J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian
kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
51(2): 149-160, June 2026
DOI: 10.14710/jitaa.51.2. 149-160

Effect of encapsulated *M. speciosa* fruit extract on growth performance, amino acid digestibility and oxidative status in high density broilers

L. Krismiyanto[1,2*], V. D. Yunianto[1], S. Sumarsih[1], and S. Sugiharto[1]
[1]*Department of Animal Science, Faculty of Animal and Agricultural Sciences,*
Universitas Diponegoro, Semarang, Central Java, Indonesia 50275
[2]*Doctoral Program of Animal Science, Faculty of Animal and Agricultural Sciences,*
Universitas Diponegoro, Semarang, Central Java, Indonesia 50275
Corresponding author e-mail: lilikkrismiyanto@lecturer.undip.ac.id

Received March 21, 2026; Accepted May 09, 2026

ABSTRACT

This study aimed to evaluate the effects of encapsulation of *Medinilla speciosa* fruit extract in feed on the oxidative status, amino acid digestibility, and performance of broiler chickens raised at high stocking densities. The *M. speciosa* contains polyphenols, flavonoids, tannins, and saponins. This study used 290 unsexed Ross strain broiler chicks aged 8 days, with an average body weight of 196.01 ± 1.12 g. *M. speciosa* fruit extract (MSFE) and encapsulated *M. speciosa* fruit extract (EMSFE) were used as sources of phytobiotics. This study was designed with four treatments and five replicates. The treatments tested were T0: normal density of 10 birds/m^2 without MSFE or EMSFE; T1: high density of 16 birds/m^2 without MSFE or EMSFE; T2: high density of 16 birds/m^2 + 0.08% MSFE; and T3: high density of 16 birds/m^2 + 0.08% EMSFE. The results showed that the addition of MSFE and EMSFE to the diet had a significant effect ($P<0.05$) on performance (feed intake, daily weight gain, and feed conversion ratio), protein and amino acid digestibility, oxidative status (malondialdehyde, superoxide dismutase, and glutathione peroxidase), as well as total lactic acid bacteria, *Escherichia coli*, and small intestine pH. The conclusion is that incorporating 0.08% encapsulation of *M. speciosa* fruit extract notably enhances the overall health, absorption of essential amino acids, and growth of broiler chickens raised in high-density conditions, suggesting its promise as an effective phytobiotic feed additive.

Keywords: Broiler chicken, Encapsulated, High Density, M. speciosa, Performance.

INTRODUCTION

The intensification of broiler chicken production has led to the adoption of high stocking densities as a strategy to improve land efficiency and economic returns. However, this practice is often associated with increased environmental stress, reduced animal welfare, disruptions to physiological balance, and decreased production performance. These conditions can trigger oxidative stress due to increased production of reactive oxygen species (ROS) that exceeds the capacity of the endogenous antioxidant system. The accumulation of ROS contributes to lipid peroxidation, damage to proteins and cell membranes, and disruption of gastrointestinal function, which ultimately results in reduced nutrient utilization efficiency and growth in broilers (Mishra and Jha, 2019).

In addition to oxidative stress, high stocking density affects the balance of gut bacteria. Suboptimal environmental conditions can increase the number of pathogenic bacteria, such as *E. coli,* and inhibit the growth of beneficial bacteria, such as lactic acid bacteria (LAB) (Akpoghelie *et al.*, 2025). This bacterial imbalance affects the pH of the digestive tract, intestinal mucosal integrity, digestive enzyme activity, and amino acid digestibility (Yadav and Jha, 2019). Given the importance of amino acids in protein synthesis and tissue growth, impaired digestibility will directly reduce broiler chicken production performance.

The use of phytogenic feed additives as growth promoters is becoming increasingly popular in modern poultry production systems. One local plant with potential for development is *M. speciosa*, which contains bioactive compounds, such as flavonoids, polyphenols, tannins, and saponins, with high antioxidant and antibacterial activities. Krismiyanto *et al.* (2025^{a}) reported that the extract from *M. speciosa* fruit contains 13.83% antioxidant activity, 4.68% total phenols, 4.11% flavonoids, 2.23% tannins, and 1.45% saponins. These compounds have the potential to reduce oxidative stress, inhibit the growth of pathogenic bacteria, and support gut bacterial balance (Sun *et al.*, 2024). However, the direct use of plant extracts often faces challenges in the form of instability of active compounds due to temperature, oxidation, and enzymatic degradation in the digestive tract, thereby limiting their bioavailability (Ahamed *et al.*, 2025).

Encapsulation technology offers an innovative approach to protecting bioactive compounds from degradation during feed processing and digestion. When active compounds are encapsulated, they can be released in a controlled manner within the digestive tract, which enhances their stability, biological efficacy, and utilization efficiency (Klojdová *et al.*, 2023). With the improved stability of antioxidant and antibacterial compounds in *M. speciosa*, it is expected that there will be an improvement in oxidative status, total BAL growth, a reduction in total *E. coli*, stabilization of intestinal pH, and increased amino acid digestibility, which will ultimately improve broiler performance, especially under high-density conditions.

Although the bioactive potential of *M. speciosa* fruit has been widely reported in the context of health, scientific information regarding the use of *M. speciosa* fruit extract encapsulated in diets of broiler chickens, particularly under high-density stress conditions, remains limited. The novelty of this study lies in the integration of oxidative stress parameters, small intestinal bacterial profile, digesta pH, amino acid digestibility, and production performance into a single integrated research design, which has not been widely reported. Therefore, this study aimed to examine the effects of *M. speciosa* extract encapsulation on oxidative stress, total BAL, total *E. coli*, small intestinal pH, amino acid digestibility, and the performance of broiler chickens reared at high stocking densities.

