

Characteristics of cecal and short-chain fatty acid production in broiler chickens fed diets with encapsulated *Medinilla speciosa* fruit extract

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ABSTRACT

This research investigated the impact of encapsulated *Medinilla speciosa* fruit extract on the cecal profile and the production of short-chain fatty acids in broiler chickens. The experiment involved 240 unsexed Ross strain broiler chickens, each 8 days old, with an average weight of 203.12 ± 2.03 g. The diets for both the starter and finisher phases were composed of yellow corn, rice bran, soybean meal, fish meal, limestone, premix, lysine, and methionine. This study used a completely randomized design with six treatments and four replicates; each unit had 10 birds. Treatments included T0 (basal diet without *Medinilla speciosa* fruit extract (MSFE) or encapsulated *Medinilla speciosa* fruit extract (EMSFE)), T1 (basal diet+0.08% MSFE), T2 (basal diet+0.02% EMSFE), T3 (basal diet+0.04% EMSFE), T4 (basal diet+0.06% EMSFE), and T5 (basal diet+0.08% EMSFE). The measured parameters included total lactic acid bacteria, *Escherichia coli*, pH, total acid, cecal length, relative weight, and SCFA production (acetate, propionate, and butyrate). Data were analyzed using analysis of variance at a 5% significance level. Duncan's test was conducted at the 5% significance level when the treatment effects were significant ($p < 0.05$). The results showed that EMSFE addition had significant effects ($p < 0.05$) on total lactic acid bacteria, *Escherichia coli*, total acid pH, and butyric acid, but not on cecal length, relative weight, acetate, and propionate. In conclusion, adding 0.04-0.08% EMSFE increased total lactic acid bacteria and butyric acid production and decreased *Escherichia coli*, pH, and total acid, while cecal length, relative weight, acetate, and propionate remained unchanged.

Introduction

The cecal is an important part of the broiler's digestive system of broilers, it is located between the small intestine and large intestine. Anatomically, the cecum of broiler chickens has a pouch-like shape and functions as a place to ferment the remaining digestive products that are not fully digested in the small intestine. Vanderghinste *et al.* (2025) stated that the cecum of broiler chickens has a length ranging from to 5-10 cm and sufficient capacity to accommodate ration nutrient material, thus allowing further digestion through bacterial activity. The cecum is important because it increases the absorption of available nutrients. Research by Dauksiene *et al.* (2021) showed that fermentation in the cecum can produce short-chain fatty acids that contribute to the provision of energy for chickens. Short-chain fatty acids, such as acetate, propionate, and butyrate, are the end products of fiber fermentation that can be absorbed by intestinal cells.

The presence of bacteria in the cecum contributes to the digestive processes. Beneficial bacteria can break down complex compounds that cannot be digested by the chicken's digestive enzymes. Healthy digestive tract conditions can support the activities of beneficial bacterial populations. Secondary metabolite compounds sourced from plants, fruits, and roots can improve body health. The herbal fruit used for the experiment in this study was sourced from *Medinilla speciosa* fruit. *Medinilla speciosa* fruit has an IC_{50} antioxidant activity of 35.46 ppm (very strong) (Surya and Luhurningtyas, 2021), total phenols of 21.67 μ g GAE/g and flavonoids of 9.21 μ g QE/g (Damayanti *et al.*, 2023). Bioactive levels in the *Medinilla speciosa* fruit can control free radicals and inhibit pathogenic bacteria. *Medinilla speciosa* fruit is presented in ration in the form of encapsulated extracts. Encapsulants serve as a dressing materials that protect the active compounds in the material so as not to experience a decrease in quality and quantity (Raza *et al.*, 2020).

The coating material used in this study was maltodextrin. Encapsula-

tion is the process of coating a material so that the content of bioactive substances in the material can be protected, absorbed and work optimally in the intestine (Donsi *et al.*, 2019). Maltodextrin is widely used in the encapsulation process because it has the ability to protect phenolic compounds against oxidation and digestive processes in the stomach (Lima *et al.*, 2022). Encapsulated active compounds can be hydrolyzed by carbohydrate enzymes in the small intestine so that the bioactive compounds in the extract can be water-soluble and acidic. Lactic acid bacteria live well at acidic pH, thus increasing nutrient absorption. Lactic acid bacteria can produce lactic acid and bacteriocins that can suppress the growth of pathogenic bacteria in competition for attachment to the intestinal mucosa. Anjana and Tiwari (2022) stated that lactic acid bacteria can suppress the number of pathogenic bacteria by forming colonies to maintain the balance of beneficial bacteria in the gut and improve body health. Healthy digestive tract conditions until the end of the intestine, especially the cecal part, can maintain the beneficial bacteria in the cecum. The generation of lactic acid and short chain fatty acids (SCFA) can significantly influence the gut and cecum, as indicated by the pH shift, which typically remains low (6-7) or below neutral. The balance of bacteria in the cecum can increase the activity of LAB in utilizing the substrate, thus providing a response to the development of size (length and weight) to be maximized.

