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The Effect of Nanoencapsulated *Phaleria macrocarpa* Fruits Extract in Drinking Water on Jejunal Histomorphology of Broiler Chickens

N. Ningsih[a,b], B. Ariyadi[c], N. D. Dono[a], Supadmo[a], & Zuprizal[a,*]
[a]Department of Animal Feed Science, Faculty of Animal Science, Universitas Gadjah Mada
[b]Politeknik Negeri Jember
Jalan Mastrip PO BOX 164 Jember, Jawa Timur 68101, Indonesia
[c]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada
Jalan Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia
*Corresponding author: zuprizal@ugm.ac.id
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ABSTRACT

The purpose of this study was to investigate the characteristics of nanoencapsulated *Phaleria macrocarpa* fruits extract (NEPM) in drinking water and its effect on microbial population and histomorphology in the jejunal wall of broiler chickens. A total number of 200 male broiler chicks were distributed into 5 treatments with 4 replicates (10 birds in each replicate). The experimental treatments were control diet (T0; negative control), diet with tetracycline (T1; positive control), diet with 2.5% of *Phaleria macrocarpa* fruits extract (T2), diet with 2.5% NEPM (T3), and diet with 5.0% NEPM (T4). The diet was yellow corn and soybean meal that contains 20.44% CP, 2917.47 kcal/kg ME, 0.84% Ca, and 0.51% Pav. Variables evaluated were characteristics of NEPM, growth performance, intestinal microbial population (lactic acid bacteria (LAB) and *Salmonella* sp.), and intestinal histomorphology (villus height, crypt depth, and villus height to crypt depth ratio (VH : CD)). Data were analyzed using ANOVA in a completely randomized design. Orthogonal contrast test were used to separate mean of data when p-value differ significantly. Results showed that the size of NEPM was 778 nm with spherical shape and positive charges with the zeta potentials of +26.5 mV. Supplementing 5% of NEPM did not affect broiler growth performance, *Salmonella* sp. or crypt depth, but increased ($p<0.05$) LAB, villi height, and VH:CD. It can be concluded that 5.0% of NEPM in the drinking water had positive effect on the jejunal histomorphology and increased population of LAB while *Salmonella* sp. was not detected on all treatments.

Keywords: broiler chicken; jejunal bacteria; jejunal morphology; nanoencapsulation; Phaleria macrocarpa fruits

INTRODUCTION

The use of antibiotics as feed additive and growth promoter (AGP's) for animal has been prohibited by the Indonesian government regulated by the Ministry of Agriculture, Republic of Indonesia (PERMENTAN) in the number of 14/PERMENTAN/PK.350/5/2017. Antibiotic is an antimicrobial agent functioned to kill microorganism, primarily pathogenic microbes in the intestine. Antibiotic was fabricated from fungi or bacteria, and has activity to enhance immune system, optimize digestive system, and improve growth performance of broiler chicken, but the specification mode of action remains unclear (Mehdi *et al*., 2018). Intestinal microbiota plays an important role to promote healthy intestinal community, immunological, and improve nutrient digestion and absorption, thus improve animal growth performance. On the other hands, uncontrolled usage of antibiotics leads to accumulation of undesirable residues in the animal tissues and their products, and cause foodborne disease to human. Therefore, researcher has been intensively investigated natural substances for their potencies as AGP's replacer in the diet. Phytogenic feed additive becomes familiar due to its beneficial effect on broiler performance attributed to their bioactive compounds (Wati *et al*., 2015).

Phytobiotic, herbs or plants extract, is supplemented in animal feed to improve poultry production. There are two major mechanisms of phytobiotic in increasing animal production as antimicrobial agents and increasing nutrient absorption (Fallah *et al*., 2013). One of the Indonesian plants that have potency to be used as phytobiotics is *Phaleria macrocarpa*. This plant is well known as a medicinal plant and contains diverse bioactive compounds, such as phenolic, benzhophenone, terpenes, and alkaloid (Alara & Olalere, 2016). These compounds have antimicrobial activities with different mode of actions. The low efficacy of *P. macrocarpa* diets-supplementation is caused by the low solubility, fast degradable, low bioavailability, and thermolabile with the temperature of animal digestion organs. Nanoencapsulation technology is one of alternative solutions to overcome these problems.

