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Research article

Effect of encapsulated *Medinilla javanensis* fruit extract on growth, protein digestibility and health of broilers

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Abstract

This study investigated the effect of encapsulated *Medinilla javanensis* fruit extract on performance, protein digestibility, and health of broilers. A total of 200 Cobb broiler chicks were divided into five treatments with four repetitions, including T0 (basal diet), T1 (basal diet + 200 mg/kg encapsulated *Medinilla javanensis* fruit extract), T2 (basal diet + 400 mg/kg encapsulated *Medinilla javanensis* fruit extract), T3 (basal diet + 600 mg/kg encapsulated *Medinilla javanensis* fruit extract), and T4 (basal diet + 800 mg/kg encapsulated *Medinilla javanensis* fruit extract) respectively. Sample collection was conducted on day 35 of age. Results showed that increasing of encapsulated *Medinilla javanensis* fruit extract levels in feeds linearly increased (P<0.05) body weight, body weight gain, intestinal lactic acid bacteria counts, protein digestibility and serum superoxide dismutase levels, while linearly decreasing (P<0.05) feed conversion ratio, intestinal Escherichia coli counts, small intestinal pH values and serum malondialdehyde levels. In conclusion, encapsulated *Medinilla javanensis* fruit extract supplementation improved growth performance, protein digestibility intestinal health and Antioxidant status of broilers. The encapsulated *Medinilla javanensis* fruit extract at a level of 600 mg/kg exerted the best performance and health of broilers.

Keywords: Antioxidant, Broiler, Herb extract, Encapsulation

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INTRODUCTION

Broiler chickens are selected chickens from a variety of chicken breeds with high productivity (Qanbari et al., 2019). Based on the data released by FAO (2020), around 133.645 million tonnes of more broiler meat are produced to meet food needs in the world. Indeed, the demand for chicken meat will continue to rise as the world's population grows. In response to this circumstance, efficiency in the poultry industry is critical so that the demand for chicken meat can be met (Hartcher and Lum, 2020). Broiler rearing systems are generally carried out intensively in closed house. Intensive rearing systems can cause broiler chickens to be in stressful conditions making them susceptible to disease and decreased growth performance (Salami et al., 2015). As a result, it can have an impact on the intestinal microbial population by increasing the population of pathogenic bacteria, which can interfere with the absorption process in the intestinal mucosa, causing protein digestion to be disrupted (Mangisah et al., 2022). In general, these negative impacts can be minimized by administering synthetic antioxidants as feed additives in broiler chickens. However, the use of synthetic antioxidants such as buttylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) is limited due to health issues (carcinogenic effects) in humans as the main consumers of broiler meat products (Candan and Bagdath, 2017).

Medinilla javanensis is an endemic plant from mainland Asia with distribution centers in Malaysia, Indonesia, and the Philippines. *Medinilla javanensis* is rich in secondary metabolites, such as saponins, cardenolides, and flavonoids (Tussanti et al., 2014). Hence, *Medinilla javanensis* fruit can be a phytobiotic and expected to be utilized as an alternative to synthetic antioxidants. Phenolic compounds are typically susceptible to environmental factors, such as temperature, humidity, light, and oxygen. Therefore, they must be protected in order for their phenolic compound to be effective as an antioxidant source (Sugiharto and Ayasan, 2022). One way to minimize the oxidation of phenolic compounds during storage is through the encapsulation method (Pang et al., 2014; Jeyakumari, 2016). Encapsulation can also mask unpleasant tastes or odors while limiting mold growth (Vincekovic et al., 2017; Sugiharto and Ayasan, 2022). Moreover, Iriyanti and Hartoyo (2019) noted that encapsulation may protect the phytogenic materials from the gastrointestinal conditions (particularly in the stomach) and thus increase the availability of bioactive components for broiler chickens. This is in response to the rapid degradation of herbs in the digestive system, which may limit the availability of herbs. In line with the above study, Yousefi et al. (2021) pointed out that encapsulation is a crucial approach for preventing the breakdown or structural changes that occur to bioactive components in herbal products during animal digestion.

One of the most important aspects of the encapsulation process for herbal extracts is determining the most suitable coating material (Öztürk and Temiz, 2018). Maltodextrin is widely used in the manufacture of microcapsules because of its low viscosity at higher concentrations. Its low viscosity results in great solubility, low risk of browning, capacity to form metrics, inhibition of crystallization, strong binding power, and good ability to suppress oxidation reactions, all of which contribute to the long shelf life of the encapsulated

products (Noor et al., 2022). Moreover, maltodextrin is also frequently utilized as a suitable coating because it is more accessible and affordable (Parikh et al., 2014).