The novelty of encapsulation of *Medinilla speciosa* fruit extract can be utilized to target the digestive organs of the cecal, and the addition of *Medinilla speciosa* fruit extract encapsulation to the ration can increase the LAB population and cecal size and reduce the *E. coli* population, lactic acid production, and SCFA in broiler chickens. This study examined the effect of encapsulated *Medinilla speciosa* fruit extract on the cecal profile and the production of short-chain fatty acids in broiler chickens.

Materials and methods

Ethical approval

This study followed a standard procedure certified by the Ethical Board of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia, under certification number 60-08/A-16/KEP-FPP.

Time, livestock and equipment

The research was conducted from June to July 2024 in digestion cages, Animal Nutrition Science Laboratory, Faculty of Animal Husbandry and Agriculture, Universitas Diponegoro, Indonesia. Total LAB and *E. coli* were analyzed at the Nutrition Laboratory, Muhammadiyah University Semarang, Indonesia. SCFA analyses were conducted at the Food and Agricultural Products Technology Laboratory, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia. Rations were analyzed proximate to the Animal Nutrition Science Laboratory, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Indonesia.

The experimental livestock used Ross strain unsexed broiler chickens aged 8 days as many as 240 birds with an average body weight of 203.12 ± 2.03 g. 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 2,993.21 kcal/kg and crude protein of 21.52%, while finisher (22–35 days) with metabolic energy content of 3,014.6 kcal/kg and crude protein of 19.56%. Purplish colored (ripe) *Medinilla speciosa* fruit was obtained from farmers on the slopes of Mount Muria, Kudus Regency, Central Java.

The materials used for extraction and encapsulation included *Medinilla speciosa* fruit extract, distilled water, 96% Merck ethanol, Sigma maltodextrin and Whatman 41 Cytiva filter paper. The equipment used for extraction and encapsulation included a sonicator, 2000 ml beaker glass, 500 ml measuring cup, digital balance with 0.1 g accuracy, stirring rod, funnel, and freeze dryer. The rearing equipment included the main cage, pens, brooder, feed and drink containers, thermohygrometer, and digital scales with 0.1 an accuracy.

Extraction and encapsulation procedure

Medinilla speciosa fruit encapsulation began with extraction based on the modified method of Gouda *et al.* (2021). The fruit was dried in an oven at 50°C until dry and pulverized into flour. *Medinilla speciosa* fruit flour was dissolved using 96% ethanol in a ratio of 1:10 (w/v), stirred until homogeneous and filtered using Whatman 41 filter paper. The filtered solution was sonicated at 37°C and room temperature at a wavelength of 50 Hz for 30 min. Sonification was performed at 45°C to evaporate the ethanol to obtain a thick extract. The next process was encapsulation based on the modified method of Agusetyaningsih *et al.* (2022) was modified, starting with the preparation of a mixture of maltodextrin and distilled water in a ratio of 3:1 (w/v). The evaporation results were mixed with the dressing solution in a ratio of 1:3 (v/v) and then dried using the freeze-drying method at -50°C until a dry form was obtained. The encapsulation products were pulverized using a mortar and pestle.

Research design and treatments

The research employed a completely randomized design, featuring six different treatments and four repetitions, leading to a total of 24 experimental units. Each experimental unit was filled with 10 animals. The treatments given to the ratio included the following:

T0= Basal Diet/BD

T1= BD + 0.08% *Medinilla speciosa* fruit extract

T2= BD + 0.02% encapsulated *Medinilla speciosa* fruit extract

T3= BD + 0.04% encapsulated *Medinilla speciosa* fruit extract

T4= BD + 0.06% encapsulated *Medinilla speciosa* fruit extract

T5= BD + 0.08% encapsulated *Medinilla speciosa* fruit extract

Chicken rearing

Two hundred forty chickens were raised at 8 days old with an average body weight of 203.12 ± 2.03 g. The ration used at the age of 1–7 days was a commercial ration, and the age of 8–35 days used the research ration plus *Medinilla speciosa* fruit encapsulation. Feeding of the treatment rations began at the age of 8 days, and drinking water was provided ad libitum.

