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Effects of Green Betel Leaf (*Piper betle* L.) Extract and Citric Acid on Nutrient Utility, Health, and Meat Quality of Broiler Chickens

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ABSTRACT

The broiler chicken industry has rapidly expanded in recent decades due to increasing demand for affordable animal protein and the species' high production efficiency. This study aimed to evaluate the effects of supplementing broiler chickens' drinking water with green betel leaf extract and citric acid on their nutrient utilization, health, and meat quality. A total of 200 unsexed day-old chicks (DOC) of Cobb 500 strain broiler chickens with an average initial weight of 42.17 ± 0.53 g were used. A completely randomized design (CRD) was implemented, comprising four treatment groups with five replications each, and each replicate consisted of 10 broiler chickens. The treatments involved administering drinking water containing a mixture of green betel leaf extract and citric acid at concentrations of 0% (T0, control), 1% of drinking water (10 ml, T1), 2% of drinking water (20 ml, T2), and 3% of drinking water (30 ml, T3). Parameters assessed included nutrient digestibility (protein, fat, and true metabolizable energy), meat quality (protein, fat, cholesterol content, water holding capacity, and drip loss), blood profiles (erythrocyte, leukocyte, hemoglobin, and hematocrit levels), and oxidative stress indicators (superoxide dismutase and malondialdehyde). Results indicated that T3 significantly had higher protein digestibility (77.22%), fat digestibility (76.00%), true metabolizable energy (3,010.09 kcal/kg), and meat protein content (21.26%) compared to T0, while reducing drip loss (27.93%) and MDA levels (7.88 nmol/mL) compared to T0. In conclusion, supplementing drinking water with 3% feed additive effectively enhances nutrient utility, health, and meat quality in broiler chickens.

Keywords: Betel leaf, Broiler, Citric acid, Meat quality, Nutrient utility

INTRODUCTION

Broiler chickens are among the most commonly raised poultry due to their rapid growth rate, short rearing period, and efficient feed conversion, making them a key commodity in the livestock industry. However, despite these advantages, broiler chickens are highly vulnerable to oxidative stress, particularly due to the overproduction of reactive oxygen species (ROS). Under normal physiological conditions, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), are natural by-products of cellular metabolism and play essential roles in cell signaling and immune defense (Chen et al., 2021). When present in excess, however, ROS overwhelm the body's antioxidant defense systems, resulting in oxidative stress. This condition leads to lipid peroxidation, protein and DNA damage, impaired immune responses, reduced feed efficiency, slower growth, and deteriorated meat quality in broiler chickens (Surai et al., 2019; Mishra and Jha, 2019; Oke et al., 2024).

Diet plays a crucial role in modulating oxidative stress, as nutritional interventions can either exacerbate or mitigate ROS production. Diets lacking in antioxidants or containing oxidized fats can increase ROS levels, whereas supplementation with natural antioxidants has been shown to reduce oxidative damage and improve overall performance. In this context, herbal additives rich in bioactive compounds have gained attention as natural alternatives to synthetic antioxidants. One such plant is green betel leaf, known for its high content of phenolic compounds, flavonoids, and tannins, which exhibit potent antioxidant, antimicrobial, and immunostimulatory effects (Lodang et al., 2020; Setyabudi et al., 2020). Active constituents such as hydroxychavicol, eugenol, and kavikol have been reported to scavenge free radicals, inhibit oxidative enzymes, and enhance the activity of endogenous antioxidants like superoxide dismutase (SOD, Sumarya et al., 2016; Nguyen et al., 2020).

In addition, citric acid commonly used organic acid in poultry production, supports gut health by lowering the pH of drinking water and the gastrointestinal tract, thereby suppressing pathogenic bacteria such as *Escherichia coli* and *Salmonella* while enhancing mineral absorption and nutrient digestibility (Vieira et al., 2017; Liao et al., 2018). The combination of green betel leaf extract and citric acid in broiler chickens' drinking water may provide a synergistic approach to mitigating oxidative stress and improving broiler chickens' performance through their combined antioxidant and antimicrobial effects. Therefore, evaluating the integrated use of these additives is important for enhancing nutrient utility, health, and meat quality in broiler chicken production.

