Buletin Peternakan 44 (1): 1-5, February 2020

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v44i1.44958

The Effect of Nano-Encapsulation *Phaleria macrocarpa* Fruits Extract in Drinking Water on the Digestive Tract and Carcass Characteristic of Broiler Chickens

Zuprizal[1], Niati Ningsih[2]*, and Taufik Ahmad Zulfian[1]

[1]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Department of Animal Science, Politeknik Negeri Jember, Jember, 68121, Indonesia

Article history
Submitted: 11 April 2019
Accepted: 2 January 2020

* Corresponding author:
Telp. +62 817-462-542
E-mail: niatiningsih@polije.ac.id

ABSTRACT

The present study was to evaluate the effects of nano-encapsulated *Phaleria macrocarpa* fruit extract (NEPM) in drinking water on the digestive organs and carcass characteristic of broiler chickens. A total number of two hundred male broiler chicks were randomly divided into five treatments with four replicates, each replicate contained ten chickens. Broiler chickens have received a basal diet supplemented with or without experimental treatments. Five 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 diets were based on yellow corn and soybean meal and contained 20.44% crude protein, 2,917.47 kcal/kg metabolizable energy, 0.84% Calcium, and 0.51% available Phosphorus. On the 35th day, one bird from each replicate pen was slaughtered. Carcass characteristics and weights of digestive organs were measured. Data were statistically analyzed using ANOVA in a completely randomized design. The orthogonal contrast test was used to separate the mean when the P-value was less than 5%. Results showed that the use of nano-encapsulated *Phaleria macrocarpa* fruits extracts up to 5.0% in the diet did not give any significant effect on the carcass, digestive organs, heart and liver weights of broiler chickens. It can be concluded that the supplementation of *Phaleria macrocarpa* fruits extracts whether nano-encapsulated or not, did not affect carcass weight, weights of digestive organs and weights of the heart and liver of broiler chickens.

Keywords: Broiler chicken, Carcass weight, Digestive tract weight, Nano-encapsulation, *Phaleria macrocarpa* fruits

Introduction

One of growth promoter alternatives to replace antibiotics is phytobiotics. Phytobiotic is the natural product originating from herb and plant extract which have pharmacological effects and widely used as natural growth promoters for broiler chicken. The main mechanism of phytobiotics as a feed additive to replaces antibiotics is with controlling gut microflora to create microflora intestinal balance, enhance digestive capacity and improving broiler growth performance and animal health, but the definite mechanism of phytobiotics is not completely clear (Fallah *et al.,* 2013). *Phaleria macrocarpa* is an indigenous medicinal herb from Indonesia and commonly known as Mahkota Dewa. It has been used as an animal feed additive. The fruit is well known to contain a lot of useful bioactive compounds, such as phenolic, benzophenone, terpenes, and alkaloid (Alara and Olalere, 2016). These phytobiotics compounds have antibacterial properties and known to be able to reduce pathogenic bacteria in the digestive tract, resulting in an increase in animal productivity with improvement of nutrient digestibility and absorption. Esfanjani and Jafari (2016); Sechi *et al*. (2016) were reported that the efficacy of bioactive compounds in biological formulations is fast release, low water solubility chemical instability, poor absorption, low bioavailability, and easily destruction against gastrointestinal conditions. Nano-encapsulation technology is one of the solutions to resolve these problems.

