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Effects of encapsulated *Peperomia pellucida* extract on growth performance, health status, and protein digestibility of broiler under high stocking density

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ABSTRACT

This study examined how adding *Peperomia pellucida* extract affects growth performance, health status, and protein digestibility of broiler under high stocking density. A total of 290 Ross strain broiler chickens, each 8 days old and weighing approximately 205.63±2.65 g, were used in this study. Their diets included yellow corn, rice bran, soybean meal, fish meal, limestone, premix, lysine, and methionine. This study used a random design with four treatments and five replicates. The treatments were: T0 (normal space with 10 bird/m^2 without *Peperomia pellucida* extract (PPE) or encapsulated *Peperomia pellucida* extract (EPPE)), T1 (high stocking density/HSD with 16 bird/m^2 without PPE/EPPE), T2 (HSD with 16 bird/m^2 plus 0.4% PPE), and T3 (HSD with 16 bird/m^2 plus 0.4% EPPE). They measured the total lactic acid bacteria, coliforms, small intestine pH, oxidative status (malonylaldehyde, superoxide dismutase, and glutathione peroxidase), protein digestibility, and performance (feed intake, daily weight gain, and feed conversion ratio). They used analysis of variance at a 5% significance level and Duncan's test when treatment effects were significant ($p<0.05$). The results showed that adding PPE or EPPE to the diet significantly affected ($p<0.05$) the total bacteria, pH of the small intestine, oxidative status, protein digestibility, and performance of broiler under high stocking density. The study concluded that adding 0.4% EPPE to the diet can improve small intestine bacteria, maintain oxidative balance, and boost protein use and performance in broiler under high stocking density.

Introduction

Broiler chickens are bred by selecting highly productive chicken breeds (Qanbari *et al*., 2019). The FAO (2020) reports that more than 133.645 million tons of broiler meat are produced to meet global food needs. With the increase in the global population, the demand for chicken meat is anticipated to grow. To meet this demand, it is essential to improve efficiency in the poultry industry (Bist *et al*., 2024). Several strategies have been implemented in the poultry industry, including cage density management, to maximize the benefits of the cage floor area used (Insawake *et al*., 2025). A high stocking density is an effort to increase the efficiency of raising broiler chickens. High stocking density causes stress and inhibits broiler growth due to decreased feed intake, resulting in suboptimal nutrient utilization and low productivity will be low (Jabbar and Youssaf, 2017). Insawake *et al*. (2025) indicate that when stocking density exceeds 14 birds per square meter, with a weight range of 30–35 kg/m^2, there is a reduction in body weight, particularly in broilers raised for 42 days. High-density intensive rearing systems can place broilers in stressful environments, making them more prone to diseases and diminishing their growth performance (Pan *et al*., 2025). Stressful conditions in chickens can alter the intestinal bacterial population by increasing pathogenic bacteria, which disrupts the absorption process in the intestinal mucosa and hinders protein digestion (Mangisah *et al*., 2022). To mitigate these adverse effects, synthetic antioxidants can be used as feed additives for broilers. However, the application of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) remain limited due to potential health risks, such as carcinogenic effects, for humans who are the primary consumers of broiler meat products (Candan and Bagdath, 2017).

Peperomia pellucida L., a wild plant originating from Asia and America, is now found globally. *Peperomia pellucida* L., known as Chinese betel, is rich in antioxidant compounds, including polyphenols, tannins, saponin glycosides, alkaloids, steroids, and terpenoids (Tuan and Men, 2024). Its extract contains phenolic compounds (627.45 mg/g), flavonoids (868.78 mg/g), and tannins (13.06 mg/g) (Fakayode *et al*., 2021). Consequently, *P. pellucida* holds potential as a phytobiotic species and could serve as an alternative to synthetic antioxidants. However, phenolic compounds are sensitive to environmental conditions such as temperature, humidity, light, and oxygen. Therefore, protecting phenolic compounds is essential to ensure they remain an effective antioxidant source (Sugiharto and Ayasan, 2023). Encapsulation is a method used to shield phenolic compounds from oxidation during storage (Jeyakumari, 2016). Furthermore, Iriyanti and Hartoyo (2019) found that encapsulation protects phytogenic substances from harsh gastrointestinal conditions, particularly in the stomach, thereby enhancing the bioavailability of active components in broiler chickens. Maltodextrin is a crucial encapsulation component due to its low viscosity, even at high concentrations. Its high solubility, low browning risk, ability to form matrices, prevention of crystallization, strong binding capacity, and effective suppression of oxidation reactions contribute to the extended shelf life of the encapsulated product (Noor *et al*., 2022).