MATERIALS AND METHODS

All procedures involving animals were approved by the Faculty of Animal and Agriculture Science, Diponegoro University, Semarang, Indonesia (approval no. 60-08/A-16/KEP-FPP).

Preparation of *M. speciosa* Fruit Extract and Encapsulation Process

The collected *M. speciosa* fruits were dried in an oven at 50°C until their moisture content was less than 14%. The dried *M. speciosa* fruit was ground into flour. The extraction procedure was as follows (Gouda *et al.*, 2021), wherein *M. speciosa* flour was dissolved in 96% ethanol at a 1:10 (w/v) ratio. The solution was placed in a sonicator at 50 Hz for 60 min. The sonicated mixture was allowed to stand for 24 h to maximize extraction. The sonicated solution was filtered using Whatman No. 41 filter paper to collect the filtrate. The filtrate was evaporated using an evaporator at 65°C for 1 h, yielding the final extract.

The extracted material was subjected to an encapsulation process following the method described by Krismiyanto *et al.* (2025^{a}). Maltodextrin was dissolved in distilled water at a 1:3 (w/v)? ratio. The extract was added to the maltodextrin solution at a 3:1 (v/v) ratio. The mixture of the maltodextrin solution and extract was placed in a freeze-dryer and dried at −40°C for 24 h, resulting in the encapsulated *M. speciosa* fruit extract product.

Animals, Experimental Design and Treatment

The experiment used 290 mixed-sex 8-day-

Table 1. Compositions and Nutrient Contents of Dietary Experiment

Ingredients	Compositions	
	Starter (8 – 21 days)	Finisher (22 – 35 days)
Yellow corn	50.12	52.81
Rice bran	15.03	17.34
Soybean meal	24.00	19.00
Fish meal	10.00	10.00
Limestone	0.30	0.30
Premix^4	0.25	0.25
Lysine	0.10	0.10
Methionine	0.20	0.20
Total	100	100
Nutrient content:		
Metabolizable content (kcal/kg)^2	2,996.85	3,026.32
Crude protein (%)^1	21.33	19.25
Amino acid^3		
Lysine (%)	1.20	1.07
Methionine (%)	0.48	0.47
Arginine (%)	1.39	1.26
Methionine+Cystine (%)	0.91	0.80
Threonine (%)	0.77	0.72
Tryptophan (%)	0.17	0.17
Ether extract (%)^1	4.74	5.18
Crude fiber (%)^1	5.51	5.59
Calcium (%)^1	1.04	1.07
Phospor (%)^1	0.68	0.74

^{1,3}Based on chemical analysis.

^{2}Calculated EM (Bolton, 1967).

^{4}Premix provided per kg of diet: calcium 25%, phosphor 1%, iron 6 g, mangan 4 g, iodine 0.075 g, copper 0.3 g, zinc 3.75 g, vitamin B_{12} 0.5 mg, and vitamin D_3 50,000 IU.

old Ross broiler chickens with an average body weight of 194.71 ± 5.32 g. The *M. speciosa* fruits were collected from the area of Muria Mountain, Kudus Regency, Central Java, Indonesia. The study was designed based on a completely randomized design with four treatments and five replicates (coefficient variation of 2.73%), resulting in 20 experimental units. The normal stocking density is 10 birds/m^2, while 16 birds/m^2 is the maximum stocking density (Agusetyaningsih *et al.*, 2022). The treatments applied were as follows: T0 (normal density of 10 birds/m^2 without MSFE or EMSFE), T1 (high density of 16 birds/m^2 without MSFE or EMSFE), T2 (high density of 16 birds/m^2 + 0.08% MSFE), and T3 (high density of 16 birds/m^2 + 0.08% EMSFE). The composition of the feed ingredients and ration levels used in the study is shown in Table 1.

Parameters Measured

The parameters observed were malondialdehyde (MDA) activity, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), lactic acid bacteria (LAB), total *E. coli*, small intestine pH, protein and amino acid digestibility, feed intake (FI), daily body weight gain (DBWG), and feed conversion ratio (FCR).

Blood Sampling and Oxidative Stress Indicators

Blood samples were collected from broilers when they were 35 days old to determine SOD, MDA, and GSH-Px levels. Approximately 3 mL of blood was collected from the brachial vein of each bird. The SOD activity was measured in erythrocyte lysate based on the inhibition of pyrogallol auto-oxidation. GSH-Px activity was measured using a coupled assay method, and MDA levels were analyzed in plasma using the thiobarbituric acid reactive substance (TBARS) method (De Leon and Borges, 2020).

Lactic Acid Bacteria, Total *E. coli*, and pH

Digesta samples from each segment of the small intestine (duodenum, jejunum, and ileum) were collected in sample pots and homogenized. The LAB counts were determined using de Man Rogosa Sharpe (MRS) (Merck, Germany) and incubated at 37°C for 48 h (Mandal *et al.*, 2021). The number of *E. coli* was counted using eosin methyline blue agar (EMBA) agar (Oxoid, UK) incubated at 37°C for 24 h (AOAC, 2023). Digesta pH was measured in the duodenum, jejunum, and ileum immediately after slaughter using a digital pH meter (Hanna Instruments, USA).