Parameter measurement

Data were measured at 35 days of age, including total LAB, *E. coli*, pH, total lactic acid, SCFA, cecal length, and weight. One animal per replicate was randomly selected based on the average body weight for dissection and collection of the digestive tract, especially the cecal. The cecal was removed from the digesta, and the contents were placed in a sterile tube. The cecal was weighed to obtain the weight (g), and its length (cm) was measured using a measuring tape. Digesta content was measured using a pH meter. Digesta was analyzed for total LAB using the total plate count method according to Manalu *et al.* (2020) through a 1 g sample added 9 ml of diluent solution (0.9% physiological NaCl) and homogenized (as dilution 1). Multilevel dilutions were performed up to 7. Samples (0.1 ml from the last three dilution series were collected and cultured in 1% CaCO₃-supplemented MRSA media using the spread plate method. The cultured samples were incubated for 48 hours at 37°C. Colonies were counted using the total plate count method. *E. coli* measurements were performed according to the Association of Official Analytical Collaboration (2006), with modifications. Digesta samples (1 ml of digesta sample were collected and 5 ml of tryptic soy broth (TSB) was added and incubated at 37°C for 24 h). The next stage was subculturing on MacConkey agar (MCA), and eosin methylene blue agar (EMBA) was incubated at 37°C for 24 h. Pink, round, concave colonies on MCA and metallic colonies on EMBA were Gram stained and tested for total *E. coli* using the total plate count method. The total BAL and *E. coli* data were processed using Log 10 transformation before statistical analysis.

The Campbell-Platt (2009) method was employed to conduct total acid testing, with the titration results expressed as a percentage of lactic acid. A 10 ml sample of digesta was thawed and placed in an Erlenmeyer flask, followed by the addition of 2–3 drops of phenolphthalein (PP) indicator. The sample was then titrated with 0.1 N NaOH until a stable pink color was achieved, indicating the endpoint. The total acid content was calculated using the following formula:

$$\text{Acid Total} = (V1 \times N \times B) / (V2 \times 1000) \times 100\%$$

The SCFA test was performed as described by Suryono and Wikan-dari (2019), in which 5 ml of digesta was centrifuged within 10 minutes at 8500 rpm. The supernatant was filtered using 45 µL of a millex. The filtrate was taken 20 µl were injected into the HPLC system. The standard solutions were acetic acid, propionic acid, butyric acid, and lactic acid at concentrations of 500, 1000, and 2500 ppm. The concentration of SCFA was determined after the retention time of each standard was compared with the retention time of the sample.

Statistical analysis

Data were analyzed for variance at the 5% significance level, and if the treatment had a significant effect, Duncan's test was conducted at the 5% significance level to determine differences between treatments (Gaspersz, 2006).

Results

Bacterial population and cecal pH

The data for total LAB, pH, and coliforms are listed in Table 1. The addition of *Medinilla speciosa* fruit extract to the diet had a significant effect ($p < 0.05$) on total LAB, pH, and coliforms in broiler chickens. Total LAB counts in T1, T3, T4, and T5 were significantly ($p < 0.05$) higher than those at T0 and T2. The pH and total acid results showed that T5 was significantly lower ($p < 0.05$) than that of the other treatments. Total coliforms in T4 and T5 were not significantly different ($p > 0.05$) from those in T3 but were significantly different ($p < 0.05$) from those in T0, T1, and T2.

SCFA production, length and relative cecal weight

Data on SCFA production (acetate, propionate, and butyrate), length, and relative weight of the ileum are listed in Table 2. The addition of *Medinilla speciosa* fruit extract to the diet had a significant effect ($p < 0.05$) on butyric acid and total SCFA, but no significant effect ($p > 0.05$) on acetate, propionate, length, and relative weight of the ileum. Butyric acid results showed that the production in T1, T3, T4, and T5 was significantly higher ($p < 0.05$) than that in T0 and T2. Butyric acid production supported by high total SCFA in T1, T4, and T5 was significantly ($p < 0.05$) higher than that at T0, T2, and T3.

Discussion

The total LAB of the cecal in T1, T3, T4, and T5 were higher than those at T0 and T2 owing to the increase in additive concentration. The treatment of the extract form in T1 at a high concentration gave the same response as T3, T4, and T5, indicating that the bioactive action of *Medinilla speciosa* fruit can be utilized until the cecal. Bioactives contained in *Medinilla speciosa* fruit include total phenols, flavonoids, tannins, and saponins and have high antioxidant activity. Flavonoids possess potent antioxidant capabilities that mitigate oxidative stress in the digestive system, thus safeguarding intestinal cells from harm (Nagar *et al.*, 2020). Flavonoids have been found to modify the composition of gut bacteria, promoting the growth of beneficial bacteria like *Lactobacillus* sp., while suppressing harmful pathogens such as *Clostridium perfringens* (Buiatte *et al.*, 2024).