This study aimed to evaluate the effects of supplementing drinking water with green betel leaf extract and citric acid on nutrient utility, health, and meat quality in broiler chickens.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals were approved by the Faculty of Animal and Agricultural Science, Diponegoro University, Semarang, Indonesia (Approval No. 61-05/A-12/KEP-FPP).

Experimental design

The experiment was carried out from December 2024 to January 2025 at the Poultry House of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. A total of 200 unsexed day-old chicks (DOC) of Cobb 500 strain broiler chickens with an average initial weight of 42.17 ± 0.53 g were used. A completely randomized design (CRD) was implemented, comprising four treatment groups with five replications each, and each replicate consisted of 10 broiler chickens. The treatments involved administering drinking water containing a mixture of green betel leaf extract and citric acid at concentrations of 0% (T0, control), 1% of drinking water (10 ml, T1), 2% of drinking water (20 ml, T2), and 3% of drinking water (30 ml, T3). A proximate analysis was conducted on the commercial feed (BR1 for starter and BR2 for finisher, produced by PT Sreeya Sewu Indonesia Tbk). Both feed and water were provided daily at 7 a.m. The broilers were housed in 1×1 m² pens, maintaining a stocking density of 10 broiler chickens/m². The ambient temperature within the pens was kept between 24°C and 32°C, and each pen was illuminated using a 50-watt bulb to ensure adequate lighting. Data on the nutrient composition of commercial BR1 and BR2 feed are listed in Table 1. Feed was analyzed for proximate and mineral content at the Laboratory of Feed and Nutrition Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia.

Table 1. Nutrient composition of commercial starter (BR1) and finisher (BR2) feed in the present study

Nutrient composition	Starter	Finisher
Metabolizable energy (kcal/kg)	3,082.56	3,116.27
Crude protein (%)	21.88	19.63
Crude fat (%)	4.59	4.74
Crude fiber (%)	4.41	4.32
Calcium (%)	1.05	1.08
Phosphorus (%)	0.76	0.81

Feed additive preparation

The feed additive was prepared using a decoction method based on Kaneria et al. (2012). Fresh green betel leaves obtained from local farms. Fresh green betel leaves were oven-dried at 50°C to reduce moisture, ground into powder, and 50 g of the powder was dissolved in 1 liter of distilled water. The mixture was homogenized, incubated in a water bath at 100°C for 30 minutes, then allowed to cool for 10-15 minutes. It was filtered to separate the solid residue, resulting in a green betel leaf extract. This extract was then mixed with 1 g of citric acid to produce the final feed additive solution. Citric acid is used to optimize the work of green betel leaf extract, so the authors do not differentiate the dosage of citric acid used. The additive was administered in volumes of 10 ml (T1), 20 ml (T2), and 30 ml (T3), depending on the treatment group. Data on the content of green betel leaf extract and citric acid are listed in Tables 2 and 3.

Table 2. Green betel leaf extract content used in the present study

Composition	Percentage
Proanthocyanidin	9.75%
Catechin	6.84%
Gallic acid	4.72%
Rutin	3.93%
Hydroxychavicol	2.87%
Quercetin glycoside	2.42%
Eugenol	2.41%
Ellagic acid	1.86%

Source: Analysis results from the Phytochemistry Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

Table 3. Citric acid content used in the present study

Content	Percentage
Oxygen	58.33%
Carbon	37.50%
Hydrogen	4.17%

Source: Analysis results from the Phytochemistry Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

Nutrient digestibility

Data on protein digestibility, fat digestibility, and true metabolizable energy were obtained using the total excreta collection method based on Hossain et al. (2013). One broiler chicken per replicate was placed in a battery cage. After a 24-hour fasting period (water only), chickens were fed diets containing Fe_2O_3 and treatment water. Fe_2O_3 is used as an indicator to differentiate the excreta, where excreta without the indicator appear green, while excreta containing the indicator turn reddish. Excreta marked red were collected and sprayed hourly with 0.2 N HCl to prevent nitrogen loss. This was repeated over 3 days. Excreta from 20 endogenous chickens (fasted for 2 days) were also collected.