So far, the application of encapsulated *Medinilla javanensis* fruit extract has never been practiced to enhance the performance and health of broilers. This study aimed to investigate the phytobiotics effect of encapsulated *Medinilla javanensis* fruit extract on the growth performance, protein digestibility, and health of broiler chickens.

MATERIALS AND METHODS

The experiment was carried out at broiler house of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro. The Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences Universitas Diponegoro approved this study (No. 59-02/A-07/KEP-FPP).

Experimental design,and treatment

A completely randomized design was used in the research. There were five treatments and four repetitions. As a result, there were a total of 20 experimental units. The following treatment was used: T0 = Basal diet, T1 = Basal diet + 200 mg/kg encapsulated *Medinilla javanensis* fruit extract, T2 = Basal diet + 400 mg/kg encapsulated *Medinilla javanensis* fruit extract, T3 = Basal diet + 600 mg/kg encapsulated *Medinilla javanensis* fruit extract, and T4 = Basal diet + 800 mg/kg encapsulated *Medinilla javanensis* fruit extract.

Production of encapsulated *Medinilla javanensis* fruit extract

The *Medinilla javanensis* fruit used in this study was obtained from the Muria mountain range in Kudus regency, Central Java, Indonesia. The dried *Medinilla javanensis* fruit was ground into a fine powder after it had been dried in an oven at 50°C. Encapsulated *Medinilla javanensis* fruit extract began with a powder fruit and a solvent of 96% ethanol in a ratio of 1:10 into a solution (Vifta et al., 2021). The mixing results were filtered through filter paper after it had been the sonification process of encapsulated *Medinilla javanensis* fruit extract. Following that, the extract was placed in a vacuum rotary evaporator at a maximum temperature of 60°C to obtain a pasta-shaped *Medinilla javanensis* fruit extract (Tusanti et al., 2014). The encapsulation was carried out with maltodextrin as a coating material. The maltodextrin was prepared by combining maltodextrin and distilled water in a 1:1 ratio (g/mL). Furthermore, encapsulated *Medinilla javanensis* fruit extract was produced by mixing the *Medinilla javanensis* fruit extract with a 1:5 maltodextrin solvent. To obtain encapsulated *Medinilla javanensis* fruit extract powder, the admixture was freeze-dried until the liquid shrank into a dry crystalline powder. Fruit extract from *Medinilla javanensis* had the following bioactive components: tannin (2.23%), polifenol (4.68%), antioxidant activity (13.83% Inhibition), and flavonoid (4.11%).

Rearing condition and feed rations

This study used 200 Cobb broiler chicks from commercial hatcheries with a mean initial body weight of 49.5±0.59 g. For 0-7 days of age, the chicks were raised with rice husk bedding and manual feeding and drinking

equipment. During these ages, the chicks were provided with pre-starter diet containing crude protein of 21-23%, 3-5% crude fiber, 5-8% crude fat, and 4-7% ash (based on feed label). The chicks were offered with formulated feed (Table 1) on day 8 onward. The purpose of using a single feed from the eighth day till harvest was to prevent the chickens from adapting to a new diet, which could lead to nutritional stress (Amrullah, 2004). During the rearing period, feed and water were provided *ad libitum*. The environmental temperature ranged from 27.3 to 34.8°C, with a relative humidity of 45 to 81% during the *in vivo* experiment. On day 4, Newcastle Disease (ND)-infectious bronchitis (IB) vaccine were administered thorugh eye drop. The Gumboro/infectious bursal disease (IBD) vaccine was further administered *via* drinking water on day 14.

From day 8, the chicks (average body weight of 152.94±0.47 g) were divided into five treatment groups (T0, T1, T2, T3, and T4), each with four replicates. The chicken group was kept at a density of 10 birds/m^2.

Table 1 Ingredients composition and nutrient content of the experimental diet.

