Original Article

Nano emulsion Formulation of Noni Leaf Extract and Maggot Oil *(Hermetia illucens)* as an Alternative to Antibiotic Growth Promoters

Daulai, MS[1*], Wijayanti, I[1], Retnani, Y[1]

1. *Department of Nutrition Science and Feed Technology, IPB University, Bogor, Indonesia*

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ABSTRACT

The extensive use of antibiotic growth promoters leads to residues and bacterial resistance. Alternative herbal products from noni leaf extract (NLE) and maggot oil are needed that can improve poultry productivity and health. Secondary metabolites from NLE and fatty acid profile obtained from maggots that can be optimized in nano emulsion form. This study was aimed to characterize and determine the optimal formula of NLE and maggot oil *(Hermetia illucens)* in nano emulsion form as an alternative to antibiotic growth promoters (AGP). nano emulsion formulations were divided into three treatments with three replicates: F1 = 2.5 g/100 mL NLE and 12.5 g/100 mL maggot oil, F2 = 7.5 g/100 mL NLE and 7.5 g/100 mL maggot oil, and F3 = 12.5 g/100 mL NLE and 2.5 g/100 mL maggot oil. nano emulsion preparation was carried out by mixing all the components of the formula were homogenized by using homogenizer *ultra turrax* at 12500 rpm for 20 minutes. Particle size, zeta potential, polydispersity index, transmittance and solubility were calculated to evaluate the nano emulsion formulation. The data were analyzed using analysis of variance (ANOVA) and the formulation of nano emulsion was optimized by using simplex lattice design. The results showed that NLE has the highest phytochemical is steroid. The formula had significant effect ($P<0.05$) on particle size, zeta potential, polydispersity index (PI), and transmittance with F1 had the lowest particle size and PI and the highest transmittance compared to other formulas, while zeta potential was stable compared to standards. Nano emulsion formulation was optimized by using 2.125 g/100 mL NLE and 12.875 g/100 mL maggot oil. Nano emulsion that was physically stable was unable to inhibit *E. coli* as indicated by the diameter of the inhibition zone.

Keywords: Antibiotic Growth Promoter, Formulation, Maggot Oil, Nano emulsion, Noni Leaf

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Corresponding Author's E-Mail:
msulaimandaulai@apps.ipb.ac.id

1. Introduction

Antibiotic growth promoters (AGPs) are antibiotics that are widely used to increase the value of feed given to livestock. The use of AGPs is believed to be able to control disease, accelerate growth and increase feed conversion (1). Based on research by (2) showed that the use of antibiotics in the form of *amoxicillin* resulted in resistance of up to 90% in *E. coli* isolates that cause digestive tract problems. The inappropriate use of antibiotics in feed can lead to decrease in the efficacy and function of antibiotic therapy and the possibility of resistance to other pathogenic bacteria that threaten animal, human and environmental health. Because of the problems associated with antibiotic use, there is a need for alternative botanical ingredients that can improve livestock productivity and health and protect against antibiotic resistance (3). Noni leaves (*Morinda citrifolia*) are one of the herbal ingredients that are rich in chemical compounds such as flavonoids, triterpenoids, and steroids that act as antibacterials. The content of antibacterial compounds in the form of flavonoids found in noni leaves reaches 2.648 mg RU/g dry matter which can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria (4). The content of active compounds from noni leaves can be optimized with a combination derived from larvae, namely maggots. Maggots or larvae (*Hermetia illucens*) can be processed into oil by extracting it by chemical extraction using solvents and mechanical separation. The extracted larvae produce oil with a percentage of saturated fatty acid profile dominated by lauric acid of 49.18%, which acts as an antibacterial and increases the immune response of livestock (5). The content of active compounds from noni leaves and fatty acids obtained from maggots can be a combination of herbal ingredients and larvae as an alternative to AGP in the form of nano emulsions. The nano-emulsion method offers advantages in increasing the bioavailability of active ingredients, controlling the release of active ingredients and improving sensory properties. Nano emulsion has a nano-size of 50-500 nm, which is able to increase the absorption of the small intestinal wall thus increasing its bioavailability (6). Research by (7) reported that the ability of the active ingredients to penetrate the nano emulsion preparation of Moringa leaf extract was faster and greater at 61.33% compared to the crude extract of 15.83%. The application of nano emulsion method can maintain long-term stability characteristics because it does not cause inherent problems, creaming, flocculation, coalescence, and sedimentation (8). Good physical stability can maintain the shelf life of the active ingredients of the extract so it is expected that the use of nano emulsion based on noni leaf extract and maggot oil will increase the efficiency and ease of application in livestock. This study aimed to characterize and determine the optimal formulation of nano emulsion based on noni leaf and maggot oil *(Hermetia illucens)* as an alternative AGP.