Blood profile and oxidative stress

Blood samples (3 mL) were collected from the brachial vein using a syringe and stored in EDTA-containing vacutainers. Samples were kept in a cooler box and analyzed in the laboratory.

Meat protein, fat, and cholesterol levels

Meat samples from the thigh and breast (50 g) were homogenized and analyzed in the laboratory to determine the levels of protein, fat, and cholesterol.

Water holding capacity

The method used to measure water holding capacity is based on Qiao et al. (2001). A 0.3 g meat sample was pressed between Whatman filter papers under a 35 kg load for 5 minutes, and the wet area was traced to determine water holding capacity.

Drip loss

The method used to measure drip loss is based on Sarkar et al. (2024). A 100 g meat sample was sealed in a plastic bag, boiled in an 80°C water bath for 1 hour, then cooled in 10°C water for 15 minutes. After drying, the sample was weighed to determine drip loss.

Data analysis

Data were analyzed using SPSS version 26.0. Data were analyzed using one-way ANOVA. If significant differences were found, Duncan's multiple range test was used to compare treatments (at a 5% significance level).

RESULT AND DISCUSSION

Nutrient digestibility

The addition of green betel leaf extract and citric acid significantly increased protein digestibility in broiler chickens compared to T0 ($p < 0.05$, Table 4). The highest digestibility was observed in T3 (3% additive), which did not differ significantly from T2 but was significantly higher than T1 and T0. T0 had the lowest value among the groups. The improvement is attributed to the synergistic action of betel leaf phytochemicals and citric acid, which enhance protease activity, optimize gut pH, suppress pathogenic bacteria, and support gut health (Lodang et al., 2020; Melaku et al., 2021).

The combination of green betel leaf extract and citric acid significantly improved fat digestibility in broiler chickens compared to T0 ($p < 0.05$, Table 4). The highest digestibility was observed in T3 (76.00%), followed by T2 and T1, while the lowest was in the control group T0 (73.53%). The improvement is attributed to enhanced lipase activity, better fat emulsification, and suppression of digestive-inhibiting bacteria. Bioactive compounds in betel leaf, such as flavonoids, tannins, and saponins, stimulate bile secretion and aid fat breakdown (Singh et al., 2023). Citric acid supports an optimal pH for lipase function and improves fatty acid solubility, enhancing absorption efficiency (Hosseini et al., 2017).

Supplementation of green betel leaf extract and citric acid significantly increased true metabolizable energy in broiler chickens ($p < 0.05$), with the highest value in T3 (3,010.09 kcal/kg) compared to T0 (2,826.34 kcal/kg) (Table 4). The enhancement is linked to improved protein and fat digestibility, increased ATP production via the Krebs cycle, and better mitochondrial efficiency (Bottje et al., 2017).

Table 4. Effects of green betel leaf extract and citric acid supplementation on nutrient digestibility in broiler chickens

Parameters	Treatments			
	T0	T1	T2	T3
Protein digestibility (%)	74.87^{c}	75.74^{b}	76.44^{ab}	77.22^{a}
Fat digestibility (%)	73.53^{c}	74.27^{bc}	75.10^{ab}	76.00^{a}
True metabolizable energy (kcal/kg)	2,826.34^{c}	2,890.25^{bc}	2,949.91^{ab}	3,010.09^{a}

$^{a-c}$ Different superscripts in the same column indicate a significant difference ($p < 0.05$). T0: Drinking water without feed additive (control), T1: Drinking water + 1% feed additive, T2: Drinking water + 2% feed additive, T3: Drinking water + 3% feed additive.