Item	(%)
Corn	53.11
Pollard	15.24
Soybean meal	20.80
MBM	10.00
CaCO3	0.30
Premixes	0.25
Lysine	0.10
Methionine	0.20
Nutrition Composition	
Metabolizable Energy (Kcal/Kg)	3090.94
Crude protein (%)	21.44
Crude fiber (%)	4.51
Crude fat (%)	3.42
Ash (%)	5.92
Water content (%)	12.42
Calcium (%)	1.21
Phosphorus (%)	0.69

Results of laboratory analysis of nutrition and feed science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro. Calculating the Bolton formula: Nitrogen-free extract = 100% - %Ash - %Crude Protein - %Crude Fiber - %Crude Fat - % Water content. Metabolizable Energy = 40.81 x (0.87[Crude Protein + 2.25 Crude Fat + Nitrogen-free extract] + 4.9).

Observed research parameters

Growth performance

Data on body weight gain (BWG) was acquired by weighing the final weight of the chicken at 35 days of age and subtracting the chicken's initial weight (8 days of age). Feed conversion ratio (FCR) was calculated by dividing the cumulative feed consumption by BWG during the period of study.

Serum superoxide dismutase (SOD) and malondialdehyde (MDA)

Serum samples were tested for superoxide dismutase (SOD) and malondialdehyde (MDA) levels. For each replication, serum samples were collected from two 35-day-old chickens. Blood was collected from wing veins of broiler. Blood was then placed into a tube without coagulant and left for up

to an hour so that the blood clots. Serum was then obtained and stored in the freezer until analysis.

The activity of MDA was measured using a thiobarbituric acid reactive substance assay (TBA). Each sample was vortexed, then treated with 8.1% sodium dodecyl for 10 minutes at room temperature; controls were treated similarly. Following incubation, 0.6% TBA and 20% acetic acid were applied to the specimen, and the tubes were placed in a water bath at 90-95°C for an hour. The supernatant was then treated with butanol:pyridine (15:1) and vortexed and centrifuged. The MDA concentrations were presented in nmol/ml units. The ability of the specimen to suppress pyrogallol auto-oxidation was used to assess SOD activity. A sample was added to a mixture of 50 mM Tris-HCl (pH 8.2), 1 mM pentaacetic acid diethylenetriamine, and 50 mM Tris-HCl (pH 8.2). The reaction was initiated by the addition of pyrogallol (final concentration, 0.2 mM), and the absorbance was calculated kinetically. The concentration of SOD was stated in units of U/ml (Agusetyaningsih et al., 2022).

Intestinal bacteria count

Digesta samples were obtained from each chicken in each replication by first slaughtering the chicken. The digesta was obtained from the ileum aseptically and then put into the sample pot. Lactic acid bacteria (LAB) and *Escherichia coli* were counted using the poure plate method, for LAB using de Man Rogosa Sharpe agar (MRSA) media, while *Escherichia coli* was using Tryptone Bile X-glucuronide agar (TBX) media. Bacterial counting was carried out after incubation for 48 hours at 44°C for LAB and at 37°C for *Escherichia coli* (Rahmah et al., 2013).

Protein digestibility

Protein digestibility was measured using the total excreta collection method using the Fe_2O_3 indicator for 4 days at 32-35 days old using 20 battery cages filled with one chicken. The feed was mixed with 0.5% Fe_2O_3. The first day the chickens were given treatment rations which added indicators for 24 hours and excreta containers were placed under the battery cages. The collection began when the red excreta first comes out until the second day it changes color. The broiler chickens were given treatment rations without indicators on the second day. The third day ration contained an indicator, while the fourth day ration did not. Every day, excreta were collected by spraying 0.2 N HCL every 2 hours (Krismiyanto et al., 2022). The excreta was dried and crushed before being analyzed for crude protein content using the Kjeldahl method. The protein digestibility was calculated using the formula:

$$\text{Crude Protein Digestibility} = \frac{\text{Crude Protein Consumption - (Extract CP - Endo CP) x 100\%}}{\text{Crude Protein Consumption}}$$

Crude Protein Consumption = (Feed Consumption; (% DM)) x % CP
Excreta Crude Protein = (Excreta weight; (% DM)) x % CP Excreta
CP = Crude Protein
DM = Dry Matter
Endo = Endogenous

pH value of gastrointestinal tract

The pH of broiler chicken digestive tracts was measured after slaughter by taking samples of the duodenum, jejunum, and ileum digesta and measuring with a digital pH meter (Eco test pH 1) for each sample.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) with a significance level of 5% to determine the treatment effect and, if there was a significant effect (P<0.05), then Duncan's test was used to determine the difference between treatments. The increasing effect of feed additive concentration on the observed parameters was also met by linear regression analysis. SAS was used for data analysis (SAS Institute Inc., Cary, NC, USA).