Peperomia pellucida is a weed plant, contains bioactive components that improve health. The novelty of this study is that encapsulated *Peperomia pellucida* extract can be used to control stress and improve body health, nutrient digestibility, and performance of broilers reared at high stocking density. High stocking density causes stress in broilers, which is characterized by reduced production performance, immune responses, and physiological health. This study aimed to determine whether *Peperomia pellucida* could suppress the negative effects of stress through antioxidant mechanisms and improve immunity.

Materials and methods

Ethical approval

All experimental procedures involving animals received authorization from the Research Ethics Committee of the Faculty of Animal and Agricultural Sciences at Universitas Diponegoro (approval number: 60-08/A-16/KEP-FPP).

Time, livestock and equipment

The research was executed between August and September 2024 within digestion enclosures situated at the Animal Nutrition Laboratory, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Indonesia. The quantitative assessments of Lactobacillus and Escherichia coli were performed at the Nutrition Laboratory of Muhammadiyah University Semarang, Indonesia. Evaluations pertaining to oxidative status were carried out at the Poultry Production Science Laboratory within the Faculty of Animal and Agricultural Science, Universitas Diponegoro, Indonesia. The analysis of the rats was conducted in proximity to the Animal Nutrition Laboratory, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Indonesia.

The experimental livestock used Ross strain unsexed broiler chickens aged 8 days as many as 290 birds with an average body weight of 205.63±2.65 g. All day old chicks have been fully vaccinated using the sprayer method. The research ration was given at the age of 8-35 days based on its phase, starter (8-21 days) with metabolic energy content of 2,990.2 kcal/kg and crude protein of 21.18%, while finisher (22-35 days) with metabolic energy content of 3,032.71 kcal/kg and crude protein of 19.42%. *Peperomia pellucida* was obtained from the environment of Universitas Diponegoro, Semarang, Indonesia.

The substances employed for extraction and encapsulation consisted of *Peperomia pellucida* fruit extract, distilled water, 96% ethanol sourced from Merck, maltodextrin obtained from Sigma, and Whatman 41 filter paper from Cytiva. The apparatus used in these processes included a Sonicator, a 2000 ml glass beaker, a 500 ml measuring cup, a digital balance with 0.1 g precision, a stirring rod, a funnel, and a freeze dryer. The rearing setup comprised a main cage, pens, brooder, containers for feed and water, Thermohygrometer, and digital scales with 0.1 accuracy.

Extraction and encapsulation procedure

The encapsulation process of *Peperomia pellucida* started with an extraction technique adapted from Gouda *et al.* (2021). First, the fruit dried in an oven at 50°C until completely dry, then ground into a fine powder. The powder was dissolved in 96% ethanol at a 1:10 (b/v) ratio. The mixture was stirred until uniform and filtered using Whatman 41 filter paper. The solution was sonicated at 37°C and room temperature at a frequency of 50 Hz for 30 minutes. To eliminate the ethanol and obtain a concentrated extract, sonication was performed at 45°C. The subsequent encapsulation process, adapted from Agusetyaningsih *et al.* (2022), involved creating a mixture of maltodextrin and distilled water at a ratio of 3:1 (b/v). The concentrated extract was mixed with the coating solution at a 1:3 (v/v) ratio, and the mixture was freeze-dried at -50°C until a dry product formed. Finally, the encapsulated product was ground using a mortar and pestle.