Encapsulating the fruit extract beneficially conserves the bioactive substances in nanometer size and refers to bioactive packing. Nanoencapsulation has several benefits, such as preserve bioactive compounds of phytobiotic, increase the affectivity of bioactive distribution in the gut, increase the physical stability of the bioactive substances, and protect them from degradation in the gut (Donsì *et al*., 2011). Nanoencapsulation can be made with ionic gelation method using chitosan and sodium tripolyphosphate (STPP). Ionic gelation method is based on different charges between the negative charges STPP and positive charges of chitosan (Sundari *et al*., 2014). Chitosan is obtained from deacetylation of the naturally polysaccharide chitin, one of the most abundant biopolymers in nature. Chitosan is known as a natural, biodegradable, biocompatible, and bioadhesive polymer. STPP is polyanion that used as cross-link agent. The interaction of chitosan with STPP leads to the formation of biocompatible cross-linked chitosan nanoparticles, which can efficiently deliver bioactive of phytobiotics as antibacterial agent in broiler intestine.

Few investigations have been reported about *P. macrocarpa* as phytobiotics to replace antibiotics, whereas *P. macrocarpa* had wide variety of useful pharmacological activities, such as anti-microbial, anti-fungal, antioxidant activity, and its non-toxic activity (Alara *et al*., 2016). Moreover, combination of *P. macrocarpa* with nanoencapsulation technology as a phytobiotic for broiler chicken had never been studied before. The purpose of this study was to investigate the characteristics of nano-encapsulated *P. macrocarpa* fruits extract, and its effect of nanoencapsulation in drinking water administration on broiler growth performance, intestinal microbial population, and micro-morphology of jejunum cell wall of broilers chickens.

MATERIALS AND METHODS

Animal, Diets, and Experimental Design

The study was conducted at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. A total number of 200 male eight days old New Lohmann MB 202 broiler chickens were distributed into five treatments and four replicates with 10 birds in each replicate pen. Each pen was equipped with a feeder and a water trough. The basal diets were formulated to meet the nutrition recommendations of broiler chickens from NRC (1994), based on yellow corn and soybean meal, which contained 20.44% crude protein (CP), 2917.47 kcal/kg metabolizable energy (ME), 0.84% Calcium (Ca), and 0.51% available Phosphorus (Pav). Chemical compositions of the diets were analyzed according to AOAC (2005). Formulation and chemical compositions for the experimental diets are shown in Table 1. The diets and drinking water were supplied *ad libitum* from days 8 to 35 (Reyes *et al*., 2018). The initial growth (0-7 days) is a critical phase for intestinal development, at the moment the segments of the gastrointestinal tract and digestive organs increase in size and weight more rapidly in relation to body weight than the other organs and tissues. Therefore, in this age, all of broilers chickens were given the same treatment in order to have the same well-functioning of gastrointestinal tract before giving the treatments. The experimental treatments were consisted of basal diet (T0; negative control), basal diet with antibiotic tetracycline (T1; positive control), and basal diet with 2.5% of *P. macrocarpa* fruits extract (T2), 2.5% nanoencapsulation of *P. macrocarpa* (NEPM) (T3), and 5.0% NEPM (T4).

Nanoencapsulation Procedure

The nanoencapsulation of *P. macrocarpa* fruits extract was formulated by ionic gelation method using 2% of *P. macrocarpa* fruits extract, 0.625% chitosan, 0.75% sodium-tripolyphosphate (STPP) (w/v), and consisted of 0.5: 1.0: 0.02 of extract, chitosan, and STPP, respectively. Initially, the fruits were extracted by macerating *P. macrocarpa* meal with 96% ethanol (1:100 w/v) in 3 days. The filtrate was then filtered using Whatman paper number 1 and was evaporated using waterbath at a temperature of 60^{o}C to remove ethanol.