Nanotechnology is one of the most popular technologies in the drug delivery system and is also used in feed additive for broiler production. Nanoparticle is a polymer with particles ranging in size from 10 to 1000 nm (1µm) which is attached to the active principle (drug or biologically active material) for pharmaceutical purposes (Homs *et al.,* 2018). The surface area of nanoparticles is

much larger than microparticle, which make the chemical reactivity improve about 1000 times (Mella and Mendo, 2010). One ecofriendly method of preparing polymeric nanoparticles is nano-encapsulation process with ionic gelation method. The ionic gelation technique of biopolymers is based on the ionic interactions between the positively and negatively charged groups. This technique involves the crosslinking agent between the positively charged primary amino groups of chitosan, and the group of polyanion, such as sodium tripolyphosphate (STPP) (Sacco *et al.,* 2016). Chitosan is a polysaccharide obtained by removing an acetyl group from chitin, which is produced in the cuticles of arthropods, the endoskeletons of cephalopods, and the cell walls of fungi, and is extensively used as drug delivery systems (Bugnicourt and Ladaviere, 2016). Chitosan is biodegradable and biocompatible with other drug delivery systems (Elgadira *et al.,* 2015). Negative ion $P_3O_{10}^{5-}$ from STPP will bind with positive ion NH^{3+} from chitosan, then allowing ionic interactions between the two (Hsieh *et al.,* 2008).

Nano-encapsulation of chitosan and STPP has the advantage that it can give protection of substances from the alteration by reaction surrounding matter and also can control release the substances on targeted sites. The previous studies Choiri *et al.* (2017) reported that supplementation of nano-encapsulation noni (*Morinda citrifolia*) fruits extract 1.5% in drinking water does not influence the productive performance of laying hens. Based on this approach, the treatments of nano-encapsulated *Phaleria macrocarpa* fruits extract was enhanced to 5.0%. The objective of the present study was to determine the effect of administration of nano-encapsulated *Phaleria macrocarpa* fruits extract in drinking water on the digestive organs and carcass characteristic and of broiler chickens.

Materials and Methods

Animal, diets, and experimental design

The study was conducted at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. Two hundred eight-days old male New Lohmann MB 202 broiler chickens were randomly assigned to five treatments with four replicates and 10 birds in each replicate pen. The basal diet (Table 1) was formulated in accordance with nutrient requirements of broilers recommended by National Research Council (NRC, 1994), based on yellow corn and soybean meal, which contained 20.44% crude protein (CP), 2,917.47 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). The diets and drinking water were supplied *ad libitum* from days 8 to 35 (Reyes *et al.,* 2018). The experimental treatments were consisted of basal diet (T0; negative control), basal diet with antibiotic tetracycline 50 mg/kg bird weight (T1; positive control), and basal diet with 2.5% of *Phaleria macrocarpa* fruits extract (T2), 2.5% nanoencapsulation of *Phaleria macrocarpa* (NEPM) (T3), and 5.0% NEPM (T4). Tetracycline, fruit extract and NEPM were added in the drinking water of the respective treatment groups. Bioactive compound of *Phaleria macrocarpa* is consist of phenolic, terpenes, alkaloids, and benzophenone, the concentration is show in Figure 1. The bioactive compound of *Phaleria macrocarpa* is consist of phenolic, terpenes, alkaloids, and benzophenone, the concentration is shown in Figure 1 (Alara and Olalere, 2016).

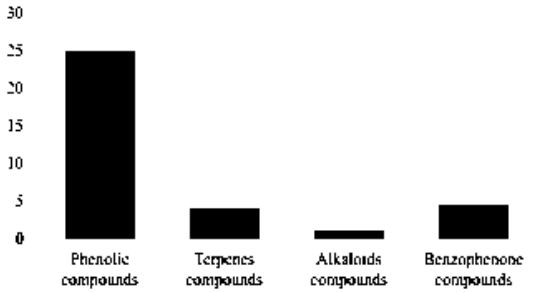

Figure 1. Bioactive compound of *Phaleria macrocarpa* (Alara and Olalere, 2016).

Nanoencapsulation procedure

Nano-encapsulation of *Phaleria macrocarpa* fruits extract (NEPM) that was used had particle size distribution of 778 nm. Zeta potential of NEPM was positive charges with value of +26.5 mV. The TEM image of NEPM (Figure 2) was displayed a homogeneous distribution and stable formulation with spherical shape and coated with transparent layer from ionic gelation between chitosan and STPP.

