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Research Article

Nanoencapsulated *Peronema canescens* Jack Leaf Extract in Drinking Water Improves Carcass Yield and Meat Quality of Broiler Chickens

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Abstract | The use of plants rich in bioactive compounds has gained attention in the post-antibiotic era, with nanoencapsulation technology developed to improve their bioavailability, stability, and solubility. This study aimed to evaluate the effect of nano encapsulated *Peronema canescens* Jack leaf extract (NEDS) in drinking water on improving the physical quality of broiler chicken meat. A total of 288 male broiler chickens were fed basal diets and provided drinking water supplemented with 25 mg/L zinc bacitracin (positive control); 0.8% (T.1); 1.7% (T.2); 3.4 % (T.3); 6.8% (T.4) NEDS; and no supplementation (negative control). The results demonstrated that supplementation of 6.8% NEDS through drinking water significantly increased carcass weight and thigh lightness ($P<0.001$), while reducing the yellowness and saturation index in the breast meat ($P<0.001$). A medium dose (1.7%) enhanced the hue angle of the thigh and reduced both redness and hardness of the meat ($P<0.001$), whereas the lowest dose (0.85%) decreased the yellowness, color saturation index of the thigh ($P<0.001$), and meat gumminess ($P<0.05$). Overall, supplementation with 1.7%, 3.4%, and 6.8% NEDS improved the FCR ratio of broiler chickens and hue angle in the breast ($P<0.05$) and reduced the reddish color of the breast ($P<0.001$). However, NEDS supplementation showed no significant effects on body weight, breast brightness, pH values (breast: 45 min, 2 h, and 24 h; thigh: 45 min and 24 h), moisture content, drip loss, pressing loss, and texture attributes including resilience, cohesiveness, springiness, and chewiness. In conclusion, supplementation of NEDS in drinking water can improve certain aspects of broiler meat appearance and carcass yield without adversely affecting overall meat quality. Among the tested doses, 6.8% NEDS was the most effective level, demonstrating the greatest improvement in carcass weight and desirable color attributes.

Keywords | Broiler chicken, Drinking water, Meat quality, Nanoencapsulated, *Peronema canescens* Jack

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INTRODUCTION

Global meat output reached 365 million tons in 2024, representing a 1.3% increase from the previous year, with the poultry industry recording the largest growth among all sectors (FAO, 2025). The demand for meat is expected to rise in line with the ongoing consumption growth, in accordance with the global predictions. Broiler chickens are a major livestock commodity and play an important role in supplying animal protein to the public.

This segment is highly preferred in the industry because of its rapid growth, efficient feed conversion, and affordable meat production (Tixier-Boichard, 2020). The public most frequently consumes breast and thigh meat because of their high nutritional value, appealing texture, and flexibility in cooking (Xu *et al*., 2024). However, the quality of broiler chicken meat is often a concern because it affects consumer acceptance, shelf life, and the additional value of processed products. Maintaining a healthy gastrointestinal system is crucial for enhancing broiler output, as it directly impacts growth performance (Ducatelle *et al*., 2023). Therefore, nutritional strategies, particularly the use of feed additives, are being employed to support gut health and consequently improve meat quality.

Over the decades, the use of antibiotic growth promoters (AGPs) has been widely applied in the poultry industry to promote and maintain livestock health (El-Fateh *et al*., 2024). However, this practice is now prohibited in many countries (Cardinal *et al*., 2019), including in the Southeast Asia region. The use of AGPs in the livestock industry leads to antibiotic residues in animal products (Muaz *et al*., 2018) and leads to the expansion of antimicrobial resistance that threatens human health (Plata *et al*., 2022). This situation has encouraged the exploration of natural alternatives that can replicate the beneficial effects of AGPs while ensuring safety and consumer acceptance. Herbal ingredients rich in bioactive compounds, particularly those with antioxidant and antimicrobial properties, have become the focus in developing feed and water additives for modern poultry production.

The woody medicinal herb *Peronema canescens* Jack (*P. canescens*), also known as Sungkai and endemic to Indonesia, is a potential candidate for replacing AGP. *P. canescens* leaves have been reported to have anti-inflammatory (Maigoda *et al*., 2023), antibacterial (Shalihin *et al*., 2024), antioxidant (Rizki *et al*., 2022), and immunomodulatory (Rahardhian *et al*., 2025). Numerous bioactive substances, including polyphenols, tannins, flavonoids, saponins, alkaloids, and diterpenoid clerodanes, are known to be present in *P. canescens* leaves. Peronemins A2, A3, B1, B2, C1, and D1 are among the clerodane-type diterpenoids that have been found to have significant pharmacological potential (Kitagawa *et al*., 1994; Ahkam *et al*., 2024). Nevertheless, many of the bioactive substances in *P. canescens* leaf extract are lipophilic, which leads to a low water solubility, restricted bioavailability, and decreased absorption in the gastrointestinal system. Furthermore, these substances are unstable and prone to quick breakdown in the gastrointestinal tract (Mohammadi *et al*., 2016), which reduces their effectiveness when administered directly to broiler chicks. By increasing the surface area to volume ratio, nanoencapsulation technology can improve the bioavailability of encapsulated materials and enable their controlled and sustained release at the target site, which is one of its many benefits (Osman *et al*., 2024). To protect bioactive compounds and enhance the functional benefits of herbal supplements in animal nutrition, nanoencapsulation may prove to be an effective and affordable method (Linh *et al*., 2022).