Meat quality

The combination of green betel leaf extract and citric acid significantly increased broiler chickens' meat protein content ($p < 0.05$), with the highest value in T3 (21.26%) compared to T0 (18.95%) (Table 5). Bioactive compounds in betel leaf support gut health and reduce oxidative stress (Hossain et al., 2017), while citric acid optimizes proteolytic enzyme activity and mineral absorption (Hosseini et al., 2017).

The combination of green betel leaf extract and citric acid significantly increased broiler chickens' meat fat content ($p < 0.05$), with the highest in T3 (7.21%) compared to T0 (5.19%) (Table 5). Bioactive compounds in betel leaf promote bile secretion and lipid absorption, while citric acid optimizes lipase activity and energy metabolism via the citric acid cycle (Han et al., 2016; Ding et al., 2022).

The combination of green betel leaf extract and citric acid significantly increased broiler chickens' meat cholesterol levels ($p < 0.05$), with the highest value in T3 (87.62 mg/100g) compared to T0 (84.66 mg/100g, Table 5). This increase may result from enhanced cholesterol absorption, elevated acetyl-CoA availability, and stimulated endogenous synthesis (Ge et al., 2019). Although betel leaf has hypocholesterolemic properties, dosage or interaction effects may have reversed its function. Despite contradicting earlier studies, cholesterol levels remained within the normal range (80-120 mg/100g; Giampietro-Ganeco et al., 2020).

The addition of green betel leaf extract and citric acid significantly improved the water-holding capacity (WHC) of broiler chicken meat ($p < 0.05$), with the highest value in T3 (62.04%) compared to T0 (59.73%) (Table 5). Bioactive compounds in betel leaf protect muscle structure, while citric acid helps regulate pH and glycolysis, enhancing protein stability (Panpipat and Chaijan, 2016; Chodkowska et al., 2022). Their synergistic effect leads to more compact muscle tissue with improved water retention, indicating better meat quality.

The supplementation of green betel leaf extract and citric acid significantly reduced drip loss in broiler chicken meat ($p < 0.05$), with the lowest value in T3 (27.93%) compared to T0 (30.20%) (Table 5). Bioactive compounds protect against heat-induced protein denaturation, while citric acid stabilizes pH and enhances protein-water interactions (Lodang et al., 2020; Unal et al., 2022). Their synergistic effects result in juicier, higher-quality meat with less moisture loss during cooking.

Blood profile and oxidative stress

The combination of green betel leaf extract and citric acid significantly increased erythrocyte levels in broiler chickens ($p < 0.05$), with the highest count observed in T3 (2.82×10^6/μL) compared to T0 ($2.34 \times 10^6/mm^3$, Table 6). The synergistic interaction of bioactive compounds found in betel leaf and citric acid contributes to improved hematological parameters by increasing iron bioavailability, promoting erythropoietin synthesis, and safeguarding bone marrow from oxidative damage, thereby supporting red blood cell formation.

The combination of green betel leaf extract and citric acid significantly increased leukocyte levels in broiler chickens ($p < 0.05$), with the highest count in T3 ($22.95 \times 10^3/mm^3$) compared to T0 ($20.41 \times 10^3/mm^3$, Table 6). Flavonoids and alkaloids stimulate immune cell production and enhance pathogen resistance (Rahayu et al., 2023), while citric acid supports leukocyte function through antioxidant protection and better metabolic activity (Krauze et al., 2021).

The combination of green betel leaf extract and citric acid significantly increased hemoglobin levels in broiler chickens ($p < 0.05$), with the highest value in T3 (11.91 g/dL) compared to T0 (9.89 g/dL, Table 6). The bioactive properties of citric acid contribute to hemoglobin synthesis by improving the absorption of essential minerals, shielding red blood cells from oxidative damage, and enhancing both the quality and functionality of erythrocytes (Elnaggar and El-Kelawy, 2024).