Protein and Amino Acid Digestibility

Protein and amino acid digestibility were determined using the total collection method with Fe_2O_3 as an indigestible marker (Suthama and Wibawa, 2016). Excreta were collected for four days starting on day 30, sprayed with 0.2 N HCl to prevent nitrogen evaporation, dried, and analyzed for crude protein. Digestibility was calculated as follows:

Protein digestibility (%) = {[protein intake − (amount of excreted protein amount of endogenous protein)] / (protein intake)} × 100 %

Amino acid digestibility (%) = {[amino acid intake − (amount of excreted amino acid − amount of endogenous amino acid)] / (Amino acid intake)} × 100 %

Where:

Protein intake = feed intake × % protein content of feed

Protein excreta = total excreta × % protein content in excreta.

Amount of protein endogenous = total endogenous × % protein content in endogenous.

Amino acid intake = feed intake × % amino acid content of feed.

Amount of amino acid excreta = total excreta × % amino acid content in excreta

Amount of amino acids endogenous = total endogenous × % amino acid content in endogenous

Broiler Chicken Performance

Broiler chickens were weighed at 8 and 35 days of age. Performance was determined based on FI, DBWG, and FCR.

FI (g) = feed given – feed refused

DBWG (g) = [(final weight − initial weight) / maintenance period]

FCR = feed intake / daily body weight gain

Statistical Analysis

Data were analyzed for variance at a 5% error rate. Duncan's multiple range test (DMRT) was conducted at a 5% significance level to determine the differences between treatments (Gasperz, 2006).

RESULT AND DISCUSSION

Growth Performance

The addition of encapsulated *M. speciosa* fruit extract had a significant effect ($P<0.05$) on feed intake in chickens raised at high stocking densities (Table 2). Feed intake in T2 and T3 was significantly higher ($P<0.05$) than in T0 and T1. The increased feed intake in T2 and T3 is associated with bioactive compounds in *M. speciosa* fruit. Bioactive activity can improve gut health, metabolic activity, and feed palatability. Bioactive compounds include flavonoids, tannins, saponins, and phenols, which possess antioxidant and antibacterial properties. These compounds can inhibit the growth of pathogenic bacteria in the digestive tract and support the growth of beneficial bacteria. A healthier digestive tract can improve the efficiency of digestion and nutrient absorption, leading to higher nutrient requirements in chickens and consequently increased feed intake (Yadav and Jha, 2019). According to Adedokun and Olojede (2019), improving the balance of gut bacteria can increase appetite and feed intake in broiler chickens because metabolic processes occur more optimally.

The addition of 0.08% *M. speciosa* fruit extract encapsulated in the diet had a significantly increased effect ($P<0.05$) on the daily weight gain of broiler chickens raised at high stocking density (Table 2). The daily weight gain in T3 was significantly ($P<0.05$) higher than that in T0, T1, and T2. This is because the encapsulation process protects the bioactive compounds contained in *M. speciosa* fruit, making them more stable and allowing for their maximum utilization within the digestive tract. When administered as an extract, some of these bioactive compounds may degrade due to the effects of temperature, oxidation, or acidic conditions in the proventriculus, resulting in fewer active compounds reaching the small intestine. Conversely, in the encapsulated form, the coating material protects the active compounds from damage during digestion in the upper gastrointestinal tract, allowing for the gradual release of active substances in the small intestine—the primary site of nutrient absorption (Krismiyanto *et al.*, 2025b). These conditions result in improved digestive health in chickens fed the encapsulation, as evidenced by an improved balance of gut bacteria and increased digestive enzyme activity. Improved digestive function enhances the digestion and absorption of nutrients, particularly proteins and amino acids, which play a crucial role in muscle tissue formation (Ravindran and Abdollahi, 2021). More efficient nutrient utilization results in increased daily weight gain.

The addition of 0.08% *M. speciosa* fruit extract capsules to the diet resulted in significantly higher growth performance ($P < 0.05$) than that in the untreated group in chickens raised at high stocking density (16 birds/m²). High stocking density is known to cause environmental stress, increased house temperature, and competition for feed and drinking water, thereby reducing the growth performance of broiler chickens. Stress resulting from high stocking density can lead to digestive tract disorders, reduced nutrient digestibility, and increased endogenous protein loss, which ultimately reduces body weight gain (Sugiharto, 2022). Antioxidant compounds play a role in reducing increased oxidative stress in chickens raised at high stocking densities, thereby allowing bodily metabolism to function more optimally. Additionally, the antibacterial activity of phytochemical compounds can help maintain the balance of gut microbiota, ensuring that digestive tract health is preserved and nutrient digestion occurs more efficiently (Obianwuna *et al.*, 2024). The administration of 0.08% *M. speciosa* fruit extract encapsulated can mitigate the negative effects of high-density rearing by improving digestive tract health, enhancing metabolism, and increasing nutrient utilization efficiency, thereby improving broiler chicken growth.

The addition of 0.08% *M. speciosa* fruit extract encapsulation to the diet had a significantly decreased effect ($P<0.05$) on the feed conversion ratio of broiler chickens raised at a high stocking density (Table 2). The feed conversion ratio in T3 was significantly ($P<0.05$) lower than that in T0, T1, and T2. This finding is supported by the daily weight gain in T3 ($P<0.05$), which showed higher values than those in the other treatments. A lower feed conversion ratio indicates that a smaller amount of feed is consumed to produce greater weight gain, thereby increasing feed utilization efficiency. This finding is related to differences in the release rates of bioactive compounds between the extract and encapsulation forms. In the extract encapsulation treatment (T3), bioactive compounds become available more rapidly in the digestive tract, thereby playing a role in enhancing enzyme activity, improving gut microbiota balance, and increasing nutrient digestibility. This more efficient nutrient utilization results in a higher feed requirement to produce body weight gain (Aftab *et al.*, 2018), leading to a better feed conversion ratio than that in the extract treatment.