It is believed that adding *P. canescens* leaf extract in nanoencapsulated form to drinking water will enhance the texture, color, and water-holding capacity of broiler meat at harvest age, particularly in the breast and thigh. As an innovative approach to replace AGPs, this study was carried out to assess the impact of applying nanoencapsulated *P. canescens* leaf extract to drinking water on the quality of 28-day-old broiler chickens.

MATERIAL AND METHODS

Nano-encapsulated *Peronema canescens* leaf jack preparation

The leaves of *Peronema canescens* Jack were collected from naturally grown plants in Ogan Komering Ilir, South Sumatra, Indonesia (3.36667°S, 105.36667°E). The leaves were sun-dried for three to seven days until completely dry and then ground into a fine powder. The extraction process followed the maceration technique described by Samreen *et al*. (2022). The nanoencapsulated *P. canescens* leaf extract (NEDS) was prepared using the ionic gelation technique, as described by Kim *et al*. (2022), with several modifications to the formulation components' ratio. A 0.1% (w/v) chitosan solution was prepared by dissolving chitosan (Shanghai Nichechem, China) in 1% (v/v) acetic acid under continuous stirring until homogeneous. A 0.1% (w/v) sodium tripolyphosphate solution (Sigma-Aldrich, Germany) was prepared as the ionic cross-linking agent, while the *P. canescens* leaf extract was added at a final concentration of 1% (w/v). First, the chitosan solution and *P. canescens* leaf extract were mixed and magnetically stirred for 10 minutes. Subsequently, the sodium tripolyphosphate solution was added dropwise over 10 minutes under constant stirring to allow nanoparticle formation. The final NEDS formulation consisted of 68% chitosan (v/v), 18% *P. canescens* leaf extract (v/v), and 14% sodium tripolyphosphate (v/v). The physicochemical characteristics of the obtained NEDS were as follows: particle size of 178.733 ± 3.286 nm, polydispersity index of 0.489 ± 0.906, encapsulation efficiency for phenols of 15.611 ± 2.647%, encapsulation efficiency for tannins of 33.294 ± 6.290%, and zeta potential of +37.800 ± 1.571 mV.

Research design

The study was conducted using 288 male day-old New Lohmann Indian River (MB 202 Platinum) strain from PT. Widodo Makmur Unggas, Yogyakarta, Indonesia. Before being allocated to each cage, the one-day-old chicks received three vaccinations: An injectable killed Newcastle Disease vaccine, a live Infectious Bursal Disease vaccine, and a live spray vaccine for Newcastle Disease and infectious bronchitis. This study was conducted in a semi-closed poultry house at the Faculty of Animal Science, Universitas Gadjah Mada. In a controlled environment building system, broiler chickens were housed at a maintenance room temperature of 30°C for chicks ages 0–3 days, which was lowered by 2°C every 3 days to achieve 20°C as necessary. The experiment consisted of six treatment groups with six replicates (8 birds per replication): Positive control = 25 mg/L of zinc bacitracin (PT. Samyou International, Sidoarjo, East Java, Indonesia); Negative control = no treatments; T.1 = 0.8% NEDS; T.2 = 1.7% NEDS; T.3 = 3.4 % NEDS; T.4 = 6.8% NEDS. The use of 25 mg/L zinc bacitracin as a positive control was adopted from Dewanti *et al.* (2024). The treatment doses of *P. canescens* leaf extract were determined based on the minimum inhibitory concentration (MIC) reported by Tamfu et al. (2022). The lowest treatment dose corresponded to one-fourth of the MIC, while the highest dose was twice the MIC against *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Each treatment was analyzed for the quantification of total phenolic and tannin compounds following the FAO (2000) method. Treatment T.1 contained 1.17 mg TAE/L of phenolics and 0.53 mg TAE/L of tannins; T.2 contained 2.35 mg TAE/L of phenolics and 1.06 mg TAE/L of tannins; T.3 contained 4.70 mg TAE/L of phenolics and 2.13 mg TAE/L of tannins; and T.4 contained 9.40 mg TAE/L of phenolics and 4.25 mg TAE/L of tannins. The treatments were administered daily through drinking water starting from day 8 of the rearing period. Feed and water were provided *ad libitum*. During the starter phase (1–7 days), birds were fed a commercial diet (7500, PT. New Hope Indonesia, Cirebon, Indonesia) containing 13% moisture, 23% crude protein, 5% crude fat, 4% crude fiber, 0.80–1.10% calcium (Ca), 0.5% phosphorus, 1.30% lysine, 0.50% methionine, 0.90% methionine + cystine, 0.20% tryptophan, and 0.80% threonine. The composition of grower diet (8–21 days) and the composition of finisher diet (22–28 days) were formulated according to Aviagen (2022) recommendations, as shown in Tables 1 and 2. Broiler management practices were generally conducted following the Indian River Broiler Management Handbook (Aviagen, 2018).