Table 1 . Ingredients and chemical composition of the basal diet

Ingredients	Proportion (%)	Chemical compositions						
		CP (%)	ME (kcal/kg)	Ca (%)	Pav (%)	Lys (%)	Met (%)	Thr (%)
Yellow corn	55.70	4.96	1881.10	0.01	0.13	0.16	0.10	0.20
Soybean meal	31.00	13.83	686.96	0.09	0.19	0.79	0.16	0.49
Meat bone meal	1.50	0.70	28.85	0.14	0.07	0.04	0.01	0.02
Rice brand	8.00	0.96	230.96	0.00	0.10	0.04	0.02	0.03
Palm oil	1.50	0.00	132.60	0.00	0.0	0.00	0.00	0.00
Vitamin premix*	0.30	0.00	0.00	0.49	0.02	0.00	0.00	0.00
L-lysine HCl (98%)	0.10	0.09	3.61	0.00	0.00	0.07	0.00	0.00
DL-methionine (99%)	0.20	0.12	7.22	0.00	0.00	0.00	0.20	0.00
$CaCO_3$	1.50	0.00	0.00	0.10	0.00	0.00	0.00	0.00
NaCl	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.00	20.66	2971.30	0.83	0.51	1.10	0.48	0.74

Note: CP: Crude protein; ME: Metabolizable Energy, Ca: Calcium; Pav: Available Phosphor; Lys: Lysine; Met: Methionine; Thr: Threonin
*Vitamin premix (Masamix-Bro) chemical composition was Vit. A: 12,500,000 IU; Vit D3: 2,500,000 IU; VitE: 10000 mg; VitK3: 2000 mg; Vit.B1: 2000 mg; Vit. B2: 4000 mg; Vit. B6: 1000 mg; Vit. B12: 12000 mcg; Vit.C: 40000 mg; Niacin: 40000 mg; Biotin: 200 mg.

Nanoencapsulation were produced by dissolving 2% of aqueous *P. macrocarpa* fruits extract with 0.625% of chitosan which has dissolved previously with acetic acids. Both 2% extract and 0.625% chitosan ware mixed using magnetic stirrer (C-MAG HS 7, IKA, Selangor, Malaysia) for 30 minutes, and then added with 0.75% aqueous STPP and mixed using stirrer for 30 minutes (Sundari *et al*., 2014).

Analysis of Characterization Nanoencapsulation

Particle size and potential zeta. Particle size and potential zeta of NEPM were determined by a Dynamic Light Scattering (DLS) method. Five mL of NEPM was analyzed for particle size with Particle Size Analyzer (PSA) (Horiba Scientiific SZ – 100, Horiba, Kyoto, Japan). Evaluation was done using scattering angle of 90°C with temperature holder of 24.8°C according to Liang *et al*. (2017).

Nano morphology. Morphological structure of NEPM was evaluated using Transmission Electron Microscopy (TEM) (JEOL JEM 1400 Plus, Jeol, Peabody, USA). The samples were prepared by dropping solutions into Copper grids coated with carbon using the auto carbon coated for 5 minutes, prior the samples drying. The samples were stayed in the copper network for 2–3 min. The samples were then immersed in 2% phosphotungstic acid stain and stained for 2–3 min. After natural drying, the samples were placed under TEM for observation according to Liang *et al*. (2017).

Broiler Growth Performance and Carcass Analysis

Feed consumption, feed conversion ratio (FCR), and water consumption were recorded daily. Body weight gain of broiler chicken was weighted weekly. At days 35, a chicken from each different replicates was randomly selected to slaughter according to Islamic law to measure carcass percentage.

Determinations of Microbial Populations

At the end of the 35 days rearing period, one bird per group with body weight closed to the median of each group was randomly selected for sample collection. Jejunal ingesta from the 4 birds were separately placed on agar plates for further analysis. These samples were used to enumerate the number of lactic acid bacteria and *Salmonella* sp. in the small intestine. Lactic acid bacteria (LAB) was cultured using de Man, Rogosa, and Sharpe agar or MRSA (Oxoid, Basingstoke, Hampshire, UK), while *Salmonella* sp. was cultured using *Salmonella shigella* Agar or SS (Oxoid, Basingstoke, Hampshire, UK).

Digesta samples with approximately 1 g were squeezed from jejunum into 9 mL peptone water solution. The solution was mixed with vortex. The suspension was prepared from dilutions 10^{-1} and serial dilutions were done (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}). One mL solution was removed from three dilutions and poured into petri dishes containing the medium. The total number of LAB and *Salmonella* sp. colony were counted after incubation in an 37°C anaerobic chamber for 48 hours. Enumeration of total bacteria was carried out manually using total plate count (TPC) method (Halimatunnisroh *et al*., 2017).