The addition of 0.08% *M. speciosa* fruit extract to the diet of chickens raised at high stocking density (16 birds/m²) showed no significant difference ($P > 0.05$) compared to the diet without the extract given to chickens raised at normal stocking density (10 birds/m²). This indicates that the administration of *M. speciosa* fruit extract improves feed utilization efficiency under high-density conditions, making it comparable to normal rearing conditions. The administration of *M. speciosa* fruit extract mitigates the negative

Table 2. Broiler Growth Performance in 8-35 Day with Encapsulated of *M. speciosa* Fruit Extract

Parameter	T0	T1	T2	T3	SE	*p value*
FI (g/bird/day)	109.84^{b}	109.42^{b}	112.04^{a}	111.87^{a}	1.43	0.000
DBWG (g/bird/day)	66.75^{b}	63.05^{c}	67.26^{b}	70.52^{a}	3.31	0.000
FCR	1.65^{b}	1.74^{a}	1.67^{b}	1.59^{c}	0.07	0.001

[abc] Mean values within row followed by different superscripts are significantly different ($P<0.05$)

T0:10 birds/m² (control); T1:16 birds/m²; T2:16 birds/m² with 0.08% MSFE; T3:16 birds/m² with 0.08% EMSFE

FI: feed intake; DBWG: daily body weight gain; FCR: feed conversion ratio.

effects of high density, as *M. speciosa* fruit contains bioactive compounds, such as flavonoids, phenolics, tannins, and saponins, that function as antioxidants and antibacterial agents. Antioxidant compounds can reduce increased oxidative stress under high-density conditions, thereby ensuring that the body's metabolism remains optimal (Sugiharto, 2022). In addition, antibacterial activity helps maintain the balance of gut microbiota, thereby making digestion and nutrient absorption processes more efficient. This improvement in gastrointestinal health allows nutrients, particularly protein and energy, to be utilized more effectively, even when chickens are raised at high stocking densities. Better nutrient utilization efficiency results in a lower amount of feed required to produce weight gain (Wang *et al.*, 2015); the feed conversion ratio in chickens fed *M. speciosa* fruit extract at high stocking densities can match that of chickens reared at normal stocking densities without the extract.

Protein and Amino Acids Digestibility

The addition of *M. speciosa* fruit extract capsules had a significantly increased effect ($P<0.05$) on protein digestibility in broiler chickens raised at high stocking densities (Table 3). Protein digestibility in T3 was significantly ($P<0.05$) higher than that in T0, T1, and T2. These results indicate that the addition of *M. speciosa* fruit extract capsules can improve protein absorption, particularly under stress conditions caused by high stocking density. High stocking density can induce heat and social stress, which can impair gastrointestinal function, such as reduced small intestinal villi height, increased mucus secretion, and increased endogenous protein loss (Goo *et al.*, 2019). These conditions result in suboptimal protein absorption, leading to reduced protein digestibility. Chickens raised at high stocking densities without any treatment, likely experience more severe physiological disturbances leading to lower protein digestibility. The encapsulated form in T3 yielded higher protein digestibility, as encapsulation technology protects bioactive compounds from degradation during digestion, allowing active ingredients to be gradually released in the small intestine—the primary site of protein absorption. The more effective release of active compounds can increase proteolytic enzyme activity, improve intestinal villi structure, and reduce endogenous protein loss (Song *et al.*, 2023). These conditions result in more protein in the diet being digested and absorbed by the body.

The digestibility of lysine, threonine, and tryptophan in T0, T2, and T3 was significantly ($P<0.05$) higher than that in T1, which was influenced by the increased digestibility of these essential amino acids, which is related to the ability of bioactive compounds in *M. speciosa* fruit to improve the condition of the digestive tract impaired by stocking density stress. Lysine, an essential amino acid, plays a role in promoting growth and protein synthesis (Holeček, 2025). Threonine plays a major role in intestinal function, the immune system, and metabolic balance (Tang *et al.*, 2021). Under environmental stress conditions, tryptophan acts as a precursor to serotonin and methionine, which are associated with stress response, appetite, and physiological stability (Höglund *et al.*, 2019). Under high stocking density (16 birds/m^2) in treatment T1, the chickens experienced stress, leading to increased protein requirements. In the encapsulated

Table 3. Protein and Essential Amino Acids Digestibility with Encapsulated of *M. speciosa* Fruit Extract

Parameter	T0	T1	T2	T3	SE	*p value*
Protein digestibility (%)	84.24^{b}	77.22^{c}	84.09^{b}	87.4^{a}	4.38	0.000
Amino acids digestibility (%)						
Lysine	78.86^{a}	74.37^{b}	78.62^{a}	78.82^{a}	3.22	0.050
Methionine	78.09^{b}	70.74^{c}	81.51^{a}	82.12^{a}	4.99	0.000
Arginine	75.71	72.97	75.03	75.97	3.03	0.417
Methionine+Cystine	71.21^{b}	71.32^{b}	76.87^{a}	77.26^{a}	4.42	0.019
Threonine	83.78^{a}	74.27^{b}	83.95^{a}	85.49^{a}	4.98	0.000
Tryptophan	82.02^{a}	75.24^{b}	82.35^{a}	83.51^{a}	4.80	0.013

^{ab} Mean values within row followed by different superscripts are significantly different ($P<0.05$)

T0:10 birds/m^2 (control); T1:16 birds/m^2; T2:16 birds/m^2 with 0.08% MSFE; T3:16 birds/m^2 with 0.08% EMSFE treatment, amino acid digestibility was higher because the encapsulation process protects bioactive compounds from damage caused by temperature, oxidation, and acidic conditions in the proventriculus, allowing active substances to be released more effectively in the small intestine. The optimal release of active compounds can improve intestinal villi structure, reduce endogenous protein loss, and increase proteolytic enzyme activity (Bedford and Apajalahti, 2022). Consequently, more lysine, threonine, and tryptophan were digested and absorbed than in chickens that did not receive the treatment.