RESULTS

Broiler chicken growth performance

Data on broiler production parameters are presented in Table 2. Linear regression demonstrated that increased levels of encapsulated *Medinilla javanensis* fruit extract in feed were followed by an increase (P<0.05) in body weight and body weight gain in broiler chickens. Enhancement levels of encapsulated *Medinilla javanensis* fruit extract also generated a linear decrease (P<0.05) in FCR. Analysis of variance depicted that the treatment had a significant effect (P<0.05) on body weight, body weight gain, and FCR, but non significant effect (P>0.05) on broiler feed consumption. Furthermore, body weight and body weight gain in the T3 and T4 groups were significantly higher (P<0.05) when compared to T0 and T1 but not different with T2. FCR was significantly lower (P<0.05) in the T4 treatment group when compared to the T0, T1, and T2 groups but not different to T3.

Table 2 Performance of broiler chickens (Days 8 to 35)

Parameter	Treatment					SEM	P Value	
	T0	T1	T2	T3	T4		A	L
DOC (g)	49.61	49.32	49.19	49.46	49.68	0.13	0.792	0.759
BW (g)	1524.77^{b}	1555.20^{b}	1614.05^{ab}	1665.59^{a}	1707.30^{a}	20.30	0.008	<0.001
BWG (g)	1374.65^{b}	1403.47^{b}	1460.75^{ab}	1510.74^{a}	1551.77^{a}	19.93	0.009	<0.001
FI (g)	2455.42	2502.82	2530.74	2560.84	2541.06^{b}	16.74	0.330	0.050
FCR	1.64^{a}	1.64^{a}	1.60^{a}	1.55^{ab}	1.50^{b}	0.01	0.015	0.001

^{abc} Means within rows with different superscripts significantly different at p<0.05 T0 (basal diet), T1 (basal diet + 200 mg/kg of encapsulated *Medinilla javanensis* fruit extract), T2 (basal diet + 400 mg/kg encapsulated *Medinilla javanensis* fruit extract), T3 (basal diet + 600 mg/kg encapsulated *Medinilla javanensis* fruit extract), T4 (basal diet + 800 mg/kg encapsulated *Medinilla javanensis* fruit extract) DOC: Day Old Chick, BW: body weight, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio, SEM: standard error of the mean. A= Analysis of variance, L= Linear Regression.

Intestinal bacteria counts and gastrointestinal pH

Data regarding the parameters of the bacterial population and pH of the gastrointestinal tract are presented in Table 3. Linear regression indicated that increased levels of encapsulated *Medinilla javanensis* fruit extract in feed were followed by enhancement (P<0.05) in the LAB population. Enhancement levels of encapsulated *Medinilla javanensis* fruit extract also resulted in a linear decrease (P<0.05) in the *Escherichia coli* bacteria population and the

small intestine pH. Analysis of variance demonstrated that the treatment had a significant (P<0.05) effect on the population of LAB, *Escherichia coli*, and the pH of the small intestine. Moreover, the LAB population in the T3 and T4 groups was higher (P<0.05) compared to T0, T1, and T2. The *Escherichia coli* population was significantly lower (P<0.05) in the T4 treatment group compared to the T0 and T1 groups. Nevertheless, no different with T2 and T3. Small intestine pH was significantly lower (P<0.05) in the T4 treatment group compared to the T0, T1, T2, and T3 groups.

Table 3 Bacteria in the ileum and the small intestine pH

Parameter	Treatment					SEM	P Value	
	T0	T1	T2	T3	T4		A	L
LAB (10^{10} cfu/g)	3.00^{c}	3.75^{c}	5.31^{b}	6.40^{a}	6.83^{a}	0.36	<0.001	<0.001
Escherichia coli (10^{2} cfu/g)	4.13^{a}	2.96^{b}	2.60^{bc}	2.33^{bc}	1.86^{c}	0.21	0.001	<0.001
Small intestine pH	6.33^{a}	6.26^{b}	6.22^{b}	6.12^{c}	5.96^{d}	0.03	<0.001	<0.001

^{abcd} Means within rows with different superscripts significantly different at p<0.05 T0 (basal diet), T1 (basal diet + 200 mg/kg of encapsulated *Medinilla javanensis* fruit extract), T2 (basal diet + 400 mg/kg encapsulated *Medinilla javanensis* fruit extract), T3 (basal diet + 600 mg/kg encapsulated *Medinilla javanensis* fruit extract), T4 (basal diet + 800 mg/kg encapsulated *Medinilla javanensis* fruit extract). SEM: standard error of the mean. A= Analysis of variance, L= Linear Regression. LAB = lactic acid bacteria.