Morphology of Jejunum

At days 35, a chicken from each different replicates was slaughtered according to Islamic law. The jejunum sample was separated from the end duodenal loop up to 1 cm proximal to the Meckel's diverticulum. Sections of approximately 6 cm were taken from the mid-jejunum, gently flushed with physiologic NaCl, and cut into 3 equal pieces. The jejunal sections were immediately fixed into 10% formalin solution for further analyses. Histological examination was prepared initially by transferring the jejunal samples with a series of alcohol with increasing concentration (70%, 80%, 90%, and 100%), xylol solution, and followed by embedding in paraffin wax. Transverse and longitudinal sections with 4 μm in thickness were prepared using microtome, stained with Hematoxyline-Eosin, and examined under the electron transmission microscope (Optilab Advance, Miconos, Yogyakarta, Indonesia). The villus height (VH) was measured from the tip of the villus to the villus-crypt junction, while crypt depth (CD) was defined as the depth of the invagination between two villi (Ariyadi *et al*., 2014).

Statistical Analysis

Characteristic of nanoencapsulated *P. macrocarpa* fruits extract was reported descriptively for particle size, zeta potential, and morphology. Data of growth performance, microbial population, and morphology of jejunal intestine were expressed as mean ± STdev, and the number of jejunal microbes was presented in colony forming unit (CFU/g) of digesta and expressed in Log 10. The data were statistically analyzed by one-way ANOVA with completely randomized design, using Statistical Package for Social Science or SPSS (SPSS GmbH, Munich, Germany). Orthogonal contrast test was used to separate data with significance difference. All indication of significance was based on a probability of less than 5%.

RESULTS

Characteristic of Nano-encapsulation

Nanoencapsulation of *P. macrocarpa* fruits extract (NEPM) was successfully obtained by ionic gelation method with combination of positive charges from chitosan and negative charges from STPP. The NEPM has pH 3.31 and visible clear solution. Using DLS measurements, NEPM showed particle size distribution of 778 nm. Zeta potential of NEPM was positive charges with value of +26.5 mV. NEPM TEM image displayed a homogeneous distribution and stable formulation with spherical shape and coated with transparent layer from ionic gelation between chitosan and STPP. The image of NEPM morphology is showed in Figure 1.

Growth Performance of Broiler Chicken

The use of different levels of nanoencapsulation of *P. macrocarpa* fruits extract did not significantly affect feed consumption, feed conversion ratio, water consumption, and carcass percentage. The data are presented in Table 2.

Microbial Population of Jejunal Intestine of Broilers Chickens

The results for bacterial populations in the jejunal intestine are presented in Table 3. There was a significant effect ($p<0.05$) of NEPM supplementation on the population of Lactic acid bacteria (LAB). Orthogonal contrast test of LAB population was showed in Table 4. Orthogonal contrast examination showed that the use of tetracycline and supplementation of nanoparticle of *P. macrocarpa* fruit extract increased ($p<0.05$) the population of LAB in the gut of broiler chickens. The results indicated that there was no significant difference between the number of LAB in the gut of broiler chickens fed diets supplemented with nonoencapsulated and nanocapsulated extracts of *P. macrocarpa*. The population of *Salmonella* sp. was not detected in any treatments.

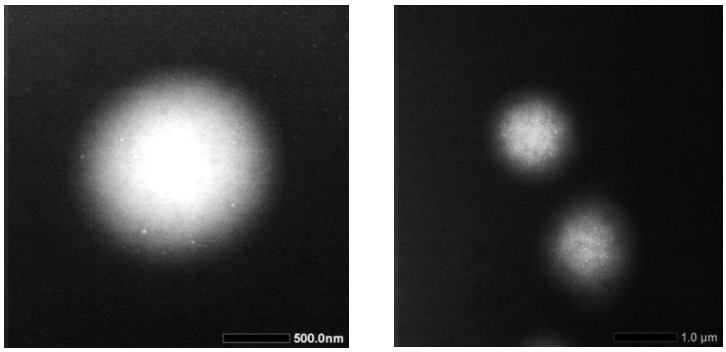
Morphology of Jejunal Intestine of Broilers Chickens

The effects of NEPM supplementation in the drinking water on histomorphology of the jejunal mucosa layer in broiler chickens are summarized in Table 5. The results showed that NEPM increased the villus height and villus height to crypt depth ratio ($p<0.01$), but did not affect crypt depth. Orthogonal contrast data of villus height are presented in Table 6 and VH:CD ratio are presented in Table 7. The data showed that antibiotic and NEPM supplementations increased villus height ($p<0.05$) and VH:CD ratio ($p<0.01$), compared with negative control diet.