This condition was reinforced at T5, where the pH and total acid con-

tent of the cecal were higher than those of the other treatments. A high total LAB count creates an acidic environment, thus increasing the thickness of the cecal digesta. Phenolic compounds with a positive hydrogen charge, especially flavonoids, have the ability to hinder the proliferation of harmful bacteria. These flavonoids can modify the permeability of bacterial cell membranes, resulting in cell rupture and subsequent cell death (Rodríguez *et al.*, 2023). Flavonoids alter the membrane permeability and potential, leading to cell lysis. Specifically, it causes depolarization of the bacterial membrane, which disrupts membrane function, making it permeable to ions and other molecules that should not pass freely (Kumar *et al.*, 2024). The total coliform results at T4 and T5 showed a higher number of colonies than at T0 and T2. Flavonoids from the phenolic group can damage the cell membrane of pathogenic bacteria. Flavonoids prevent bacteria from producing the energy needed to perform vital functions, effectively inhibiting the growth and body functions of pathogens.

The extract in T1 gave the same response as T3, T4 and T5, because the extract level of 0.08% was the same as T5, but the presentation process was different. Encapsulation of the extract is given with the aim that the material is utilized up to the cecal; it is likely that the extract in T1 has a usable amount in the cecal. Encapsulation increases the stability and bioavailability of the sensitive compounds. Flavonoids are powerful antioxidants and carotenoids degrade rapidly in the presence of light and oxygen. Techniques like high-pressure homogenization and dispersion enhance both the physical stability and the absorption rate within the digestive system (Perry and McClements, 2020). Bioactives maintain their viability and function until they reach a certain location in the digestive system, where their effects are needed. Fani and Singh (2021) stated that Biopolymer systems remain effective as they pass through the gastrointestinal tract and are released in the gut, where they offer health advantages.

Butyric acid production in T1, T3, T4, and T5 was significantly ($p < 0.05$) higher than that in T0 and T2, which could be supported by the total cecal LAB (Table 1). Butyric acid plays an important role in shaping the lactic acid bacterial population in the chicken cecal, especially by improving gut health and beneficial bacterial populations. Butyric acid functions as the primary energetic substrate for colonization and promotes the proliferation of advantageous gut microbiota, such as lactic acid bacteria, which results in enhanced nutrient assimilation and the overall well-being of poultry (Molnár *et al.*, 2020). Butyric acid enhances the integrity of the intestinal barrier, mitigates inflammatory responses, and promotes

Table 1. The selected caecal bacterial population, pH and acid total of cecal broiler chickens fed with encapsulated *Medinilla speciosa* fruits extract.

Parameter	Treatments						P-value
	T0	T1	T2	T3	T4	T5	
LAB ¹	4.82±0.17 ^b	5.76±0.27 ^a	5.01±0.34 ^b	5.55±0.57 ^a	5.80±0.31 ^a	6.04±0.20 ^a	0
<i>E. coli</i> ¹	3.44±0.50 ^a	2.63±0.24 ^{bc}	3.05±0.41 ^{ab}	2.73±0.20 ^{bc}	2.44±0.19 ^c	2.24±0.21 ^c	0.00
pH	6.99±0.10 ^a	6.77±0.04 ^b	6.80±0.06 ^b	6.79±0.05 ^b	6.71±0.02 ^b	6.47±0.10 ^c	0
Acid total ²	0.14±0.01 ^c	0.32±0.01 ^c	0.26±0.03 ^d	0.34±0.04 ^c	0.42±0.04 ^b	0.47±0.04 ^a	0

¹Log CFU/g, ²%, T0: Basal Diet/BD, T1: BD+MSFE 0.08%, T2: BD+EMSFE 0.02%, T3: BD+EMSFE 0.04%, T4: BD+EMSFE 0.06%, T5: BD+EMSFE 0.08%, Mean±SEM, LAB: lactic acid bacteria, CFU: colony forming unit, MSFE: *Medinilla speciosa* fruit extract, EMSFE: Encapsulated *Medinilla speciosa* fruit extract.