Data collection

Broiler growth performance

All chickens were weighed collectively each week using a TM-A 150 kg scale (Altraman, West Jakarta, Indonesia). Beginning throughout the treatment period, feed and water wastes were measured each morning before the addition of feed and the replacement of drinking water. The final body weight was recorded for each replication group, 12 hours after feed was withdrawn while drinking water had remained available. FCR was calculated by dividing the total feed intake by the body weight gain (Jie *et al.*, 2024). Carcass weight was determined by weighing the broiler after the removal of blood, feathers, head, neck, shanks, and internal organs (including the lungs and kidneys) (Junior *et al.*, 2024). The research steps are outlined in the experimental design flowchart (Figure 1).

Meat color traits

Meat color traits were calculated based on lightness level (L*), redness intensity (a*), and yellowness intensity (b*), measured using a colorimeter (Minolta CR-300, Minolta Camera Co. Ltd., Japan) referring to the CIELAB color space (Spínola *et al.*, 2025). Each sample was read three times and the average was recorded. Based on Al-Baadani *et al.* (2023) meat color analysis was continued by calculating the hue angle and saturation index.

$$\text{Hue Angle} = \arctan(b^*/a^*) \text{ or } \tan^{-1}(b^*/a^*)$$
$$\text{Saturation Index (Chroma)} = \sqrt{(a^{*2} + b^{*2})}$$

pH characteristics

pH of the meat was measured in the breast and thigh sections at 45 minutes, 2 hours, and 24 hours. The pH measurement was performed based on the modified method of Kim *et al.* (2019). One gram of chicken breast meat was homogenized with 40 mL of distilled water for 30 seconds using a homogenizer at a speed of 13.000 rpm (T 25 digital ULTRA-TURRAX, IKA Werke GmbH & Co., Staufen, Germany), and the pH of the homogenate was measured directly by a pH meter (FE20, Mettler Toledo, Greisensee, Switzerland). The reading was taken three times.

Water holding capacity traits

Moisture content in the meat was analyzed in the breast and thigh sections. Moisture content was determined by drying the sample at 105 °C until a constant weight was achieved (AOAC, 2011). The water holding capacity observed included pressing loss, thawing loss, and drip loss. Muscle samples from the chest and thigh, measuring 1x1 cm were weighed on filter paper. The samples were then pressed using a pressing machine with a force of 35 kg for 5 minutes. The compressed samples were weighed again. The compression loss was measured based on the following equation (Weng *et al.*, 2022):

$$\text{Pressing loss (\%)} = [(W1\text{-}W2)/ W1] \times 100\%$$

The sample was weighed prior to freezing to determine its initial weight. After that, the sample was thawed overnight at room temperature, dried with paper towels, and weighed the next morning to measure the thawing loss (Pontalti *et al.*, 2025). Twenty-four hours after death, the thigh and breast muscles were weighed, put in plastic bags, hung on hooks, and kept for three days at 2 °C. The samples were hung, wiped with absorbent paper, and then weighed once more. The weight difference, which was reported as a percentage of the starting muscle weight, correlated with drip loss (Belloir *et al.*, 2019).

Table 1: Ingredients and composition of the basal diets.

Feed ingredients	Composition of grower diet (%)	Composition of finisher diet (%)
Yellow corn	56.11	59.95
Soybean meal	28.08	25.03
Rice bran	6.00	6.00
Meat bone meal	5.30	4.00
Palm oil	2.50	3.00
Limestone	0.60	0.60
Dicalcium phosphate	0.50	0.60
L-Lysine HCl	0.25	0.24
NaCl	0.25	0.25
DL-Methionine	0.20	0.15
L-Threonine	0.12	0.09
Mineral premix[1]	0.07	0.08
Vitamin premix[2]	0.02	0.02
Total	100	100
Calculated value		
Metabolizable energy (kcal/kg)	3052.71	3113.29
Crude Protein (%)	21.10	19.34
Digestible lysine (%)	1.16	1.06
Digestible methionine (%)	0.48	0.42
Digestible threonine (%)	0.88	0.78
Calcium (%)	0.91	0.80
Available Phosphorus (%)	0.42	0.38

[1]Supplied per kilogram diet: Mn, 180 mg; Mg, 50 mg; Fe, 160 mg; Zn, 175 mg; Cu, 12 mg; I, 0.98 mg; Co, 0.36 mg; Se, 0.3 mg. [2]Supplied per kilogram diet: Vitamin A, 5,000 IU; Vitamin D3, 900 IU; Vitamin E, 80 mg; Vitamin K3, 10 mg; Vitamin B1, 10 mg; Vitamin B2, 18 mg; Vitamin B6, 20 mg; Vitamin B12, 0.1 mg; Vitamin C,10 mg; Ca-d-Pantothenate, 40 mg; Niacinamide, 120 mg; Folic Acid, 4 mg; Biotin, 0.1 mg.