DISCUSSION

Characteristics of Nanoencapsulation

The particle size of nanoencapsulation of *P. macrocarpa* was 773 nm and these results appropriate with



A B

Figure 1. Morphology of nano-encapsulation in 500 nm scale (A) and in 1000 nm scale (B)

Table 2. The performance (mean ± STdev) of broiler chickens fed with different percentages of nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM)

Variables	Treatments					p-value
	T0	T1	T2	T3	T4	
Feed consumption (g/bird/35 days)	2624.56 ± 72.73	2711.98 ± 44.79	2711.41 ± 42.47	2668.28 ± 34.23	2729.95 ± 68.26	0.092
Weight gain (g/bird/35 days)	1596.56 ± 97.58	1618.64 ± 84.48	1656.18 ± 42.73	1635.58 ± 27.33	1674.75 ± 30.30	0.471
Feed conversion ratio	1.61 ± 0.07	1.64 ± 0.06	1.60 ± 0.02	1.61 ± 0.02	1.61 ± 0.02	0.813
Carcass (%)	66.53 ± 2.95	67.60 ± 4.31	65.61 ± 2.85	66.47 ± 5.32	68.01 ± 0.15	0.881
Water consumptiom (L/bird/35 day)	7.91 ± 0.56	7.70 ± 0.32	7.54 ± 0.25	7.20 ± 0.68	7.52 ± 0.11	0.272

Note: T0: negative control without feed additive, T1: Positive control with antibiotic tetracycline, T2: 2.5% of *Phaleria Macrocarpa* fruits extract non-encapsulated, T3: 2.5% NEPM, T4: 5% NEPM.

Table 3. Microbial population of jenunal intestine (mean ± STdev) of broiler chickens fed with different percentage of nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM)

Species	Treatments					p-value
	T0	T1	T2	T3	T4	
BAL (Log cfu/g)	6.81 ± 0.64	7.15 ± 0.20	8.64 ± 0.25	8.80 ± 0.08	9.21 ± 0.45	<0.01
Salmonella sp.	No growth	No growth	No growth	No growth	No growth	-

Note: T0: negative control without feed additive, T1: Positive control with antibiotic tetracycline, T2: 2.5% of *Phaleria Macrocarpa* fruits extract non-encapsulated, T3: 2.5% NEPM, T4: 5% NEPM.

nanoparticle requirement, that should less than 1 mm. The height of nanoparticle size from this study attributed to the height of percentage between chitosan and STPP. The height of concentration between chitosan and STPP led to the increased particle size caused by agglomeration of chitosan. Several factors that influence the particles size of nanoparticles are the molecular weight of chitosan, the concentration of STPP, the weight ratio of TPP to chitosan, reaction temperature, and pH (Ningsih *et al*., 2017; Lee *at al*., 2016).

The TEM image revealed that NEPM had spherical shape. The present research is in agreement with Alves *et al.* (2016) who previously reported spherical appearance of nanoencapsulation of gallic acid. In addition, Blaiszik *et al*. (2008) also stated that nanoencapsulation with self-healing material produced spherical shape, closed to monodisperse in capsule diameter, and smooth non-porous shell wall.

Chitosan-tripolyphosphate nanoparticles mixed with each other led spontaneously formed compact complexes with an overall positive surface charge. Ionization process from amino group of chitosan stimulate to positive zeta potential. Positive zeta potential might ease nanoparticle to interact with mucus that has negative charges, and makes the drug delivery more effective, thus improve the availability of the drug in the tissue. Positive zeta potential showed a stronger electrostatic interaction with mucus or with mucosa surface. Zeta potential of >25 mV showed the stable formulation and had high affinity on the cell membrane (Motiei *et al*., 2017; Honary & Zahir, 2012). In the present experiment, positive zeta potential of the NEPM was +26,5 mV, indicated a stable formulation.