Protein consumption and digestibility

Data regarding protein consumption and digestibility parameters are presented in Table 4. Linear regression showed that the increased levels of encapsulated *Medinilla javanensis* fruit extract in feed were followed by an increase (P<0.05) in protein digestibility. Analysis of variance indicated that treatment had a significant effect (P<0.05) on protein digestibility, but the treatment had no significant impact (P>0.05) on protein consumption in broiler chickens. Furthermore, protein digestibility in the T4 group was higher (P<0.05) compared to T0, T1, T2, and T3.

Table 4 Protein consumption and digestibility

Parameter	Treatment					SEM	P Value	
	T0	T1	T2	T3	T4		A	L
Protein consumption (g)	27.50	26.80	25.53	26.95	27.89	0.33	0.194	0.699
Protein digestibility (%)	73.21^{d}	75.97^{c}	79.88^{b}	82.06^{b}	84.77^{a}	1.00	<0.001	<0.001

^{abcd} Means within rows with different superscripts significantly different at p<0.05 T0 (basal diet), T1 (basal diet + 200 mg/kg of encapsulated *Medinilla javanensis* fruit extract), T2 (basal diet + 400 mg/kg encapsulated *Medinilla javanensis* fruit extract), T3 (basal diet + 600 mg/kg encapsulated *Medinilla javanensis* fruit extract), T4 (basal diet + 800 mg/kg encapsulated *Medinilla javanensis* fruit extract). SEM: standard error of the mean. A= Analysis of variance, L= Linear Regression.

Antioxidant activity

Data on blood serum MDA and SOD parameters are presented in Table 5. Linear regression showed that the increased levels of encapsulated *Medinilla javanensis* fruit extract in feed were followed by an increase (P<0.05) in blood serum SOD levels. Enhancement levels of encapsulated *Medinilla javanensis* fruit extract in feed also resulted in a linear decrease (P<0.05) in blood serum MDA levels. Analysis of variance depicted that had a significant (P<0.05) effect on MDA and SOD blood serum levels of broiler chickens. Moreover, broiler serum MDA levels in the T3 and T4 groups were lower (P<0.05) compared to T0, T1, and T2. Broiler blood serum SOD levels in groups T3 and T4 were higher (P<0.05) compared to T0 but not different with T1 and T2.

Table 5 Antioxidant Activity

Parameter	Treatment					SEM	P Value	
	T0	T1	T2	T3	T4		A	L
MDA (nmol/ml)	3.44[a]	3.38[a]	3.17[a]	2.72[b]	2.67[b]	0.09	0.001	<0.001
SOD (U/ml)	13.77[b]	16.32[ab]	17.35[ab]	19.39[a]	19.90[a]	0.69	0.013	<0.001

[ab] Means within rows with different superscripts significantly different at $p<0.05$ T0 (basal diet), T1 (basal diet + 200 mg/kg of encapsulated *Medinilla javanensis* fruit extract), T2 (basal diet + 400 mg/kg encapsulated *Medinilla javanensis* fruit extract), T3 (basal diet + 600 mg/kg encapsulated *Medinilla javanensis* fruit extract), T4 (basal diet + 800 mg/kg encapsulated *Medinilla javanensis* fruit extract). SEM: standard error of the mean. A= Analysis of variance, L= Linear Regression, MDA= malondialdehyde, and SOD = superoxide dismutase.

DISCUSSION

This current experiment was carried out in an opened-broiler house over the dry period so that all the chicks may experience heat stress. In fact, broilers reared in high-temperature conditions (above 30°C for 10 hours per day) may not have the ideal body weight (Thuekeaw et al., 2022). This could explain why the broiler chickens raised, particularly for the control group, had a lower body weight than the Cobb broiler strain's standard body weight. In average, the final body weight of chickens in the present study was 1,692 g, which is below the standard body weight of Cobb strain (Islam et al., 2018). The current study found that encapsulating *Medinilla javanensis* fruit extract in feed increased broiler chicken growth performance linearly. The FCR value was also reduced linearly by treatment. Sugiharto and Ayasan (2022) stated that encapsulation of herbal ingredients could positively affect nutrient digestibility, physiological conditions, and health, thereby improving growth performance and feed efficiency in broiler chickens. Encapsulation is a technique for protecting and improving the availability of herbal ingredients in order to provide more positive benefits to the performance and health of chickens (Sugiharto, 2021; Sugiharto and Ayasan, 2022). Aside from the encapsulation effect, herbal products have been shown to improve the growth rate and feed efficiency of broiler chickens. Pawesti et al. (2022) found that encapsulated *Cosmos caudatus* K. leaf extract increased body weight and improved feed conversion ratio in broiler chickens. The beneficial effect of using herbal ingredients is due to the various active components found in these ingredients (Sugiharto, 2021).