Texture traits

The modified Aguirre *et al.* (2018) approach was used to perform texture analysis on each replicate of cooked chicken breast meat samples that were cut with a knife into 1x1 cm pieces. A texture analyzer (TA. XT Plus, Texture Technologies, Hamilton, MA) fitted with a 76.2 × 10 mm cylindrical probe to compress the data was used to measure texture. To accomplish 50% compression, a 50 kg load cell was tested at 3.0 mm/s for pre-testing, 1.0 mm/s for testing, and 3.0 mm/s for post-testing.

Data analysis

The data collected were analyzed using ANOVA in a completely randomized design with SPSS Statistics software version 29.0. A one-way arrangement was used to check the effects of different treatments, and Duncan's post-hoc test was used to compare the means of the groups. The significance level was set at $P \leq 0.05$. The statistical model used was:

$$Yij = \pi + T_i + e_{ij}$$

Yij represents value of the concerned treatment, π is the mean for the concerned treatment, T_i is the fixed effect of the treatments, and e_{ij} is the random error.

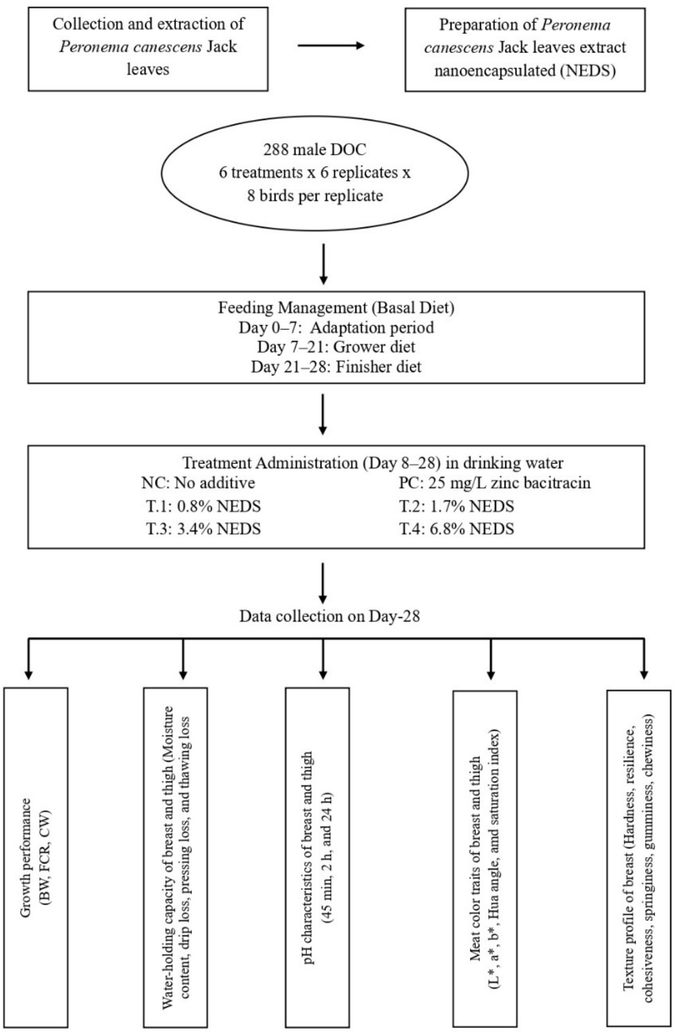

Figure 1: Flowchart of the experimental design.

RESULTS AND DISCUSSION

Broiler growth performance

Table 2 showed the results of growth performance traits in broiler chickens receiving nano-encapsulated *Peronema canescens* Jack leaf extract in drinking water. The table indicated that the administration of NEDS did not have a significant effect on the body weight of 28-days old chickens.

Table 2: Growth performance traits of broiler chickens receiving nano-encapsulated *Peronema canescens* Jack leaf extract in drinking water (g).

Variables	T.1	T.2	T.3	T.4	NC	PC	Level of sig	
							SEM	P value
BW (g)	1,582.26	1,618.26	1,638.11	1,662.57	1,592.67	1,607.61	9.05	0.09
CW (g)	1,079.50[c]	1,180.83[b]	1,222.16[b]	1,339.00[a]	991.00[d]	1,208.83[b]	20.94	<0.001
FCR	1.46[ab]	1.45[b]	1.43[b]	1.42[b]	1.53[a]	1.50[ab]	0.01	0.02

[a-c]Means within a row with different superscripts are different (P < 0.05). Abbreviation: SEM, standard error of the mean; BW, body weight; CW, carcass weight; FCR, feed conversion ratio; g, gram. NC (no treatments); T.1 (0.8 % NEDS); T.2 (1.7% NEDS); T.3 (3.4% NEDS); T.4 (6.8 % NEDS); PC (25 mg/L zinc bacitracin).