The digestibility of methionine and methionine + cysteine in T2 and T3 was significantly (P<0.05) higher than that in T0 and T1. This was influenced by the increased digestibility of these sulphur-containing amino acids, which is related to the role of bioactive compounds in *M. speciosa* fruit in improving the physiological condition of the digestive tract and reducing increased oxidative stress under high-density conditions. High stocking density can cause heat stress and physiological stress, which disrupts gastrointestinal function, reduces proteolytic enzyme activity, and increases endogenous protein loss. These conditions result in lower digestibility of amino acids, including methionine and cysteine. Additionally, methionine is an amino acid highly sensitive to stress because it plays a role in protein synthesis, feather formation, and as a methyl group donor in metabolism; thus, methionine requirements increase under environmental stress conditions (Lugata *et al.*, 2022). Administration of *M. speciosa* fruit extract or capsules can improve intestinal mucosal integrity, thereby increasing the absorption surface area and digestive enzyme activity, resulting in higher digestibility of proteins and sulphur-containing amino acids, such as methionine and cysteine. Furthermore, the antibacterial activity of phytochemical compounds helps maintain the balance of the intestinal microbiota, thereby making the digestive process more efficient (Obianwuna *et al.*, 2024).

The addition of *M. speciosa* fruit extract or encapsulated extract had no significant effect (P>0.05) on arginine digestibility in high-density broiler chickens, as arginine digestibility is relatively stable and less susceptible to changes in gastrointestinal conditions or environmental stress compared to some other essential amino acids. Arginine is an essential amino acid with a high digestibility rate in corn- and soybean meal-based diets; therefore, most of the arginine in the feed can already be optimally digested by broiler chickens (Nogueira *et al.*, 2021). Under these conditions, the administration of phytobiotics, such as *M. speciosa* fruit extract, does not provide a significant improvement because arginine digestibility is already near its maximum level. Additionally, arginine is not heavily involved in intestinal mucus components; therefore, the loss of endogenous protein due to high-density stress does not significantly affect arginine digestibility compared to amino acids, such as threonine or methionine.

Oxidative Stress Indicators

The addition of *M. speciosa* fruit extract capsules to the diet had a significant effect (P<0.05) on serum malondialdehyde (MDA) levels in broiler chickens raised at high stocking densities (Table 4). Serum malondialdehyde levels in T3 were significantly higher (P<0.05) than those in T0, T1, and T2. High stocking density can increase heat stress, competition, and metabolic activity, thereby increasing the production of free radicals in the body (Sugiharto, 2022). An increase in free radicals triggers lipid peroxidation in cell membranes, producing malondialdehyde as the end product. Encapsulated extracts of *M. speciosa* fruit, containing antioxidant compounds such as flavonoids and phenolics, are

Table 4. Oxidative Status with Encapsulated of *M. speciosa* Fruit Extract

Parameter	T0	T1	T2	T3	SE	*p value*
MDA (U/ml)	3.76^b	4.86^a	3.46^c	3.13^d	0.68	0.000
SOD (U/ml)	42.95^b	35.18^c	48.22^b	55.83^a	8.55	0.000
GSH-Px (U/ml)	404.57^b	368.55^c	407.79^b	421.94^a	21.82	0.000

^{ab}Mean values within row followed by different superscripts are significantly different (P<0.05)

T0:10 birds/m² (control); T1:16 birds/m²; T2:16 birds/m² with 0.08% MSFE; T3:16 birds/m² with 0.08% EMSFE

MDA: malondialdehyde; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase immediately available in the digestive tract, allowing for faster absorption and utilization to suppress free radical formation. This results in oxidative stress being suppressed earlier, leading to relatively lower MDA levels compared to treatments using the extract in its unencapsulated form. Treatment T1 indicates that high density can increase metabolic rate and tissue oxygen consumption, thereby increasing free radical production. If antioxidants are not released from the encapsulant or the extract is not released quickly enough to counteract this increase in free radicals, the level of lipid peroxidation remains high, as reflected in increased serum MDA levels (Gonçalves *et al.*, 2021).

The addition of *M. speciosa* fruit extract capsules to the diet had a significant effect ($P<0.05$) on serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels in broiler chickens raised at high stocking densities (Table 4). SOD and GSH-Px levels in T3 were significantly higher ($P<0.05$) than those in T0, T1, and T2, which may be influenced by increased activity of both enzymes associated with heightened oxidative stress under high-density conditions, followed by the body's adaptive response, as well as the effect of antioxidant compounds from *M. speciosa* fruit administered in encapsulated form. An increase in free radicals stimulates the endogenous antioxidant defence system, including SOD and GSH-Px enzymes, to work more actively in neutralizing reactive oxygen species (Surai, 2016). Therefore, chickens reared at high stocking densities generally exhibit higher antioxidant enzyme activity than those reared at normal stocking densities. The administration of *M. speciosa* fruit extract capsules can help reduce oxidative stress because they contain flavonoids, phenolic compounds, and natural vitamins with antioxidant properties. However, as an extract, some of the active compounds may degrade during digestion, resulting in suboptimal absorption of active ingredients. In contrast, in the encapsulated form, bioactive compounds are protected by a coating, making them more stable against temperature, oxidation, and the acidic conditions of the proventriculus and allowing them to be released gradually in the small intestine. This more effective release enables greater absorption of antioxidant compounds and allows for more optimal stimulation of the endogenous antioxidant system (Kikusato, 2021).