Table 4. Orthogonal contrast of lactic acid bacteria population in jenunal intestine of broiler chickens fed with different percentages of nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM)

Treatments	LAB (Log cfu/g)
T0	6.81 ± 0.64
T1	7.15 ± 0.20
T2	8.64 ± 0.25
T3	8.80 ± 0.08
T4	9.21 ± 0.45
p-values for main effect of diets	<0.01
p-values for contrast	
T0 vs T1, T2, T3, T4	<0.01
T1 vs T2, T3, T4	<0.01
T2 vs T3, T4	0.143

Note: LAB: Lactic acid bacteria, T0: negative control without feed additive, T1: Positive control with antibiotic tetracycline, T2: 2.5% of *Phaleria macrocarpa* fruits extract non-encapsulated, T3: 2.5% NEPM, T4: 5.0% NEPM

Broiler Growth Performance

There was no significant difference in growth performance of broiler chicken. Our finding agreed

Table 5. Morphology of jejunal intestine (mean ± STdev) of broiler chickens fed with different percentages of nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM)

Item	Treatments					p-value
	T0	T1	T2	T3	T4	
Villus height (μm)	550.04 ± 60.10	607.99 ± 126.77	782.82 ± 104.47	1173.77 ± 179.65	1428.90 ± 176.46	<0.01
Crypt depth (μm)	121.52 ± 9.07	98.70 ± 27.01	128.09 ± 10.41	102.60 ± 31.87	123.71 ± 18.50	0.24
V:C ratio	4.52 ± 0.19	6.25 ± 0.56	6.10 ± 0.44	12.45 ± 4.36	11.66 ± 1.44	<0.01

Note: V:C Ratio= Villus height : Crypts depth ratio. T0: negative control without feed additive, T1: Positive control with antibiotic tetracycline, T2: 2.5% of *Phaleria Macrocarpa* fruits extract non-encapsulated, T3: 2.5% NEPM, T4: 5% NEPM.

Table 6. Orthogonal contrast of villus height jejunal intestine of broiler chickens given nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM)

Treatments	Villus height (μm)
T0	550.04 ± 60.10
T1	607.99 ± 126.77
T2	782.82 ± 104.47
T3	1173.77 ± 179.65
T4	1428.90 ± 176.46
p-values for main effect of diets	<0.01
p-values for contrast	
T0 vs T1, T2, T3, T4	<0.01
T1 vs T2, T3, T4	<0.01
T2 vs T3, T4	0.054

Note: T0: negative control without feed additive, T1: Positive control with antibiotic tetracycline, T2: 2.5% of *Phaleria macrocarpa* fruits extract non-encapsulated, T3: 2.5% NEPM, T4: 5.0% NEPM

Table 7. Orthogonal contrast of villus height to crypt depth ratio jejunal intestine of broiler chickens given nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM)

Treatments	Villus height to crypt depth ratio
T0	4.52 ± 0.19
T1	6.25 ± 0.56
T2	6.10 ± 0.44
T3	12.45 ± 4.36
T4	11.66 ± 1.44
p-values for main effect of diets	<0.01
p-values for contrast	
T0 vs T1, T2, T3, T4	0.004
T1 vs T2, T3, T4	0.166
T2 vs T3, T4	0.053

Note: T0: negative control without feed additive, T1: Positive control with antibiotic tetracycline, T2: 2.5% of *Phaleria macrocarpa* fruits extract non-encapsulated, T3: 2.5% NEPM, T4: 5.0% NEPM

with research by Sundari *et al*. (2014) who observed that feed consumption, feed conversion, and carcass weight was not affected by nanoencapsulation of turmeric extract. The possible reason may be attributed to the characteristic of nanoencapsulation of *P. macrocarpa* fruits extract. Bunglavan *et al*. (2014) found that several factors that influence the activity of nano-encapsulation in the digestive tract are physicochemical characteristics such as solubility charge and size. Increasing the particle size of nanoencapsulation up to 778 nm has an impact on particle distribution on the intestinal mucosa of broiler chicken, the distribution more slowly and less effective than small particle size. Parera *et al*. (2015) and Katouzian & Jafari (2016) reported that nanoparticle with <500 nm shows the best rapidity flow in the intestinal mucosa. Higher particle size means the compound will be more slowly distributed and release (50 > 200 > 500). There are very limited findings about phytobiotic *P. macrocarpa* to support this result. Therefore, further research is needed to investigate the effects of nanoencapsulation of *P. macrocarpa* with better characteristics.