Research Design and Treatments

The study employed a completely randomized design with four treatments and five repetitions, resulting in 20 experimental units. The treatments applied to the ratio were as follows:
T0= normal space (10 birds/m²) without *Peperomia pellucida* extract (PPE) or encapsulated *Peperomia pellucida* extract (EPPE).
T1= HSD (16 birds/m²) without PPE/EPPE;
T2= HSD (16 birds/m²) + PPE (0.4%) in feed
T3= HSD (16 birds/m²) + EPPE (0.4%) in feed

Chicken rearing

The chickens were kept in pens with litter floors and organized into 20 experimental units of 10 birds each. During the first week (days 1 to 7), commercial feed was combined with the experimental diet to facilitate adaptation. From days 8 to 35, the chickens received the experimental basal diet enhanced with encapsulated *Peperomia pellucida* extract according to their treatment group. Feed was distributed based on daily consumption, and water was always available (Table 1).

Table 1. Nutrient composition of starter-finisher broiler diets.

Feed Stuff	Composition (%)	
	Starter (8-21 days old)	Finisher (22-35 days old)
Yellow Corn	51.16	52.01
Rice Bran	13.99	18.14
Soybean Meal	24	19
Fish Meal	10	10
Limestone	0.3	0.3
Premix	0.25	0.25
L-Lisin	0.1	0.1
DL-Metionin	0.2	0.2
Total	100	100
Nutrient Content:		
Metabolizable Energy (kcal/kg)[2)]	2,990.20	3,032.71
Crude Protein (%)[1)]	21.18	19.42
Crude Far (%)[1)]	4.67	4.7
Crude Fiber (%)[1)]	5.45	4.49
Calcium (%)[1)]	1.04	1.07
Phosphor (%)[1)]	0.71	0.75

[1)]Ration were analyzed for proximate and mineral contents at the Animal Nutrition and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (2024). [2)]EM Content Calculated Based on Bolton Formula (1967).

Parameters Measurement

Broiler Performance

The evaluation of broiler performance encompassed metrics such as body weight (BW), daily body weight gain (DBWG), feed intake (FI), and feed conversion ratio (FCR). Empirical data were gathered through the quantification of body weight and feed consumption, commencing from the initial week and extending to the conclusion of the experimental phase.

Oxidative status

MDA and SOD samples were collected taken from the blood serum of two 35-day-old chickens in each replicate to examine MDA and SOD activity. SOD activity was tested based on the ability of the sample to suppress pyrogalol auto-oxidation. The mixture consisted of 50 mM Tris-HCl (pH 8.2), 1 mM pentaacetic acid diethylenetriamine, and sample. The reaction was initiated by the addition of pyrogalol (final concentration: 0.2 mM), and the absorbance was calculated kinetically. SOD concentration was expressed as U/ml. MDA level was measured using the reactive material assay for thiobarbituric acid (TBA). Each sample was vortexed and supplemented with 8.1% sodium dodecyl sulfate. Then, the samples were left at room temperature for 10 minutes. Controls were treated in the same manner. After incubation, the samples were supplemented with 20% acetic acid and 0.6% TBA, and the tubes were placed in a water bath for 1 hour at 90-95°C. Then, butanol and pyridine (15:1) were added to the supernatant, and the mixture was vortexed and centrifuged. The MDA concentration was expressed in nmol/mL (Agusetyaningsih *et al.*, 2022).

The glutathione peroxidase (GPx) assay was performed using the GSH-Px activity assay kit. The procedure for the GPx activity assay using the GSH-Px Assay Kit begins with heating the microplate reader or UV-Vis spectrophotometer for more than 30 minutes and setting the wavelength to 340 nm. The working reagents were then incubated at 25°C (for most species) or 37°C (for mammals) for 30 minutes. The blank well contained 20 µL of deionized water and 160 µL of working reagent, while the test well contained 20 µL of sample, 160 µL of working reagent, and 20 µL of H_2O_2 solution. After all the materials were added, the mixture was homogenized, and the absorbance was measured at a wavelength of 340 nm at 10 s (A1 for blank and A3 for test) and 70 s (A2 for blank and A4 for test). The absorbance changes were calculated as ΔA_blank = A1–A2 and ΔA_test = A3–A4. Blank well measurements only need to be taken 1-2 times to ensure the accuracy of the experimental results.