The levels of SOD and GSH-Px at T2 did not differ significantly ($P > 0.05$) from those at T0, indicating that administering *M. speciosa* fruit extract mitigated the effects of oxidative stress caused by high stocking density, thereby restoring the body's antioxidant status to levels comparable to those under normal rearing conditions. An increase in free radicals stimulates the activity of the endogenous antioxidant system, including SOD and GSH-Px enzymes, to neutralize reactive oxygen species (Surai, 2016). In chickens reared at high stocking densities without treatment, this condition generally causes an increase in antioxidant enzyme activity in response to greater oxidative stress. With low levels of free radicals, the need for activation of endogenous antioxidant enzymes is reduced; therefore, SOD and GSH-Px activity remains at normal levels. This results in SOD and GSH-Px values in high-density chickens administered the extract not being significantly different from those of chickens reared at normal density without treatment.

Total Lactic Acid Bacteria, *E. coli*, and Small Intestine pH

The addition of *M. speciosa* fruit extract capsules to the diet had a significant effect ($P<0.05$) on total LAB, *E. coli*, and small intestine pH in broiler chickens raised at high stocking density (Table 5). Total LAB in T2 and T3 was significantly ($P<0.05$) higher than in T0 and T1. This improvement was due to the increase in total LAB associated with the bioactive compounds in *M. speciosa* fruit, which can improve the balance of gastrointestinal microflora, particularly in the small intestine. The administration of *M. speciosa* fruit encapsulates or extracts can increase total LAB, as they contain flavonoids, phenolics, tannins, and saponins with selective antibacterial properties. These compounds can inhibit the growth of pathogenic bacteria without disrupting beneficial bacteria, thereby allowing LAB to thrive. Additionally, phenolic compounds also act as natural prebiotics that support the growth of beneficial microflora in the small intestine (Plamada and Vodnar, 2021). Total LAB in the extract and encapsulated treatments under high-density conditions may be higher than in untreated samples under normal density condi-

Table 5. The Selected Small Intestine Bacterial Population and pH with Encapsulated of *M. speciosa* Fruit Extract

Parameter	T0	T1	T2	T3	SE	*p value*
BAL (Log CFU/g)	6.69^{b}	6.65^{b}	7.41^{a}	7.69^{a}	0.51	0.000
E. coli (Log CFU/g)	4.41^{a}	4.49^{a}	3.93^{b}	3.64^{c}	0.37	0.000
pH	6.4^{b}	6.54^{a}	6.32^{b}	6.12^{c}	0.17	0.000

^{ab} Mean values within row followed by different superscripts are significantly different (P<0.05)

T0:10 birds/m² (control); T1:16 birds/m²; T2:16 birds/m² with 0.08% MSFE; T3:16 birds/m² with 0.08% EMSFE

tions. This indicates that phytobiotic supplementation from *M. speciosa* fruit is not only capable of reducing the negative effects of high density but can also improve the balance of small intestinal microflora beyond normal levels due to the presence of additional bioactive compounds that function as antibacterial and antioxidant agents. Total BAL in T1 showed a lower count compared to other treatments, influenced by environmental stress that disrupted the balance of small intestinal bacteria, characterized by an increase in pathogenic bacteria and a decrease in beneficial bacteria such as lactic acid bacteria. Stress can reduce the secretion of digestive enzymes and damage the intestinal mucosa, making the digestive tract less optimal for the growth of beneficial microflora (Yadav and Jha, 2019). In chickens raised at high density without treatment, these conditions resulted in lower total LAB.

The total *E. coli* count in the T3 treatment was significantly (P<0.05) lower than in the other treatments; this may be due to the fact that the reduction in total *E. coli* is associated with the greater effectiveness of bioactive compounds from *M. speciosa* fruit in the encapsulated form in inhibiting the growth of pathogenic bacteria in the small intestine. Bioactive compounds (flavonoids, phenols, tannins, and saponins) can damage bacterial cell walls, **disrupt** membrane permeability, and inhibit the enzymatic activity of pathogenic bacteria, such as *E. coli*. When administered as an extract, some active compounds may degrade owing to the effects of temperature, oxidation, and acidic conditions in the proventriculus, resulting in fewer active substances reaching the intestine and suboptimal antibacterial effects. Encapsulated extract from *M. speciosa* fruit can improve gut microbiota balance by increasing total beneficial bacteria, such as lactic acid bacteria (LAB). Increased LAB levels can lower the pH of the digestive tract through the production of organic acids, making the intestinal environment less suitable for *E. coli* growth (Rodjan *et al.*, 2018). This condition resulted in lower total *E. coli* counts in the encapsulated treatment group. The total *E. coli* in T0 was similar (P>0.05) to that in T1; this result indicates that the increase in stocking density at that level has not yet significantly affected the balance of gut microbiota, particularly on total *E. coli*. High stocking density can increase environmental stress, temperature, humidity, and competition among chickens, which may impair physiological conditions and disrupt the microbial balance of the digestive tract (Sugiharto, 2022). Stressful conditions can lead to reduced immunity and changes in the intestinal environment that support the growth of pathogenic bacteria, including *E. coli*. However, the absence of significant differences in this study is likely due to the fact that the high stocking density used remained within the tolerance limits of broiler chickens, thus not causing significant physiological disturbances.