In contrast to this finding, dietary nanoencapsulated thyme essential oil supplementation increased the final weight of broiler chickens (Nouri, 2019). To our knowledge, no prior research has been conducted on *P. canescens* leaf extract in poultry, comparators used a variety of herbal plants that were nanoencapsulated using comparable methods. Carcass weight increased (P<0.001) with the highest dose of NEDS (6.8%) in drinking water compared to other NEDS doses, positive controls, and negative controls. In line with the report of Hasanain *et al.* (2024) dietary encapsulated *Andrographis paniculata* and *Moringa oleifera* leaves extracts supplementation increased carcass weight in broiler chickens. This inconsistency between body weight and carcass weight may be attributed to improved nutrient utilization and muscle deposition efficiency induced by the bioactive compounds in *P. canescens* extract. It is suspected that NEDS enhances protein synthesis and reduces visceral fat accumulation, leading to a higher proportion of carcass weight relative to total body weight. Among the bioactive compounds in NEDS, saponins have been reported to act as lipase inhibitors, and inhibiting lipase activity after ingestion is a viable strategy for reducing fat absorption (Marrelli *et al.*, 2016). These findings suggest that carcass weight may serve as a more sensitive indicator of NEDS effects on growth performance than body weight. The addition of NEDS in drinking water contributes adaptively to an increase in the metabolic rate of broiler chickens, caused by the immunomodulatory effects of *P. canescens* leaf extract. This statement was consistent with the findings of Ahkam *et al.* (2024), which stated that *P. canescens* leaves have immunomodulatory effects. One of the kaempferol compounds that was isolated from the leaf extract of *P. canescens* Jack may have immunomodulatory properties (Rahardhian *et al.*, 2025). Because of *P. canescens* leaves immunomodulatory attributes, broiler chickens have stronger immune systems and are more resilient to environmental stressors and infections. Under these conditions, the health of broiler chickens becomes more stable, allowing the feed consumed to be absorbed properly and accumulated as muscle.

This study reported that the feed conversion ratio improved (i.e., lowered) (P<0.05) with NEDS supplementation at doses above 1.7% in the drinking water of broiler chickens. As nanoencapsulation technology can more effectively deliver bioactive substances to target organs in the digestive tract, it is probably the reason for the decrease in the feed conversion ratio of broiler chickens with the additional NEDS, except for the supplementation of NEDS with a dose of 1.7%. The encapsulation layer protects various bioactive compounds such as flavonoids, tannins, and saponins from damage caused by stomach pH or digestive enzymes, so that these compounds remain intact during their journey through the digestive tract and can be released regularly in the small intestine (Gómez-Gaete *et al.*, 2024). In this area, these compounds function as antibacterial agents that reduce the number of harmful bacteria such as *Escherichia coli* and *Clostridium perfringens* (Iqbal *et al.*, 2020), thereby reducing nutrient competition between the host and harmful microbes. Improved intestinal conditions increase the integrity and length of intestinal villi, which results in an increase in surface area for nutrient absorption (Oso *et al.*, 2019). Due to more efficient nutrient utilization, more of the feed consumed can be used for body tissue growth. Consequently, even though feed consumption decreased, the final body weight remained higher and the feed conversion ratio improved.

Meat color traits

Results of the color analysis of breast and thigh meat sections include lightness (L*), redness (a*), yellowness (b*), hue angle (HA), and saturation index (SI) can be seen in Table 3. NEDS application did not affect the lightness (L*) of the breast meat, but the highest dose of NEDS (6.8%) increased the brightness of the thigh meat (P<0.001). In contrast to the results, the study by Hassan *et al.* (2024) recorded higher L* values with nano-thyme administration than with antibiotic treatment. a* value in breast meat decreased with the application of 1.7%, 3.4%, and 6.8% NEDS in drinking water compared to lower doses of NEDS (0.8%) and the control treatments (P<0.001). Different results were found in the study by Hasanain *et al.* (2024), which reported an increase in the a* value of the breast meat with the addition of nanoencapsulated moringa leaf extract. Different from the breast meat test, the a* test of the thigh meat showed that the administration of

1.7% NEDS in drinking water could reduce a* compared to other treatments (P<0.001). Rahman and Kim (2016) found that the pigment myoglobin was typically linked to the redness of meat, which was an important feature of some meat products. The study additionally reported that pigment plays a major role in determining the intensity of red color through its chemical form, such as oxymyoglobin, which gives a bright red color, deoxymyoglobin, which is purplish red, and metmyoglobin, which is brownish.

Supplementation highest dose of NEDS (6.8%) in drinking water resulted in a substantial decrease (P<0.001) in yellowing tests on the breast meat. In the industrial sector, yellowness color assessment is a crucial step in determining the extent of freshness that influences consumer perception, particularly in poultry meat (Hayat *et al*., 2024). In line with our findings, dietary antibiotics in broiler chicken increased the yellowness value of the breast more than adding nano-thyme or nano-ginger (Hassan *et al*., 2024). Yellowness testing on the thigh also reported a significant decrease (P<0.001) with the administration of 3.4% and 6.8% doses of NEDS. The b* score for chicken breast meat usually ranges from 9 to 15. The b* feature represents the yellow axis when (b*>0) and represents the blue axis when (b*<0) (Hernández *et al*., 2016). The lower b* value with the administration of NEDS is potentially due to bioactive compounds in NEDS such as tannins, saponins, and flavonoids, which have antioxidant activity (Rizki *et al*., 2022). This activity inhibits the oxidation of lipids and carotenoid pigments, thereby reducing the b* value of broiler chicken meat fed with bioactive compounds (Gao *et al*., 2021). This occurs because these antioxidant substances maintain pigments and reduce oxidative degradation, which usually leads to meat turning more yellow.