Total intestinal bacteria

The method of calculating total bacteria started from the slaughter process carried out on the 36th day by taking one broiler chicken in each experimental unit. The next step was to slaughter the chicken in the neck vein and clean the feathers. The next process is to take The small intestine was collected, and the ileal digesta was collected and put into a 10 g pot. Total LAB and coliforms were counted using the pour plate method for LAB using de Man Rogosa Sharpe agar (MRSA) media, while Escherichia coli with Tryptone Bile X-glucuronide agar (TBX) media, in dilutions of 1 g samples, were taken and then placed in a Petri dish. The media was poured into a Petri dish to a volume of 15 ml, then closed and placed on the table by shaking in the form of figure eight and allowed to settle until solid. The solid samples were incubated upside down in an incubator for 48 h at 44°C for LAB and at 37°C for coliforms. After the incubation period was completed, the colonies that formed were counted. Each colony can be considered to originate from one cell that divides into many cells, although it also originates from other cells that are located close together. Colony counts were determined using a Quebec colony counter (Rahmah *et al*., 2016).

Protein Digestibility

Protein digestibility was measured by collecting total excreta in combination with Fe_2O_3 indicator for 4 days at 32-35 days of age. feed was mixed with 0.5% Fe_2O_3. On the first day, the chickens were fed the treatment ration with the indicator for 24 h, and the excreta container was placed at the bottom of the battery cage. The collection began when the first red excreta appeared until the second day changed color. On the second day, broilers were fed the treatment ration without an indicator. The third day ratio with indicator and the fourth day ratio without an indicator. Excreta were collected daily by spraying 0.2 N HCl every 2 h (Krismiyanto *et al*., 2022). The collected excreta were dried and crushed for crude protein content analysis using the Kjeldahl method.

Measurement of pH of the small intestine

To measure pH levels in the digestive system of broiler chickens, samples were collected from the duodenal digesta, jejunum, and ileum at the time of slaughter. These samples were then analyzed using a digital pH meter (Eco test pH 1) for each individual sample.

Statistical Analysis

Data were analyzed with SPSS version 25.0 (SPSS Inc.) at the 5% significance level, and if the treatment showed a significant impact, Duncan's test was applied at the same significance level to identify differences among treatments (Gaspersz, 1992).

Results

Growth performance

Data on broiler production performance parameters are presented in (Table 2). Analysis of variance showed that treatment with encapsulated *Peperomia pellucida* extract had a significant effect (P<0.05) on body weight, feed intake, daily weight gain, and FCR. The Duncan test results showed that feed intake in T3 was significantly higher (p<0.05) than that in T0, T1, and T2. Feed intake in T1 was significantly lower (p<0.05) than that in the other treatments. Daily weight gain in T3 was significantly higher (p<0.05) than that at T0, T1, and T2. This result synergizes with FCR in T3, which was significantly lower (p<0.05) compared to T0, T1, and T2.

Table 2. Broiler performance on day 35.

Variables	Treatment				SEM	P-value
	T0	T1	T2	T3		
Feed intake (g/bird/day)	100.88^c	98.55^d	103.56^a	102.05^b	0,094	0
Daily body weight gain (g/bird/day)	63.06^b	59.98^c	64.37^b	66.68^a	0.32	0
Feed conversion ratio	1.60^b	1.64^b	1.61^b	1.53^a	0.01	0.00

T1: Normal space (10 bird/m^2) with BD without PPE/EPPE 0.4%, T2: High stocking density (16 bird/m^2) with BD without PPE/EPPE 0.4%, T3: High stocking density (16 bird/m^2) with BD+PPE 0.4%, T4: High stocking density (16 bird/m^2) with BD+EPPE 0.4%; PPE: *Peperomia pellucida* extract; EPPE: encapsulated *Peperomia pellucida* extract; BD: basal diet.