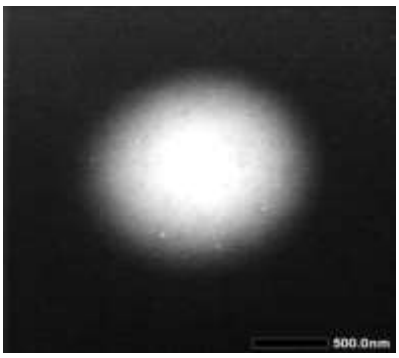

Figure 2. Morphology of nano-encapsulation in 500 nm scale.

The nanoencapsulation of *Phaleria macrocarpa* fruits extract was formulated by ionic gelation method using 2% of *Phaleria macrocarpa* fruits extract, 0.625% chitosan, 0.75% sodium-tripolyphosphate (STPP) (w/v), and consist of 0.5, caecal junction). Each part of the small intestine, caeca, and colon was emptied by gentle pressure and weighed.

Table 1. Ingredients and chemical composition of the basal diet

Ingredients	Proportion (%)
Yellow corn	55.70
Soybean meal	31.00
Meat bone meal	1.50
Rice brand	8.00
Palm oil	1.50
Vitamin premix*	0.30
L-lysine HCl (98%)	0.10
DL-methionine (99%)	0.20
$CaCO_3$	1.50
NaCl	0.20
Total	100.00
Calculated values	
Crude protein %	20.66
Metabolizable energy (ME Kcal/Kg diet)	2,971.30
Calcium %	0.83
Available P%	0.51
Lysine %	1.10
Methionine %	0.48
Threonine%	0.74
Methionine + Cystine %	0.98

Noted: *Vitamin premix (Masamix-Bro) chemical composition per kg was Vit.A:12,500,000IU; Vit D_3:2,500,000IU; Vit E:10,000mg; VitK_3:2,000mg; Vit.B_1:2,000mg; Vit.B_2:4,000mg; Vit.B_6:1,000mg; Vit.B_{12}:12,000mcg; Vit.C:40,000mg; Niacin:40,000mg; Biotin:200mg.

Table 2. The performances of male broilers at 35 days of age as influenced by *Phaleria macrocarpa* fruits extract nano-encapsulation in drinking water (mean ± SD)

Variables	Treatments					
	T0	T1	T2	T3	T4	P-*value*
Weight gain (g kg BW^{-1})	1,596.56±97.58	1,618.64±84.48	1,656.18±42.73	1,635.58±27.33	1,674.75±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

SD : Standard Deviation

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.

Statistical analysis

The data on effects of NEMD on the weights of digestive organs, and carcass characteristics were subjected to analysis of variance (ANOVA) based on a completely randomized design using Statistical Package for Social Science or SPSS (SPSS GmbH, Munich, Germany). Orthogonal contrast test were used to separate means with significance difference. All indications of significance were based on a probability of less than 5.0%.

Results and Discussion

Table 3 shows that the supplementation of different levels of nano-encapsulation of *Phaleria macrocarpa* fruits extract in the drinking water did not significantly affect the weights and size of proventriculus, ventriculus, liver and heart. These results were in agreement with a previous study Bhattacharyya *et al*. (2015) which reported that herbal phytobiotics had no effects on the relative weight of the digestive organs of the commercial broiler. Erhan *et al*. (2012) similarly reported that addition of natural feed additive pennyroyal (*Mentha pulegium* L.) levels (0, 0.25 or 0.50%) had no effects on the weight of liver and heart. In contrast, Kiczorowska *et al*. (2016) reported that a reduced proventriculus weight with diet phytobiotics of *Boswellia serrate,* but not influence the efficiency of digestive tract to digest the dry and organic matter. Rahayu *et al*. (2008) investigated that the weight of the heart, liver, and other digestive tract was influenced by the genotype, the function of organs, and not influenced by sexes and 1 to 10 weeks of age. Due to these facts, the treatmentS did not modified the function of proventriculus, ventriculus, liver, and heart, therefore not give significantly different on the weight of digestive tract.