Measurements of the hue angle of breast meat showed an increase (P<0.001) with the supplementation of all NEDS doses except the lowest dose (0.8%). In contrast to the results of the breast hue angle test, Table 3 showed hue angle of thigh meat only increased with the administration of 3.4% NEDS in drinking water compared to other doses and controls (P<0.001). The saturation index value in the chest decreased (P<0.001) with the highest dose of NEDS (6.8%). In contrast to the saturation index value in the chest, which decreased with the highest dose (6.8%), an interesting finding can be seen in the saturation index value in the thigh, which decreased (P<0.001) with the lowest dose (0.8%) of NEDS. Saturation index value describes the color intensity of meat (Ahmed *et al*., 2024), with a range of 10-15 considered a normal SI value reflecting moderate color saturation.

pH characteristics

The results in Table 4 showed that NEDS administration did not affect the pH of breast (45 minutes, 2 hours, and 24 hours) and the pH of thigh (45 minutes and 24 hours). However, interesting results were noted at the 2-hour test, where the pH of thight was decreased with 3.4% NEDS dose administration and increased with 6.8% NEDS dose (P<0.05). The same results were reported by previous researchers, Hassan *et al*. (2024) reported no effect of the pH value of chicken breast meat at 2 and 24 hours with the addition of encapsulated herbal ingredients. At 2 hours postmortem, the decrease in thigh muscle pH observed at the 3.4% NEDS dose may be related to enhanced glycolytic

Table 3: Meat color traits of breast and thigh muscles in broiler chickens receiving nano-encapsulated *Peronema canescens* Jack leaf extract in drinking water.

Variables	T.1	T.2	T.3	T.4	NC	PC	Level of sig	
							SEM	P value
Breast								
L*	58.43	60.97	59.91	58.14	57.46	59.27	0.491	0.353
a*	3.05^{b}	2.37^{c}	2.05^{c}	2.10^{c}	3.65^{ab}	4.23^{a}	0.160	<0.001
b*	13.56^{b}	12.92^{bc}	11.46^{cd}	10.59^{d}	16.84^{a}	15.64^{a}	0.417	<0.001
Hua angle	77.29^{ab}	79.57^{a}	79.84^{a}	78.76^{a}	77.71^{a}	74.82^{b}	0.435	0.004
Saturation index	13.91^{b}	13.14^{bc}	11.65^{cd}	10.81^{d}	17.24^{a}	16.21^{a}	0.434	<0.001
Thigh								
L*	57.59^{bc}	59.59^{ab}	59.59^{ab}	61.06^{a}	53.03^{cd}	54.22^{d}	0.568	<0.001
a*	3.41^{b}	2.63^{c}	3.45^{b}	3.79^{b}	4.99^{a}	5.65^{a}	0.194	<0.001
b*	10.81^{c}	11.90^{bc}	12.85^{b}	13.00^{b}	14.67^{a}	12.84^{b}	0.283	<0.001
Hua angle	72.28^{bc}	77.45^{a}	75.04^{ab}	73.60^{bc}	71.07^{c}	66.15^{d}	0.740	<0.001
Saturation index	11.36^{d}	12.19^{cd}	13.32^{bc}	13.55^{bc}	15.50^{a}	14.05^{ab}	0.298	<0.001

$^{a-d}$Means within a row with different superscripts are different (P < 0.05). Abbreviation: SEM, standard error of the mean; L*, lightness; a*, redness; b*, yellowness. NC (no treatments); T.1 (0.8 % NEDS); T.2 (1.7% NEDS); T.3 (3.4% NEDS); T. 4 (6.8 % NEDS); PC (25 mg/L zinc bacitracin).

Table 4: pH characteristics of breast and thigh muscles in broiler chickens receiving nano-encapsulated *Peronema canescens* Jack leaf extract in drinking water.

Variables	T.1	T.2	T.3	T.4	NC	PC	Level of sig	
							SEM	P value
Breast								
pH 45 minute	5.859	5.944	5.945	5.952	5.954	5.881	0.021	0.684
pH 2 hour	5.841	5.934	5.934	5.919	5.909	5.940	0.020	0.771
pH 24 hour	5.842	5.911	5.921	5.910	5.867	5.841	0.018	0.711
Thigh								
pH 45 minute	6.251	6.336	6.132	6.356	6.317	6.267	0.028	0.223
pH 2 hour	6.185[bc]	6.266[abc]	6.128[c]	6.354[a]	6.295[ab]	6.244[abc]	0.022	0.041
pH 24 hour	6.286	6.271	6.234	6.388	6.289	6.314	0.020	0.401

[a-c]Means within a row with different superscripts were different (P < 0.05). Abbreviation: SEM, standard error of the mean. NC (no treatments); T.1 (0.8 % NEDS); T.2 (1.7% NEDS); T.3 (3.4% NEDS); T.4 (6.8 % NEDS); PC (25 mg/L zinc bacitracin).

Table 5: Water-holding capacity traits of breast and thigh muscles in broiler chickens receiving nano-encapsulated *Peronema canescens* leaf Jack extract in drinking water.