Total bacteria and pH of the small intestine

The bacterial population parameters and the pH of the small intestine are presented in Table 3. An increase in the level of encapsulated *Peperomia pellucida* extract in the feed was followed by an increase (P<0.05) in the LAB population. Increased levels of encapsulated *Peperomia pellucida* extract also resulted in a decrease (P<0.05) in the coliform bacterial population and small intestine pH. Analysis of variance showed that the treatments had a significant effect (P<0.05) on the LAB population, coliform, and pH of the small intestine. In addition, the LAB population at T3 was significantly (P<0.05) higher than that at T0, T1, and T2. The coliform population in T3 was significantly (P<0.05) lower than that in T0, T1, and T2. The pH of the small intestine at T3 was lower (P<0.05) than that at T0, T1, T2, and T3.

Table 3. The selected intestinal bacteria population and pH.

Variables	Treatment				SEM	P-value
	T0	T1	T2	T3		
BAL (Log cfu/g)	6.81^c	6.13^d	7.61^b	8.04^a	0,036	0
pH	6.42^{ab}	6.50^a	6.31^b	6.16^c	0.02	0.00
Coliform (Log cfu/g)	4.32^b	4.66^a	3.86^c	3.60^d	0.04	0

T1: Normal space (10 bird/m^2) with BD without PPE/EPPE 0.4%, T2: High stocking density (16 bird/m^2) with BD without PPE/EPPE 0.4%, T3: High stocking density (16 bird/m^2) with BD+PPE 0.4%, T4: High stocking density (16 bird/m^2) with BD+EPPE 0.4%; PPE: *Peperomia pellucida* extract; EPPE: encapsulated *Peperomia pellucida* extract; BD: basal diet.

Oxidative status and protein digestibility

Data on blood serum MDA, SOD, and GSh Px levels are presented in Table 4. Analysis of variance showed that the addition of PPE or EPPE to the diet had a significant effect (P<0.05) on the levels of MDA, SOD, and GSh Px in the broiler blood serum. Furthermore, serum MDA levels at T3 were lower (P<0.05) than those at T0, T1, and T2. Broiler blood serum SOD levels at T2 and T3 were higher (P<0.05) than at T0 and T1. The GSh Px levels at T2 and T3 were higher (P<0.05) than those at T0 and T1. Protein digestibility levels in T3 were not significantly different (P>0.05) from T0 and T2, but were significantly (P<0.05) higher than those in T1.

Table 4. Oxidative status and protein digestibility.

Variables	Treatment				SEM	P-value
	T0	T1	T2	T3		
MDA (U/ml)	3.50[b]	4.60[a]	3.20[c]	2.90[d]	0.04	0
SOD (U/ml)	50.25[b]	42.06[c]	58.50[a]	63.86[a]	0.99	0
GSH Px (U/ml)	414.22[b]	378.20[c]	417.44[a]	431.59[a]	2.02	0
Protein Digestibility (%)	81.90[a]	76.84[b]	81.85[a]	85.39[a]	0.65	0.00

T1: Normal space (10 bird/m^2) with BD without PPE/EPPE 0.4%, T2: High stocking density (16 bird/m^2) with BD without PPE/EPPE 0.4%, T3: High stocking density (16 bird/m^2) with BD+PPE 0.4%, T4: High stocking density (16 bird/m^2) with BD+EPPE 0.4%; PPE: *Peperomia pellucida* extract; EPPE: encapsulated *Peperomia pellucida* extract; BD: basal diet.

Discussion

The results of broiler performance show that the use of herbal products in this study, namely *Peperomia pellucida* extract encapsulated in feed, can improve broiler growth performance. This phenomenon is due to the phenolic compounds of *Peperomia pellucida* extract, namely flavonoids, which play an important role in increasing the LAB population, improving the morphology or structure of the small intestine (increasing villi height and the ratio of villi height to kripta depth) of broiler intestines, so that it can support the process of absorption of nutrients, especially better proteins, and also play a role in minimizing free radicals and increasing SOD levels to support broiler health.