The small intestinal pH in the T3 group was significantly (P<0.05) higher than that in the other treatment groups. This finding is because a decrease in small intestinal pH is associated with increased activity of beneficial bacteria, particularly LAB, as well as the more optimal effectiveness of the bioactive compounds of *M. speciosa* fruit in the encapsulated form. Bioactive compounds can inhibit the growth of total *E. coli* and support the development of total LAB in the digestive tract (small intestine). Lactic acid bacteria produce organic acids, such as lactic acid and acetic acid, which can lower intestinal pH, resulting in a more acidic intestinal environment (Wang *et al.*, 2018). Low pH conditions inhibit the growth of pathogenic bacteria, such as *E. coli*, and support the digestion of nutrients. In the extract treatment, some bioactive compounds may have degraded because of the effects of temperature, oxidation, and acidic conditions in the proventriculus, resulting in fewer active compounds reaching the small intestine. Consequently, the effects on the balance of intestinal microflora and the production of organic acids were not as optimal as in the encapsulated treatment. High-density conditions in T1 can increase stress, which has the potential to disrupt the balance of the small intestine's microflora. Administration of *M. speciosa* extract capsules can mitigate these negative effects through antioxidant and antibacterial activities, thereby maintaining a stable intestinal environment and supporting the formation of a lower pH.

CONCLUSION

Incorporating 0.08% encapsulated *M. speciosa* fruit extract notably enhances the overall health, absorption of essential amino acids, and growth of broiler chickens raised in high-density conditions, suggesting its potential as an effective phytobiotic feed additive.

ACKNOWLEDGEMENTS

The authors express their gratitude for the financial support received from the Institute of Research and Community Service at Universitas Diponegoro in Semarang, Indonesia, through grant number 609-73/UN7.D2/PP/VIII/2023. The authors are thankful for the funding provided by the Institute of Research and Community Service at Universitas Diponegoro in Semarang, Indonesia, under the same grant number.

REFERENCES

Adedokun, S.A., and O.C. Olojede. 2019. Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. Front. Vet. Sci. 5, 348. https://doi.org/10.3389/fvets.2018.00348

Aftab, U., M.R. Bedford, and D. Creswell. 2018. Prospects of improving efficiency of feed utilisation in broiler. Worlds. Poult. Sci. J. 74, 427–442. https://doi.org/10.1017/S0043933918000466

Agusetyaningsih, I., E. Widiastuti, H.I. Wahyuni, T. Yudiarti, R. Murwani, T.A. Sartono, and S. Sugiharto. 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. https://doi.org/10.2478/aoas-2021-0043

Ahamed, M.S., A. Asghar, N. Hayat, B.L. Muqaddas, S. Saif, S.M.S. Shahriar, R. Riaz, M.A. Ashraf, B. Khalid, and A. Sadia. 2025. Optimizing bioactive compounds extraction: advanced food safety protocols for enhanced bioavailability and processing stability. S. Asian J. Life Sci. 13, 58–65. https://dx.doi.org/10.17582/journal.sajls/2025/13.58.65

Akpoghelie, P.O., G.I. Edo, A.N. Mafe, E.F. Isoje, U.A. Igbuku, A.B.M. Ali, E. Yousif, J.O. Owheruo, S. Oberhiri, and A.E.A. Essaghah. 2025. Food, health, and environmental impact of lactic acid bacteria: The superbacteria for posterity. Probiotics Antimicrob. Proteins 17, 2819–2855. https://dx.doi.org/10.17582/journal.sajls/2025/13.58.65

AOAC International. 2023. Official Methods of Analysis of AOAC International. 22nd Edition. Rockville, Maryland, USA.

Bedford, M.R., and J.H. Apajalahti. 2022. The role of feed enzymes in maintaining poultry intestinal health. J. Sci. Food Agric. 102, 1759–1770. https://doi.org/10.1002/jsfa.11670

Bolton, W. 1967. Poultry nutrition. HMSO, London, England.

De Leon, J.A.D., and C.R. Borges. 2020. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J. Vis. Exp. JoVE 10–3791. https://doi.org/10.3791/61122

Gasperz, V. 2006. Teknik Analisis dam Penelitian Percobaan, 3rd ed. CV Tarsito, Bandung.

Gonçalves, A., B.N. Estevinho, and F. Rocha. 2021. Methodologies for simulation of gastrointestinal digestion of different controlled delivery systems and further uptake of encapsulated bioactive compounds. Trends Food Sci. Technol. 114, 510–520.

Goo, D., J.H. Kim, G.H. Park, J.B. Delos Reyes, and D.Y. Kil. 2019. Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. Animals 9, 107. https://doi.org/10.3390/ani9030107

Gouda, M., A.E.D. Bekhit, Y. Tang, Y. Huang, L. Huang, Y. He, and X. Li. 2021. Recent innovations of ultrasound green technology in herbal phytochemistry: A review. Ultrason. Sonochem. 73, 105538.

Höglund, E., Ø. Øverli, and S. Winberg. 2019. Tryptophan metabolic pathways and brain serotonergic activity: a comparative review. Front. Endocrinol. (Lausanne). 10, 435368. https://doi.org/10.3389/fendo.2019.00158

Holeček, M. 2025. Lysine: Sources, Metabolism, Physiological Importance, and Use as a Supplement. Int. J. Mol. Sci. 26, 8791. https://doi.org/10.3390/ijms26188791

Kachungwa Lugata, J., A.D.S.V. Ortega, and C. Szabó. 2022. The role of methionine supplementation on oxidative stress and antioxidant status of poultry-a review. Agriculture 12, 1701. https://doi.org/10.3390/agriculture12101701

Kikusato, M. 2021. Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. Anim. Biosci. 34, 345. https://doi.org/10.5713/ab.20.0842

Klojdová, I., T. Milota, J. Smetanová, and C. Stathopoulos. 2023. Encapsulation: a strategy to deliver therapeutics and bioactive compounds?. Pharmaceuticals 16, 362. https://doi.org/10.3390/ph16030362

Krismiyanto, L., V.D. Yunianto, S. Sumarsih, and S. Sugiharto. 2025a. Evaluation of Medinilla speciosa fruit as natural feed additive for poultry through inhibition, resistance and antioxidant capacity tests. J. Adv. Vet. Res. 15, 838–842.