Supplementation with green betel leaf extract and citric acid significantly increased hematocrit levels in broiler chickens ($p < 0.05$), with T3 (27.20%) showing the highest value compared to T0 (23.99%, Table 6). Bioactive compounds in citric acid promote iron absorption, protect erythrocyte membranes from oxidative stress, and stimulate erythropoietin production, thus improving both quantity and quality of red blood cells (Islam et al., 2021).

Dietary supplementation with green betel leaf extract and citric acid significantly increased blood SOD levels in broiler chickens ($p < 0.05$), with the highest activity observed in T3 (2.717 U/gHb) compared to T0 (2.460 U/gHb, Table 6). This enhancement is attributed to the synergistic effects of flavonoids and citric acid in inducing SOD gene expression and stabilizing the enzyme (Tang et al., 2019; Ebeid and Al-Homidan, 2022).

Supplementation with green betel leaf extract and citric acid significantly reduced blood MDA levels in broiler chickens ($p < 0.05$), with the lowest value in T3 (7.88 mmol/mL) compared to T0 (9.91 nmol/mL, Table 6). The effect is linked to enhanced antioxidant capacity from flavonoids, tannins, and citric acid, which act as free radical scavengers, metal chelators, and membrane stabilizers, improving cellular integrity and metabolic health (Sahin et al., 2016; Magied, 2019).

Table 5. Effects of green betel leaf extract and citric acid supplementation on broiler chickens' meat quality

Parameters \ Treatments	T0	T1	T2	T3
Meat protein content (%)	18.95[c]	20.17[b]	20.65[ab]	21.26[a]
Meat fat content (%)	5.19[a]	5.83[ab]	6.50[bc]	7.21[c]
Meat cholesterol content (mg/100g)	84.66[a]	85.66[b]	86.44[bc]	87.62[c]
Water holding capacity (%)	59.73[c]	60.27[bc]	60.78[b]	62.04[a]
Drip loss (%)	30.20[c]	29.27[bc]	28.42[ab]	27.93[a]

[a-c] Different superscripts in the same column indicate a significant difference ($p < 0.05$). T0: Drinking water without feed additive (control), T1: Drinking water + 1% feed additive, T2: Drinking water + 2% feed additive, T3: Drinking water + 3% feed additive.

Table 6. Effects of green betel leaf extract and citric acid supplementation on blood profile and oxidative stress in broiler chickens

Parameters \ Treatments	T0	T1	T2	T3
Erythrocyte ($10^6/mm^3$)	2.34[b]	2.48[b]	2.70[a]	2.82[a]
Leukocyte ($10^3/mm^3$)	20.41[c]	21.30[bc]	22.23[ab]	22.95[a]
Hemoglobin (g%)	9.89[c]	10.51[bc]	11.18[ab]	11.91[a]
Hematocrit (%)	23.99[c]	25.08[bc]	26.15[ab]	27.20[a]
SOD (U/gHb)	2,460[c]	2,549[bc]	2,636[ab]	2,717[a]
MDA (nmol/mL)	9.91[c]	9.13[bc]	8.28[ab]	7.88[a]

[a-c] Different superscripts in the same column indicate a significant difference ($p < 0.05$). T0: Drinking water without feed additive (control), T1: Drinking water + 1% feed additive, T2: Drinking water + 2% feed additive, T3: Drinking water + 3% feed additive. SOD: Superoxide dismutase, MDA: Malondialdehyde.

CONCLUSION

The addition of 3% green betel leaf extract and citric acid as a feed additive can improve nutrient utility, health, and meat quality in broiler chickens. Further research on molecular mechanisms behind the synergistic effects of green betel leaf extract and citric acid, such as their impact on gut microbiota, antioxidant enzyme expression, and nutrient absorption pathways, is warranted.

DECLARATIONS

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Author's contributions

Adam Satria Pratama Nasution conducted the research, collected and analyzed the data, and drafted the manuscript. Luthfi Djauhari Mahfudz and Cahya Setya Utama reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Competing interests

The authors have declared no conflict of interest.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all authors.

Availability of data and materials

All data generated during the study are included in this article. Any additional information is available upon reasonable request from the authors.

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