In the case of *Medinilla javanensis* fruit, several secondary metabolites, particularly flavonoids, were reported to be able to inhibit pathogenic bacteria, thereby supporting digestive enzyme activity and thus increasing nutrient digestibility in broiler chickens (Xue et al., 2021). According to Wijayanti and Ardigurnita (2018), quercetin is the flavonoid that is present in *Medinilla* sp. in the greatest concentration. In line with this, Ouyang et al. (2016) reported that giving alfalfa flavonoids (15 mg/kg feed) improved the rate of body weight gain and FCR in broiler chickens. Flavonoids can also improve the microbial population balance in the gastrointestinal tract, particularly in the intestine. As a result, flavonoid has a positive effect on broiler chicken function and intestinal health (Prihambodo et al., 2021). Flavonoids have been shown to improve the antioxidant status and physiological conditions of broiler chickens as a source of antioxidants (Sugiharto, 2021). In terms of energy allocation in the body, improving physiological conditions can reduce the allocation of

energy derived from feed for maintenance and recovery activities, allowing energy to be used more efficiently for chicken growth (Kogut et al., 2017).

With regard particularly to the dose of encapsulated *Medinilla javanensis* extract, the dose of this phytobiotic used in this current work was regarded sufficient to have significant effects on the parameters measured. Reference regarding the optimal dose for the use of encapsulated *Medinilla javanensis* extract in broiler chicken feed has not been found in the literature. However, referring to the dosage of phytobiotic materials in broiler chickens, Prihambodo et al. (2021) reported that the dosage varies from 10-7,200 mg/kg feed, depending on the types of phytobiotics.

The LAB population in the ileum increased linearly with increasing levels of *Medinilla javanensis* fruit extract encapsulation in feed. The treatment also reduced the value of the *Escherichia coli* population and the pH of the small intestine in a linear fashion. This finding agreed with Sugiharto and Ayasan (2022), who stated that encapsulation could increase the Antioxidant and antibacterial potential of herbal components in broiler chickens. The secondary metabolite content of *Medinilla javanensis* fruit, particularly flavonoids, had antibacterial properties that could reduce the population of pathogenic bacteria (Vifta et al., 2021). Masjid et al. (2020) found that nano-encapsulated *Melia azedarach Linn*. leaf extract increased the population of beneficial microbes, specifically LAB, in the intestines of broiler chickens. Increased LAB population leads to an increase in bacterial metabolites such as organic acids, which can lower the pH of the small intestine. The acidic conditions in the intestine inhibit the growth of pathogenic bacteria such as *Escherichia coli* (Sugiharto, 2016). For the record, *Escherichia coli* grows best at an alkaline pH (Wilks and Slonczewski, 2007).

Protein is one of the most essential nutrients not only for the growth, but also for the immune system development and many other biological functions in broiler chickens. For this reason, protein digestibility becomes priority to be determined in most broiler trials. It was apparent in this current study that protein digestibility increased linearly as the level of encapsulated *Medinilla javanensis* fruit extract in feed was increased. It was apparent in this study that the control group (T0) showed the lowest protein digestibility. It was very possible that high temperatures (33-35°C) in broiler house during rearing may have a negative impact on digestive function by the intestine, thereby reducing protein digestibility in broiler chickens. This was corroborated by the higher population of *Escherichia coli* in the intestine of T0 group compared to that in the other treatment groups. According to Thuekeaw et al. (2022), high-temperature conditions can increase the population of *Escherichia coli* in the digestive tract so that it has a negative impact on digestive function in broiler chickens. The exact mechanism by which *Medinilla javanensis* extract increased protein digestibility in broiler chickens remains unknown. Yet, the potential of *Medinilla javanensis extract* to enhance the population of lactic acid bacteria can make the intestinal conditions more acidic, hence aiding in the digestion and absorption of protein in the intestine (Pearlin et al., 2020). Herbal products have generally been shown to improve the intestinal morphology (increased villi height and ratio of villi height to crypt depth) of broiler chicken intestines. Such improvement may also, therefore, improves the digestion and absorption of nutrients, particularly protein, by broiler chickens (Sugiharto, 2021).