The increase in broiler performance results in this study is in accordance with Sugiharto and Ayasan (2023), who mentioned that the provision of encapsulated herbal ingredients can have a positive effect on nutrient digestibility and physiological and health conditions, thus having an impact on improving the growth performance and feed efficiency of broiler chickens. Encapsulation is a technique aimed at protecting and improving the availability of herbal ingredients to provide more positive benefits to chicken productivity and health (Sugiharto, 2021; Sugiharto and Ayasan, 2023). In *Peperomia pellucida*, several secondary metabolites contained in *Peperomia pellucida*, especially flavonoids, have been reported to inhibit pathogenic bacteria and thus support digestive enzyme activity, which has a positive effect on increasing nutrient digestibility in broiler chickens (Xue *et al*., 2021).

The population of lactic acid bacteria in the small intestine increased as the encapsulation level of *Peperomia pellucida* extract in the feed increased. The treatments also linearly decreased the coliform population values in the small intestine. This condition is in accordance with the decrease in pH of the small intestine. This phenomenon was due to the increased percentage of absorption of herbal components, namely phenolic compounds, from the encapsulated *Peperomia pellucida* extract. Encapsulated phenolic compounds have a protective layer to protect phenolic compounds from the acidic environment in the proventriculus and gizzard, which can cause degradation of phenolic compounds and protect phenolic compounds from mechanical processes in the gizzard. The proventriculus secretes hydrochloric acid and digestive enzymes that make the proventriculus and gizzard acidic. This finding aligns with that of Sugiharto and Ayasan (2023), who stated that encapsulation can enhance the bioavailability of herbal components with antibacterial and antioxidant properties for broiler chickens. Encapsulation is a technique that protects and improves the availability of herbal ingredients (Sugiharto, 2021; Sugiharto & Ayasan, 2023).

MDA levels in broiler serum decreased with the encapsulation of *Peperomia pellucida* extract in feed. This finding aligns with the research of Hardjo *et al*. (2025), who reported that administering 200 mg/kg of body weight of *P. pellucida* extract reduced MDA levels in mouse serum. MDA is a metabolite of lipid peroxidation by free radicals and a marker of free radical activity in cells. High MDA levels indicate oxidative stress due to free radicals (Iriyanti and Hartoyo, 2019). MDA products form when free radicals enter the blood and attack polyunsaturated fatty acids (PUFAs) or unsaturated fatty acids. Cell membranes containing PUFAs, such as membrane-crossing proteins, can move more flexibly. However, when PUFAs in cell membranes are exposed to free radicals, lipid peroxidation occurs. This produces MDA products that disrupt the process of molecular movement in cell membranes. Lindblom *et al*. (2019) stated that oxidative stress occurs in vivo when reactive oxygen species (ROS) and free radical products overcome the capacity of antioxidants and antioxidant enzymes to convert these species to less reactive species, resulting in tissue-damaging free radicals binding to lipids. Then, they become lipid peroxyl radicals and finally, cyclic endoperoxides, which is the final step in forming MDA.

The level of superoxide dismutase (SOD) in broiler serum increased linearly as the level of *Peperomia pellucida* extract encapsulation in the feed increased. Superoxide dismutase (SOD) is an enzyme in the defense system consisting of copper-zinc superoxide dismutase (Cu, Zn-SOD), which catalyzes the conversion of the superoxide anion free radical into hydrogen peroxide and oxygen molecules, and manganese superoxide dismutase (Mn-SOD), which inhibits the function of superoxide in mitochondria (Iriyanti and Hartoyo, 2019). The increase in SOD levels in chicken serum administered with encapsulated *P. pellucida* extract is thought to be due to the activity of phenolic compounds, especially flavonoids, which minimize free radicals and increase SOD levels. This finding aligns with Al-Zharani *et al.*'s (2023) research, which demonstrated that administering flavonoids at a dose of 80 mg/kg body weight can elevate SOD levels in rat serum.

