to efforts against antimicrobial resistance. Further studies focusing on carcass traits, immune responses, and cost-effectiveness are needed to confirm their value in wider poultry production.

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NOVELTY STATEMENT

This study is the first to evaluate the effects of fresh betel leaf (*Piper betle* L.) supplementation, rather than extracts or dried forms, on the growth performance and respiratory health of Ho chickens under smallholder conditions in Vietnam. Unlike previous reports that focused mainly on in vitro properties or on single bioactive compounds, our research demonstrates the practical applicability of incorporating locally available fresh leaves at inclusion levels of 0.5–1.5% into poultry diets. The findings not only establish a direct link between dietary betel leaf and reduced respiratory disorder incidence but also highlight its dual role in enhancing feed efficiency and supporting sustainable, antibiotic-free production systems in tropical environments.

AUTHOR'S CONTRIBUTION

NTC: Wrote the draft, and performed the experiments. NMT: Resources, software and methodology. PN: Conceived, designed, and analyzed the data. All authors reviewed and approved the final manuscript.

Ethical approval

This study did not require formal ethical approval, as no invasive procedures were performed. However, all practices involving animal care, handling, and sample collection were conducted in accordance with the Law on Animal Husbandry (No. 32/2018/QH14) issued by the National Assembly of the Socialist Republic of Vietnam. The welfare of the animals was ensured throughout the experimental period.

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Generative AI and AI-assisted technology statement

Generative AI tools were not used to generate any scientific content. Any AI assistance was limited to minor language editing, and all ideas, interpretations, and conclusions are solely those of the authors.

Conflict of interest

The authors state that there are no conflicts of interest, whether financial, academic, or personal, that could have affected the conduct or reporting of this study.

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