The weights of the various parts of the intestinal tract (Duodenum, Jejenum, Ileum, Seca, and Colon) were not affected by the supplementation of nano-encapsulated of *Phaleria macrocarpa* fruits extract in drinking water, control negative and control positive with antibiotic tetracycline, presented in Table 4. Similarly, Bozkurt *et al*. (2009a) found no significant different on the relative weights of the small intestines with supplemented a prebiotic, mannan oligosaccharide, and an essential oil. The small intestine of poultry is the main organ for digestion and absorption of nutrients. The supplementation of *Phaleria macrocarpa* fruits extract whether nano-encapsulated or not, did not influence the characteristics of intestinal tract (Duodenum, Jejenum, Ileum, Seca, and Colon) of broiler chickens. There has been a limited study on the evaluation of the effects on nano-encapsulation of phytobiotics on the intestinal tract of broiler chicken. The main reason for no significant effect of the intestinal tract is might be related to the characteristic of nano-encapsulation of *Phaleria macrocarpa* fruits extract. The characteristics of *Phaleria macrocarpa* that used in this research

Tabel 3. Relative weight (g kg BW^{-1}) of organs (proventriculus, ventriculus, limpa, and heart) of male broilers at 35 days of age as influenced by *Phaleria macrocarpa* fruits extracts nano-encapsulation in drinking water (mean ± SD)

Variables	Treatments					P-*value*
	T0	T1	T2	T3	T4	
Proventriculus (g kg BW^{-1})	8.447±0.80	9.11±3.20	8.826±1.87	9.041±2.03	9.123±1.38	0.988
Ventriculus (g kg BW^{-1})	25.217±6.66	25.267±4.04	23.353±2.89	22.376±5.75	27.25±4.17	0.668
Lymph(g kg BW^{-1})	1.894±0.66	2.763±1.11	1,781±0.23	1.969±0.516	2.702±0.27	0.122
Heart (g kg BW^{-1})	41.652±6.39	47.545±4.05	48.808±4.45	45.921±9.25	49.132±4.34	0.427

SD: Standard Deviation
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 4. Relative weight (g kg BW^{-1}) of the intestinal tract of male broilers at 35 days of age as influenced by *Phaleria macrocarpa* fruits extract nano-encapsulation in drinking water (mean ± sd)

Variables	Treatments					P-*value*
	T0	T1	T2	T3	T4	
Duodenum (g/bird/35day)	16.189±0.39	14.735±3.320	15.594±1.06	16.203±1.29	16.238±3.47	0.858
Jejunum (g/bird/35day)	34.683±2.46	31.509±4.97	34.210±8.98	34.054±4.11	36.075±8.76	0.894
Ileum (g/bird/35day)	27.752±2.71	22.418±2.69	30.024±4.54	26.788±1.80	29.729±1.17	0.305
Caeca (g/bird/35day)	7.591±2.04	9.516±2.11	9.548±2.79	7.438±1.39	7.038±1.30	0.264
Colon (g/bird/35day)	6.431±3.25	4.213±1.52	4.194±2.00	3.465±0.45	3.177±0.53	0.168

SD : Standard Deviation.
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 5. Carcass characteristics (g kg BW^{-1}) of male broilers at 35 days of age as influenced by *Phaleria macrocarpa* fruits extract nano-encapsulation in drinking water (mean ± SD)

Variables	Treatments					P-*value*
	T0	T1	T2	T3	T4	
Slaughter weight (g kg BW^{-1})	1,798.75±109.85	1,777.50±81.68	1,770.50±75.83	1,773.75±79.44	1,848.00±31.00	0.631
Carcass weight (g kg BW^{-1})	1,196.50±86.49	1,199.00±26.74	1,162.75±92.02	1,180.50±127.60	1,255.50±21.42	0.587
Carcass percentage (%)	66.53±2.95	67.60±4.31	65.61±2.85	66.47±5.32	68.01±0.15	0.881
MBR	3.615±0.48	3.260±0.53	3.612±0.97	3.740±0.86	4.275±0.14	0.644