MDA levels in broiler serum decreased linearly with the addition of encapsulated *Medinilla javanensis* fruit extract to feed. The findings supported the findings of Sa'adah et al. (2019), who discovered that the supplement *Medinilla javanensis* fruit extract 1000 mg/kg body weight reduced MDA levels in rat serum. Malondialdehyde (MDA) is a by product of free radical lipid peroxidation. It is a compound that can demonstrate free radical activity in cells, so that if MDA levels are high, free radicals cause oxidative stress (Irianti et al., 2021). The activity of the active components in the *Medinilla javanensis* fruit extract, particularly the flavonoids, was thought to be responsible for the reduction in MDA levels in broiler serum in this experiment. Iskender et al. (2016) agreed, reporting that active ingredients in herbal products such as flavonoids (heperidin, naringin, and quercetin) could reduce lipid peroxidation, as evidenced by a decrease in MDA levels in serum.

The level of superoxide dismutase (SOD) in broiler serum increased linearly as the amount of encapsulated *Medinilla javanensis* fruit extract in feed increased. Superoxide dismutase (SOD) is a defense system enzyme that consists of copper zinc-superoxide dismutase (Cu, Zn-SOD) that catalyzes superoxide anion free radicals into molecular oxygen and hydrogen peroxide and manganese superoxide dismutase (Mn-SOD) that acts as the main Antioxidant in inhibiting superoxide work in mitochondria (Irianti et al., 2021). The increase in SOD levels in the serum of chickens given encapsulated *Medinilla javanensis* fruit extract is thought to be due to the activity of phenolic compounds, particularly flavonoids, which play a role in reducing free radicals and increasing SOD levels. The results agreed with the findings of Luo et al. (2014), who discovered that supplementing flavonoids at a dose of 100 mg/kg body weight can improve SOD levels in rat serum. Mavrommatis et al. (2021) found that supplementing grape dregs extract 0.2% of feed rations to broiler chickens increased SOD mRNA levels in chicken livers.

Apart from its positive role in the growth and health of broiler chickens, herbal products usually contain antinutrient components which can pose a negative impact on broiler chickens. One of the antinutritive components in encapsulated *Medinilla javanensis* is tannin. In this study, the content of tannin (contributed from the encapsulated *Medinilla javanensis* extract) in the treated feeds was around 0.0018%, which was considered as safe for broiler chickens. Indeed, Hidayat et al. (2021) suggested that the safe limit for using tannins in broiler feeds is 0.56%. With regard to the cost of feeds used in this study, supplementation of encapsulated *Medinilla javanensis* extract resulted in increased feed cost for broiler chickens. The feed cost for T0 was 7,525.72 IDR/kg, T1 9,438.99 IDR/kg, T2 11,352.25 IDR/kg, T3 13,265.52 IDR/kg, and T4 15,178.78 IDR/kg. Feed cost per kg live body weight for T0 was 12,342.18 IDR, T1 15,479.94 IDR, T2 18,163.60 IDR, T3 20,561.55 IDR, and T4 22,768.17 IDR. Hence, although the dietary supplementation of encapsulated *Medinilla javanensis* extract improve growth performance, yet its application need to be considered with caution as it may increase the cost of broiler production. Further research is needed to reduce the cost of production of encapsulated *Medinilla javanensis* extract so that its application for broiler chickens would be economically feasible.

CONCLUSIONS

Using encapsulated *Medinilla javanensis* fruit extract in feed improves growth rate, FCR, protein digestibility, Antioxidant status, and bacterial population in the broiler chickens' intestines. Encapsulated *Medinilla javanensis* fruit extract at a level of 600 mg/kg is recommended for application in feed.

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AUTHOR CONTRIBUTIONS

Vitus Dwi Yunianto Budi Ismadi, Lilik Krismiyanto, and Sugiharto Sugiharto contributed to the design of the study and manuscript preparation. Achmad Muzakky Dityana performed the experiments and contributed significantly to the analysis and manuscript preparation.

CONFLICT OF INTEREST

This research did not exist any conflict of interest.

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