Variables	T.1	T.2	T.3	T.4	NC	PC	Level of sig	
							SEM	P value
Breast								
Moisture content	69.986	73.191	72.444	70.350	71.185	70.99	0.386	0.121
Drip loss	3.129[c]	4.346[bc]	6.313[ab]	4.608[bc]	4.166[c]	6.63[a]	0.324	0.005
Pressing loss	51.354	57.611	58.652	57.547	57.568	58.34	0.797	0.068
Thawing loss	8.003[b]	9.530[ab]	8.865[b]	10.774[ab]	12.203[a]	11.89[a]	0.476	0.034
Thigh								
Moisture content	71.177	70.537	71.593	73.140	71.965	71.624	0.451	0.712
Drip loss	2.205	1.661	1.921	1.403	2.427	2.383	0.176	0.496
Pressing loss	54.607	54.347	56.538	56.906	55.216	54.423	0.654	0.671
Thawing loss	5.278[c]	7.065[abc]	6.138[bc]	7.546[ab]	6.355[bc]	8.738[a]	0.313	0.017

[a-c]Means within a row with different superscripts are different (P < 0.05). Abbreviation: SEM, standard error of the mean. NC (no treatments); T.1 (0.8 % NEDS); T.2 (1.7% NEDS); T.3 (3.4% NEDS); T.4 (6.8 % NEDS); PC (25 mg/L zinc bacitracin).

activity resulting from accelerated metabolic energy turnover. In contrast, the higher pH detected at the 6.8% NEDS dose may reflect a reduced postmortem glycolytic rate influenced by the stronger antioxidant effects of *P. canescens* bioactive compounds. The tannins contained in NEDS possess antioxidant activity, acting to alleviate oxidative stress by scavenging reactive oxygen species (ROS) and modulating antioxidant enzyme systems in birds (Choi and Kim, 2020). This non-linear response suggests that NEDS modulates metabolic activity in a dose-dependent manner. Tannin intake has been reported to exert both beneficial and adverse effects depending on the dosage administered to broiler chickens (Ramah *et al*., 2020). More broadly, the absence of significant pH differences at other measurement times in both breast and thigh muscles is presumed to result from uniform pre-slaughter handling of all chickens, which minimized potential variations in stress-induced pH decline. Therefore, the observed pH stability indicates that NEDS supplementation does not disrupt the normal biochemical processes involved in muscle acidification. Pre-slaughter handling and the physiological condition of broilers are known to be important factors affecting meat quality, including carcass acidity (Vlad *et al*., 2025).

Water holding capacity traits

Table 5 displayed findings of the water-holding capacity trait testing. There was no significant difference in moisture content between the thigh and breast tests. In line with the results of this study, Hassan *et al*. (2024) showed that nanoencapsulation of thyme and ginger did not have a significant effect on the moisture content of the broiler chicken thighs. The greater surface area of nanoparticles improves tissue penetration and cellular absorption, promotes contact with the digestive tract, and prolongs their retention time (Abd El-Hack *et al*., 2018). The bioactive ingredients in NEDS can function more effectively in preserving the equilibrium of water and electrolyte metabolism in broiler chickens due to this absorption efficiency, which also helps to avoid excessive fluid retention in muscle tissue.

Drip loss of the breast meat showed a decrease (P<0.05) with the lowest dose of NEDS (0.8%) and without treatment compared to other treatments. Different results were recorded in the study by Partovi *et al.* (2019), which reported that the application of nanocurcumin increased drip loss in broiler chicken breasts compared to the negative control. Water content affects water binding capacity in meat (Mir *et al*., 2017). The treatment with NEDS can reduce drip loss, possibly because of the role of bioactive compounds in *P. canescens* leaf extract, such as phenols, which can work to improve water retention by modifying the gel properties of proteins, making the water in the meat bind more strongly to the muscle tissue (Yang *et al*., 2023). Drip loss of the thigh part did not show any difference with the addition of NEDS in the water drinker.

Pressing loss of the breast and thigh did not show a significant effect with NEDS treatments. High moisture content indicates that muscle tissue contains more free water that is not strongly bound in the muscle protein structure (Kong *et al*., 2022). When pressure is applied, this free water is easier to remove from the tissue, making pressing loss values very important in evaluating meat quality. Because the thigh has a denser and more elastic muscle structure, as well as a higher composition of fat and connective tissue, it is believed that there was no effect of pressing loss between treatment groups. As a result, the ability to retain water under pressure was comparatively consistent across all treatment groups (Lee *et al*., 2023).

Compared to other doses and the control, NEDS doses of 0.8% and 3.4% in drinking water reduced thawing loss in the breast (P<0.05). The thawing loss analysis results for the thigh only indicated a decrease in NEDS doses of 0.8% (P<0.05). The supplementation of NEDS can reduce thawing loss because the bioactive compounds contained in NEDS help maintain the structural integrity of muscle cells during the freezing and thawing process. Stronger cell membranes and more stable muscle tissue shield muscle fibers from excessive damage caused by ice crystal formation during freezing (Wang *et al*., 2024b). This reduces thawing loss because the water held in muscle tissue stays well-bound and is difficult to escape. The results of this study are supported by research conducted by Akuru *et al.* (2020), which reported that the addition of pomegranate peel extract (containing tannin compounds) reduced thawing loss in broiler chicken breasts. Based on thawing loss results, the addition of NEDS can retain at least some of the water that is lost during thawing. This is believed to be occurring because NEDS assists in strengthening cell membranes and protein structures, which inhibits excessive fluid loss during thawing and lowers water loss in meat. Flavonoids have the capacity to form complexes with proteins either covalently or non-covalently, strengthening protein networks and enhancing the stability of meat structure (Wang *et al*., 2024a).