Krismiyanto, L., V.D. Yunianto, S. Sumarsih, and S. Sugiharto. 2025b. Characteristics of cecal and short-chain fatty acid production in broiler chickens fed diets with encapsulated Medinilla speciosa fruit extract. J. Adv. Vet. Res. 15, 881–884.

Mandal, A., R.K. Mandal, Y. Yang, B. Khatri, B.W. Kong, and Y.M. Kwon. 2021. In vitro characterization of chicken gut bacterial isolates for probiotic potentials. Poult. Sci. 100, 1083–1092.

Mishra, B., and R. Jha. 2019. Oxidative stress in the poultry gut: Potential challenges and interventions. Front. Vet. Sci. 6, 1–5. https://doi.org/10.3389/fvets.2019.00060.

Nogueira, B.R.F., N.K. Sakomura, M. de P. Reis, B.B. Leme, M.P. Létourneau-Montminy, and G. da S. Viana. 2021. Modelling broiler requirements for lysine and arginine. Animals 11, 2914. https://doi.org/10.3390/ani11102914

Obianwuna, U.E., X. Chang, V.U. Oleforuh-Okoleh, P.N. Onu, H. Zhang, K. Qiu, and S. Wu. 2024. Phytobiotics in poultry: revolutionizing broiler chicken nutrition with plant-derived gut health enhancers. J. Anim. Sci. Biotechnol. 15, 169. https://doi.org/10.1186/s40104-024-01101-9

Plamada, D., and D.C. Vodnar. 2021. Polyphenols—gut microbiota interrelationship: A transition to a new generation of prebiotics. Nutrients 14, 137. https://doi.org/10.3390/nu14010137

Ravindran, V., and M.R. Abdollahi. 2021. Nutrition and digestive physiology of the broiler chick: state of the art and outlook. Animals 11, 2795. https://doi.org/10.3390/ani11102795

Rodjan, P., K. Soisuwan, K. Thongprajukaew, Y. Theapparat, S. Khongthong, J. Jeenkeawpieam, and T. Salaeharae. 2018. Effect of organic acids or probiotics alone or in combination on growth performance, nutrient digestibility, enzyme activities, intestinal morphology and gut microflora in broiler chickens. J. Anim. Physiol. Anim. Nutr. (Berl). 102, e931–e940. https://doi.org/10.1111/jpn.12858

Song, X., M. A. Anas, A. Kurniawati, C. Hanim, M.A. Aprianto, A.M.A. Madani, Q. Wang, and H. Chen. 2023. Effects of reduced-protein diets with protease supplementation on growth, carcass yield, intestinal morphology, organ development, nutrient digestibility, and blood biochemical of broiler chickens. Transl. Anim. Sci. 7, txad098. https://doi.org/10.1093/tas/txad098

Sugiharto, S. 2022. Dietary strategies to alleviate high-stocking-density-induced stress in broiler chickens–a comprehensive review. Arch. Anim. Breed. 65, 21–36. https://doi.org/10.5194/aab-65-21-2022

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti, and F.D. Putra. 2017. Intestinal microbial ecology and hematological parameters of broiler fed cassava waste pulp fermented with *Acremonium charticola*. Vet. world 10, 324. https://doi.org/10.14202/vetworld.2017.324-330

Sun, Y., X. Wang, L. Li, C. Zhong, Y. Zhang, X. Yang, M. Li, and C. Yang. 2024. The role of gut microbiota in intestinal disease: from an oxidative stress perspective. Front. Microbiol. 15, 1328324. https://doi.org/10.3389/fmicb.2024.1328324

Surai, P.F. 2016. Antioxidant systems in poultry biology: superoxide dismutase. J. Anim. Res. Nutr. 1, 8. https://doi.org/10.21767/2572-5459.100008

Suthama, N., and P.J. Wibawa. 2016. Digestibility evaluation of microparticle protein derived from fish meal and soybean meal in broiler chicken, in: Seminar International: The Sixth International Conference on Suatainable Animal Agriculture for Developing Counries. Malang. pp. 19–21.

Tang, W., W. Li, Y. Yang, X. Lin, L. Wang, C. Li, and R. Yang. 2021. Phenolic compounds profile and antioxidant capacity of pitahaya fruit peel from two red-skinned species (*Hylocereus polyrhizus* and *Hylocereus undatus*). Foods 10, 1183. https://doi.org/10.3390/foods10061183

Wang, C., Y. Cui, and X. Qu. 2018. Mechanisms and improvement of acid resistance in lactic acid bacteria. Arch. Microbiol. 200, 195–201. https://doi.org/10.1007/s00203-017-1446-2

Wang, Y., Y.J. Ru, G.H. Liu, W.H. Chang, S. Zhang, H.J. Yan, A.J. Zheng, R.Y. Lou, Z.Y. Liu, and H.Y. Cai. 2015. Effects of different rearing systems on growth performance, nutrients digestibility, digestive organ weight, carcass traits, and energy utilization in male broiler chickens. Livest. Sci. 176, 135–140.

Yadav, S., and R. Jha. 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J. Anim. Sci. Biotechnol. 10, 2. https://doi.org/10.1186/s40104-018-0310-9