SD: Standard Deviation, MBR: Meat bone 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.

have particle size distribution 778 nm and zeta potential was positive charges with the value of +26.5 mV. It has an impact on particle distribution on the intestinal mucosa of broiler chicken, the distribution was more slowly and less effective than small particle size, therefore the function of nano-encapsulation not optimal.

The effect of nano-encapsulation of *Phaleria macrocarpa* fruits extract in drinking water on the carcass characteristics of broilers chickens are presented in Table 5. Carcass characteristics (slaughter weight, carcass, weight, carcass percentage, and meat bone ratio) were unaffected by supplementation of nano-encapsulation of *Phaleria macrocarpa* fruits extract in drinking water, and with control negative and control positive with antibiotic tetracycline. The results associated with weight gain, presented in Table 2. No significant effect on the weight gain is correlated with the results of carcass characteristics. In a recent study, Sundari *et al.* (2014) reported that nano-encapsulation of turmeric extract did not improve carcass weight of broiler chicken. Furthermore Bozkurt *et al.* (2009b) reported that the slaughter characteristics of broiler chicken both of male and female were not affected by the feed additive. In the study of Parera *et al*. (2015) and Katouzian and Jafari (2016), it was reported that higher particle size means the compound will be more slowly distributed and released (50 > 200 > 500nm), nanoparticle with <500 nm shows the best rapidity flow in the intestinal mucosa. It was possible that the nano-encapsulation size may be too large to allow any increase in the rate of absorption, and did not give significant effect on carcass characteristics.

Conclusions

It can be concluded that supplementation of 5.0% *Phaleria macrocarpa* fruits extract whether nano-encapsulated or not, did not affect carcass, digestive organs, heart and liver weights of broiler chickens. Nano-encapsulation may not be useful in increasing absorption of the extract under these circumstances.

Acknowledgment

The authors are grateful to the *Ministry of Research*, *Technology and Higher Education* of the Republic of *Indonesia* for the financial support.

References

Alara, O. R. and O. A. Olalere. 2016. A critical overview on the extraction of bioactive compounds from *Phaleria macrocarpa* (Thymelaceae). Nat. Prod. Chem. Res. 4: 1-4.

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th edn. Assoc. Off. Anal. Chem., Arlington.

Bhattacharyya, A., P. K. Shukla, and M. Shukla. 2015. Effect of poly herbal phytobiotic on the growth, immunocompetence, development of digestive organs and carcass characteristics of commercial broilers. J. Anim. Res. 5: 347-351.

Bozkurt, M., K. Küçükyılmaz, A. U. Çatlı, and M. Çınar. 2009a. The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. S. Afr. J. Anim. Sci. 39: 197 – 205.

Bozkurt, M., K. Küçükyılmaz, A.U. Çatlı, and M. Çınar. 2009b. Effect of dietary mannan oligosaccharide with or without oregano essential oil and hop extract supplementation on the performance and slaughter characteristics of male broilers. S. Afr. J. Anim. SCI. 39: 223 – 232.

Bugnicourt, L. and C. Ladaviere. 2016. Interests of chitosan nanoparticles ionically cross-linked with tripolyphosphate for biomedical applications. Prog. Polym. Sci. 60: 1 – 17.

Choiri, Z., N. D. Dono, B. Ariyadi, C. Hanim, R. Martien, and Zuprizal. 2017. Effect of nano-encapsulation of noni (*Morinda citrifolia*) fruits extract on jejunal morphology and microbial populations in laying hens. Pak J Nutr. 17: 34–38.