Protein digestibility increased linearly with the amount of *Peperomia pellucida* extract encapsulated in the diet. Studies have shown that providing herbal products positively impacts the microbial population in the gut and the morphology of broiler intestines, including an increase in villus height and the villus-to-crypt depth ratio. This contributes positively to the process of digestion and absorption of nutrients, particularly proteins, by broiler chickens (Sugiharto, 2021). This is also supported by data on the increasing population of lactic acid bacteria (LAB) and the reduction in the population of Escherichia coli. According to Krismiyanto *et al*. (2015), this affects the health of the digestive tract, which causes an increase in protein digestibility.

Phenolic compounds from *Peperomia pellucida* extract, namely flavonoids, play an important role in increasing the LAB population, which plays a role in turn improving the morphology or structure of the small intestine. Lactic acid bacteria in the cecum ferment indigestible carbohydrates in the feed components to produce SCFA. Short-chain fatty acids are defined as a group of fatty acids consisting of fewer than six carbons, mainly acetate, propionate, and butyrate. These three fatty acids account for more than 95% of total SCFA, with a ratio of 60:20:20; however, this proportion is not constant, as it depends on many factors, such as feed components, microbiota composition, and fermentation site (Liu *et al*., 2021). Acetate, butyrate, and propionate are used as energy sources by the small intestinal epithelial cells. Small intestinal cells can use SCFAs as a fuel to maintain normal function and support nutrient absorption. Short-chain fatty acids, particularly butyrate, play a role in the maintenance and restoration of the small intestinal mucosa. Butyrate provides energy to small intestinal epithelial cells and helps maintain the integrity of the mucosal layer, which is essential for optimal nutrient absorption (Martin-Gallausiaux *et al*., 2021).

Conclusion

The addition of encapsulated *Peperomia pellucida* extract as much as 0.4% in the diet can improve the composition of small intestinal bacteria, maintain oxidative status, improve protein digestibility and growth performance of broiler under high stocking density.

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Conflict of interest

The authors have no conflict of interest to declare.

References

Agusetyaningsih, I., Widiastuti, E. Wahyuni, H.I., Yudiarti, T., Murwani, R., Sartono, T.A., Sugiharto, S., 2022. Effect of encapsulated Cosmos caudatus leaf extract on the physiological conditions, immune competency, and antioxidative status of broilers at high stocking density. Ann. Anim. Sci. 22, 653 – 662.

Al-Zharani, M., Mubarak, M., Rudayni, H.A., Al-Doaiss, A.A., Abd-Elwahab, M.M. Al Eissa, M.S., 2023. Quercetin as a dietary supplementary flavonoid alleviates the oxidative stress induced by lead toxicity in male wistar rats. Nutrients. 15, 1888.

Bist, R. B., K. Bist, S. Poudel, D. Subedi, X. Yang, B. Paneru, S. Mani, D. Wang, L. Chai. 2024. Sustainable poultry farming practices: A critical review of current strategies and future prospects. Poult. Sci. 103, 1 – 28.

Candan, T., Bagdath, A., 2017. Use of natural antioxidants in poultry meat. J. Sci. 13, 279-291.

Fakayode AE, Imaghodor FI, Fajobi AO, Emma-Okon BO, Oyedapo, O.O., 2021. Phytonutrients, antioxidants and anti-inflammatory analysis of *Peperomia pellucida*. J. Med. Pharm. Allied Sci. 10, 3517-3523.

FAO (Food and Agriculture Organization of the United Nation), 2020. Food and agriculture data. Available online: https://www.fao.org/faostat/

Gaspersz, V., 1992. Design and Analysis of Experiments. Tarsito, Bandung Indonesia.

Hardjo, M., Taib, L. P., Surahmat, S., Soraya, G. V., Cangara, M. H., Natzir, R., 2025. Effect of *Peperomia pellucida* L. Kunt Extract in Inhibiting the Increase of Alanine Aminotransferase Enzyme and Changes in Liver Histopathology of Alcohol-Fed Wistar Male Rats (Rattus norvegicus). Egyptian Journal of Chemistry 68, 209-216.