Table 2. Short chain fatty acids (SCFA) production, relative length dan weight of cecal on broiler chickens fed with encapsulated *Medinilla speciosa* fruits extract.

Parameter	Treatments						P-value
	T0	T1	T2	T3	T4	T5	
Acetate ¹	36.43±0.41	38.28±1.19	36.80±2.74	38.36±3.20	38.49±1.55	39.08±1.24	0.38
Butyrate ¹	3.00±0.12 ^b	5.71±0.32 ^a	3.62±0.23 ^b	5.16±1.50 ^a	5.66±0.58 ^a	6.34±1.25 ^a	0.00
Propionate ¹	9.77±0.96	11.3±1.29	11.06±1.67	11.39±0.84	11.46±1.90	11.44±0.59	0.44
Total SCFA ¹	44.19±1.30 ^c	50.27±1.92 ^a	46.45±3.18 ^{bc}	49.89±3.12 ^b	50.59±2.29 ^a	51.84±2.15 ^a	0.00
Length ²	7.75±0.38	8.25±0.27	8.73±0.78	8.75±0.41	8.90±0.10	9.18±0.85	0.07
Weight ³	0.23±0.006	0.27±0.029	0.27±0.033	0.3±0.036	0.31±0.026	0.32±0.027	0.13

¹μmol/g, ²cm/kg, ³%, T0: Basal Diet/BD, T1: BD+MSFE 0.08%, T2: BD+EMSFE 0.02%, T3: BD+EMSFE 0.04%, T4: BD+EMSFE 0.06%, T5: BD+EMSFE 0.08%, Mean±SEM, MSFE: *Medinilla speciosa* fruit extract, EMSFE: Encapsulated *Medinilla speciosa* fruit extract.

the synthesis of mucin along with antibacterial peptides (Onrust *et al.*, 2015). Flavonoids are polyphenolic compounds that are metabolized by lactic acid bacteria (LAB) in the cecal, leading to the production of beneficial metabolites, such as butyric acid, which plays an important role in maintaining gut health. Yang *et al.* (2023) stated that flavonoids undergo metabolic transformation by gut microbiota, which facilitates their conversion into low molecular weight phenolic acids, thereby enhancing their bioavailability. This phenomenon results in the establishment of a hypoxic cecal environment. Lactic acid bacteria synthesize butyric acid via specific metabolic pathways. Butyric acid constitutes a primary energy substrate for intestinal epithelial cells, consequently promoting the proliferation of lactic acid bacteria and improving overall gut health (Zhou *et al.*, 2023), including the cecal.

Flavonoids have different effects on SCFA production. Flavonoids can have specific effects on certain types of bacteria; therefore, not all bacteria producing acetic and propionic acids are affected by flavonoids. Huang *et al.* (2018) stated that flavonoids have the capacity to modulate specific metabolic pathways in bacterial organisms; consequently, the synthesis of butyric acid is subject to greater influence compared to the production of acetic and propionic acids. Acetic acid can be generated through the fermentation of carbohydrates by bacteria like Enterobacteriaceae, while propionic acid can be formed through the same process by bacteria such as Propionibacterium (Polansky *et al.*, 2016). The synthesis of acetic and propionic acids can be affected by several elements, such as the role of digestive enzymes like amylase and lipase. These enzymes are capable of breaking down simple sugars and fatty acids, which act as substrates for gut microbiota, ultimately resulting in the production of short-chain fatty acids (SCFAs). Increased activity of amylase and lipase can influence the concentrations of acetic and propionic acids.

The length and relative weight of the cecal showed similar results, which could be due to the production of acetic and propionic acids that were not different. The amount of acetate and propionate produced is only used as an energy source by beneficial bacteria. Fan *et al.* (2023) stated that the number of intestinal bacteria determines SCFA production. SCFA production was also affected in the LAB group; if acetate and propionate were not balanced with butyrate, it did not change the size of the cecal. Mátis *et al.* (2022) stated that SCFAs, especially butyrate, enhance the intestinal barrier's integrity and influence immune responses, which can impact the structure of the cecal. Maintaining a proper balance of SCFAs is crucial for enhancing gut health; an imbalance can lead to changes in bacterial composition, affect cecal size, and result in enteritis (Liu *et al.*, 2021).

Conclusion

The addition of *Medinilla speciosa* fruit extract encapsulation level 0.04-0.08% in the ration increased total lactic acid bacteria and butyric acid production and reduced *Escherichia coli*, pH, and total acid, although the length and relative weight of the cecal and the production of acetate and propionate showed similar results.

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Conflict of interest

The authors have no conflict of interest to declare.

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