Elgadira, M. A., Md. S. Uddin, S. Ferdosh, A. Adam, A. J. K. Chowdhury, and Md. Z. I. Sarker. 2015. Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: A review. J. Food Drug. Anal. 23: 619-629.

Erhan, M. K., S. C. Bolukbası, and H. Urusan. 2012. Biological activities of pennyroyal (*Mentha pulegium* L.) in broilers. J. Liv. Sci. 146: 189–192.

Esfanjani, A. F. and S. M. Jafri. 2016. Biopolymer nano-particles and natural nano-carriers for nano-encapsulation of phenolic compounds. Colloids and Surfaces B: Biointerfaces. 146: 532–543.

Fallah R., A. Azarfar, and A. Kiani. 2013. A review of the role of five kinds of alternatives to in feed antibiotics in broiler production. J. *Vet. Med*. Anim. Health. 5: 317-321.

Homs, M. G. Calderó, M. Monge, D. Morales, and C. Solans. 2018. Influence of polymer concentration on the properties of nano-emulsions and nanoparticles obtained by a low-energy method. Colloids Surf. A. 536: 204 – 212.

Hsieh, F. M., C. Huang, T. F. Lin, Y. M. Chen, and J. C. Lin. 2008. Study of sodium tripolyphosphate-crosslinked chitosan beads entrapped with *Pseudomonas putida* for phenol degradation. Process Biochem. 43: 83–92.

Katouzian, I. and S. M. Jafari. 2016. Nano-encapsulation as a promising approach for targeted delivery and controlled release of vitamins. Trends Food Sci. Technol. 53: 34 – 48.

Kiczorowska, B., A. R. M. Al-Yasiry, W. Samolińska, A. Marek, and E. Pyzik. 2016. The effect of dietary supplementation of the broiler chicken diet with Boswellia serrataresin on growth performance, digestibility, and gastrointestinal characteristics, morphology, and microbiota. J. Liv. Sci. 191: 117–124.

Mella, R. M. and O. H. Mendo. 2010. Review nanotechnology on animal production. Trop. Subtrop. Agroecosyst. 12: 423–429.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. Ed Rev ke-9. Academy Press, Washington DC.

Parera, G., M. Zipser, S. Bonengel, W. Salvenmoser, and A. Bernkop-Schnürch. 2015. Development of phosphorylated nanoparticles as zeta potential inverting systems. Eur. J. Pharm. Biopharm. 97: 250–256.

Rahayu, H. S. I., I. Zulkifli, M. K. Vidyadaran, A. R. Alimon and S. A. Babjee. 2008. Carcass Variables and Chemical Composition of Commercial Broiler Chickens and the Red Jungle Fowl. Asian-Aust. J. Anim. Sci. Vol. 21, No. 9: 1376 – 1382.

Reyes, F. C. C., A. T. A. Aguirre, E. M. Agbisit, F. E. Merca, G. L. Manulat, and A. A. Angeles. 2018. Growth performances and carcass characteristics of broiler chickens fed akasya [*Samanea Saman* (Jacq.) Merr.] pod meal. Trop. Anim. Sci. J. 41: 46-52.

Sacco, P., S. Paoletti, M. Cok, F. Asaro, M. Abrami, M. Grassid, and I. Donati. 2016. Insight into the ionotropic gelation of chitosan using tripolyphosphate and pyrophosphate as cross-linkers. Int. J. Biol. Macromol. 92: 476–483.

Sechi, M., D. N. Syed, N. Pala, A. Mariani, S. Marceddu, A. Brunetti, H. Mukhtar, and V. Sanna. 2016. Nanoencapsulation of dietary flavonoid fisetin: Formulation and in vitro antioxidant and α-glucosidase inhibition activities. Mater. Sci. Eng. C. 68: 594–602

Sundari, Zuprizal, T. Yuwanta and R. Martien. 2014. Effect of nanocapsule level on broiler performance and fat deposition. Int. J. Poult. Sci. 13: 31-35.