Texture characteristics

Table 6 presents the effects of NEDS supplementation in drinking water on the texture characteristics of broiler breast meat. Based on the data presented in the Table 6, the administration of NEDS did not influence the resilience, cohesion, springiness, and chewiness variables. In contrast to the previous variables, the hardness of the breast decreased with the application of 1.7% NEDS compared to other doses, positive control, and negative control (P<0.001). Furthermore, a decrease in gumminess was recorded on the lowest dose of NEDS (0.8%), compared to other treatments (P<0.001). In contrast to the results of this study, provision of nanocurcumin did not affect the texture of broiler chicken breast meat, including hardness (Partovi *et al*., 2019). The absence of effect on meat texture, possibly because its effect was more on increasing antioxidant capacity, which affected meat color without changing protein structure or connective tissue.

Table 6: Texture characteristics of breast meat in broiler chickens receiving nano-encapsulated *Peronema canescens* Jack leaf extract in drinking water.

Variables	T.1	T.2	T.3	T.4	NC	PC	Level of sig	
							SEM	P value
Hardness	3.312[cd]	2.919[d]	3.928[ab]	3.282[cd]	4.286[a]	3.558[bc]	0.107	<0.001
Resilence	0.226	0.227	0.274	0.254	0.255	0.263	0.005	0.059
Cohession	0.546	0.578	0.600	0.557	0.566	0.595	0.006	0.093
Springiness	0.662	0.685	0.675	0.737	0.677	0.656	0.009	0.118
Gumminess	1.747[c]	1.948[bc]	2.229[ab]	1.986[abc]	2.405[a]	2.294[a]	0.063	0.014
Chewiness	1.526	1.415	1.751	1.606	1.548	1.574	0.409	0.315

[a-d]Means within a row with different superscripts are different (P < 0.05). Abbreviation: SEM, standard error of the mean. NC (no treatments); T.1 (0.8 % NEDS); T.2 (1.7% NEDS); T.3 (3.4% NEDS); T.4 (6.8 % NEDS); PC (25 mg/L zinc bacitracin).

The primary mechanism of NEDS is thought to be through increasing antioxidant activity, which inhibited lipid and pigment oxidation, thereby affecting meat color stability (Domínguez *et al*., 2019). However, this process did not directly interact with structural components that determine texture, such as myofibrillar proteins. Nevertheless, the precise biochemical pathways underlying these effects remain unclear, a limitation of the current study that requires further investigation.

CONCLUSION

Supplementation of nanoencapsulated *Peronema canescens* Jack leaf extract in broiler chicken drinking water improved carcass quality and meat physical characteristics. Administration of NEDS at a dose of up to 6.8% increased carcass weight and enhanced meat appearance by increasing thigh lightness and reducing yellowness and redness values in the breast, without adversely affecting pH, moisture content, or texture parameters. However, this study was limited to evaluating only meat physical quality and did not examine other parameters such as gut health, immune response, and antioxidant activity. Therefore, further research is recommended to investigate the biological mechanisms of NEDS in broilers and to optimize dosage levels for broader application, while confirming its potential as a natural supplement to improve broiler meat quality.

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NOVELTY STATEMENT

This study is the first innovation to evaluate the effect of administering *Peronema canescens* Jack leaf extract in nanoencapsulated form through drinking water on the meat quality of 28-day-old broiler chickens. This approach offers a new strategy for improving meat quality by enhancing the stability and effectiveness of naturally occurring bioactive compounds.

AUTHOR'S CONTRIBUTION

The final version of the work was reviewed and approved by all authors. BPM gathered and analyzed the data. The experimental results were examined and validated by HLS. The experiment was managed by APB. The manuscript was conceived by BPM dan NDD. The study framework was developed by ZZ. The experimental design was validated by BA, RM, CH, and RR, who also contributed to the paper revision.

Ethical approval statement

The study was conducted at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia, from July to October 2024. All experimental procedures involving animals were reviewed and approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (Approval No. 67/EC-FKH/int./2024) and were carried out in strict accordance with the Guidelines for the Care and Use of Laboratory Animals.

Generative AI and AI-assisted technology statement

The authors declare that the use of generative artificial intelligence (AI) and AI-based technologies, such as Grammarly and ChatGPT, is limited to assisting with English grammar and reducing writing errors to improve the quality of the manuscript. There is no AI technology used in the creation, analysis, or interpretation of scientific data. The entire content in this manuscript is entirely the responsibility of the authors.

Statement of conflict of interest

The authors declare no financial interests or personal relationships that could have influenced the work reported in this manuscript.

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