Insawake, K., Songserm, T., Songserm, O., Plaiboon, A., Homwong, N., Adeyemi, K. D., Rasmidatta, K., Ruangpanit, Y., 2025. Effects of isoquinoline alkaloids as an alternative to antibiotic on oxidative stress, inflammatory status, and cecal microbiome of broilers under high stocking density. Poult. Sci. 104, 1 – 12.

Iriyanti, N., Hartoyo, B., 2019. Encapsulated fermeherbafit bioavailability and the application to broilers. J. Agric. Sci. Technol. A. 9, 157-165.

Jabbar, A., A. Yousaf., 2017. Effect of age wise incubation programme on broiler breeder hatchability and post hatch performance. J. Anim. Feed and Res. 7, 13 – 17.

Jeyakumari, A., Zynudheen, A.A., Parvathy, U., 2016. Microencapsulation of bioactive food ingredients and controlled release, a review. MOJ Food Process. Technol. 2, 1-9.

Krismiyanto, L., Suthama, N., Wahyuni, H.I., 2015. Bacterial presence and caecum development due to the addition of inulin from dahlia tubers (*Dahlia variabilis*) in crossbred starter chickens. Indonesian J. Anim. Sci. 24, 54 – 60.

Lindblom, S. C., Gabler, N. K., Bobeck, E. A., Kerr, B. J., 2019. Oil source and peroxidation status interactively affect growth performance and oxidative status in broilers from 4 to 25 d of age. Poult. Sci. 98, 1749-1761.

Liu, L., Li, Q., Yang, Y., Guo. A., 2021. Biological function of short-chain fatty acids and its regulation on intestinal health of poultry. Front. Vet. Sci. 8, 1-13.

Mangisah, I., Rizqiati, H., Suthama, N., 2022. The effect of feeding lactobacillus casei and dahlia tuber extract mixture on intestinal bacteria, nutrient digestibility, immune organ and growth of broilers. Adv. Anim. Vet. Sci. 10, 2555-2561.

Martin-Gallausiaux, C., Marinelli, L., Blottière, H., Larraufie, P., Lapaque. N.2021. SCFA: Mechanisms and functional importance in the gut. Proceed. Nutr. Soc. 80, 37-49.

Noor, E., Yusron, M., Sitorus, R.D.M.C., 2022. Proteus penneri encapsulation with maltodextrin and sodium alginate using a spray-drying method. Food. Sci. Technol. 42, e49621.

Pan, H., Bi, J. Hu, H. Huang, Y. Li, A., Zhang, H. Wan, Y. Zhan, K. Wang, K. Zhao, Z., Bai, X. 2025. Chinese herbal medicine improves antioxidant capacity of chicken liver at high stocking density involved gut-liver microbiota axis based on multi-omics technologies. Poult. Sci 104, 1 – 12.

Qanbari, S., Rubin, C.J., Maqbool, K., Weigend, S. Geibel, J. Kerje, S. Wurmser, C., Peterson, A.T., Brisbin, I.L. Preisinger, R. Fries, R. Simianer, H., Andersson, L., 2019. Genetics of adaptation in modern chicken. Genetica. 15, 1-21.

Rahmah, A.Z., Suthama, N., Yunianto, V.D. 2016. Total lactic acid bacteria and Escherichia coli in broiler chickens supplement a mixture of herbs in the ration. Anim. Agric. J. 2, 39-47.

Sugiharto, S. 2021. Herbal supplements for sustainable broiler production during post antibiotic era in Indonesia – an overview. Livest. Res. Rural Dev. 33.

Sugiharto, S., Ayasan, T., 2023. Encapsulation as a way to improve the phytogenic effects of herbal additives in broilers – an overview. Ann. Anim. Sci. 23, 53-68.

Tuan, C. T., Men, T.T., 2024. *Peperomia pellucida*'s ingredients, antioxidant properties, and safe usage as food and herbal medicine. J. Microb. Biotechn. 34, 2321 – 2330.

Xue, F., Wan, G. Xiao, Y. Chen, C., Qu, M., Xu, L. 2021. Growth performances, gastrointestinal epithelium and bacteria responses of Yellow-feathered chickens to kudzu-leaf flavonoids supplement. AMB Expr. 11, 1-10.