Microbial Populations

Increasing LAB populations due to NEPM supplementation was similar with that reported by Natsir *et al.* (2013) who found that supplementation of phytobiotic, encapsulated or non-encapsulated, increased the number of LAB in intestine of Lohmann broiler chickens. Similarly, Ürüşan & Bölükbaşı (2017) showed that supplementation of non-encapsulated turmeric powder increased the colony of BAL in the gut of Ross 308 commercial line broiler chickens.

P. macrocarpa contains variety of bioactive compounds such us phenolic and garlic acids that can speed up the growth of LAB and control the growth of pathogenic bacteria. This action might be closely related to the reduction of intestinal pH. Organic acids are able to kill pathogenic bacteria through the interruption of the lipid membrane formation on the bacterial cell and increase the plasma acidity. This condition promote bacteria that intolerant with the low pH cannot adapt with the more acid environment (Fascina *et al*., 2012). Alara & Olalere (2016) reported that phenolic compounds of eight berries inhibited the growth of selected gram-negative bacteria and were not active against gram positive LAB. Increasing LAB population in broiler chicken indicated the better gastrointestinal conditions, because LAB potentially improved nutrient digestibility especially with cellulose content (Herdian *et al*., 2018).

In the current study, *Salmonella* sp. was not found in all treatments. It was similar with Choiri *et al.* (2017) that reported no population of *Salmonella* sp. on the intestinal mucose of laying hens that received nanoencapsulated noni extract in drinking water. Result in this study might be related with the used of coarse litter. The coarse wood components in litter may play a significant role in reducing the colonization and population of *Salmonella* sp by providing antimicrobial agents against exclusion microorganisms or by giving stimulation of the gizzard and proventriculus (Santos *et al*., 2008).

Morphology of Jejunal Intestine

The effects of NEPM supplementation in drinking water on the jejunal morphology of broiler chicken contributed in improving villus height and VH:CD ratio. Both antibiotics and phytobiotic have the ability to stop the growth and even kill pathogenic microbes, reduce the number of toxins in the intestine, lower the secretion of the intestinal barrier, and trigger the growth of villus on the wall of the intestine (Awad *et al*., 2012). Villus height and crypt depth exhibit the intestinal health, as well as nutrient digestion and absorption. Longer villi and shorter crypt leads to the higher performance that associated with the absence of microbial toxins. A study by Rahman et al. (2017) showed that supplementation of onion (*Allium cepa L.*) extract/powder modified the gut microbiota, increased villus health, increased nutrient digestibility and absorption. Iriyanti *et al*. (2018) also reported that the small intestine functions as feed digestion and nutrients absorptions that were influenced by the epithelial intestine and the conditions and amount of microvilli.

Nanoencapsulated trial showed higher CH:CD ratio than nonencapsulated group as this results may be due to nanoencapsulated can defend the bioactive compounds of *P. macrocarpa* than the bioactive can efficiently distribute and release in animal gut as antibacterial activity. Donsi *et al*. (2011) reported that nanocapsules of essential oils were able to delay the microbial growth or completely inactivate the microorganisms and can increase the growth of intestinal morphology. On the other hand, supplementation of NEPM was no significant effect on crypt depth. This result similarly with Chori *et al*. (2017) reporting that no significant effect on crypt depth of laying hens with supplementation of nanoencapsulation of noni extract.

CONCLUSION

In conclusion, the present study discovered that NEPM could be beneficially used as an alternative for antibiotics in the diets of broiler chickens. NEPM can significantly enhance the population of the lactic acid bacteria, the growth and proliferation of absorptive cell on the jejunum of broiler chickens, and the population of *Salmonella sp* was not found in all treatments.

CONFLICT OF INTEREST

The researchers state that no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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