2. Materials and Methods

2.1. Data Collection

The research was conducted empirically, namely laboratory research for 3 months from December 2022 to February 2023. The preparation of noni leaf extraction was carried out in the Laboratory of Meat and Livestock nutrition, followed by qualitative phytochemical analysis in the Laboratory of the Department of Chemistry. The manufacture and characterization of nano emulsions were carried out in the integrated laboratory of the Department of Nutrition Science and Feed Technology, IPB University and the Integrated Laboratory and Research Center, University of Indonesia. Maggot oil was obtained from Magalarva® with fatty acid profile that presented in Table 1.

2.2. Noni Leaf Extraction

The noni leaf extraction process was initiated by soaking noni leaf powder dissolved with 96% ethanol at the ratio of 1: 5 for 3 x 24 hours. The maceration solution was filtered with filter paper and the solution was evaporated using a rotary vacuum evaporator at a temperature of 500 C until a thick extract was obtained. The results of the thick extracts are subjected to qualitative phytochemical tests in the form of alkaloid, flavonoid, steroid, triterpenoid, tannin and saponin tests.

2.3. Preparation of Nano emulsion from Noni Leaf Extract and Maggot Oil

Nano emulsion formulation refers to the suitability of the minimum inhibition zone against *E. coli* bacteria. Based on the results of research conducted by (4), the minimum inhibitory concentrations (MIC) in noni leaf extract against *E. coli* bacteria were 1.25 g/100 mL and the best inhibition was obtained at a concentration of 10 g/100 mL. The determination of the concentration of maggot oil adapts to the results of research from (9) with MIC in maggot oil against *E. coli* bacteria of 1.25 g/100 mL and the best concentration for the maggot oil inhibition zone in the formula of 12.5 g/100 mL. The nanoemulsion was modified from the reference of (10) with a formula ratio of water phase (distilled water), oil phase (noni leaf extract and maggot oil) and emulsion (Tween 80) of 80:15:5 as shown in Table 2**.** The extraction results of noni leaves and maggot oil obtained from Magalarva® were prepared as the oil phase. The water phase was prepared by mixing water and emulsion in the form of Tween 80 in a glass beaker . The preparation of the nano emulsion was carried out by mixing all the materials in the form of aqueous phase, oil phase and emulsion,which were homogenized with a homogenizer (*ultra turrax*) at 12500 rpm for 20 minutes.

2.4. Characterization of Nano emulsion Preparation of Noni Leaf Extract and Maggot Oil

2.4.1. Particle Size, Polydispersity Index, and Zeta Potential

The determination of particle size, polydispersity index (PI), and zeta potential was performed in up to 3 replicates using a particle size analyzer (Horiba SZ-100) to determine the quality of nano emulsion preparations.

Table 1. Fatty Acid Profile of Maggot Oil (Magalarva®)

Fatty Acids	Content (%)
Saturated Fatty Acids	
Caprate (C 10:0)	1.20
Laurate (C 12:0)	39.02
Miristate (C 14:0)	8.31
Palmitate (C 16:0)	17.89
Stearate (C 18:0)	2.86
Unsaturated Fatty Acids	
Oleic (C 18:1)	14.69
Linoleic (C 18:2)	10.75
Linolenic (C 18:3)	1.31

Tabel 2. Nanoemulsion Formula Design

Material	Formula		
	F1 (1:5)	F2 (3:3)	F3 (5:1)
Noni Leaf Extract (gram)	2.5	7.5	12.5
Maggot Oil (gram)	12.5	7.5	2.5
Tween 80 (mL)	5	5	5
Distilled Water (mL)	ad 100	ad 100	ad 100

The measurements were performed according to the principle of dynamic light scattering, that is, ,the sample suspension is irradiated with a laser and the scattered light is used to determine the determination of the average particle size , which is obtained from the Z-average value in units of nm (10).

2.4.2. Transmittance Percentage

To determine the transmittance percentage, 1 mL of nano emulsion was dissolved in 100 mL of distilled water. The transmittance percentage was measured using a UV-Vis spectrophotometer at a wavelength of 650 nm and distilled water as a blank.

2.4.3. Nano emulsion Formulation Optimization of Noni Leaf Extract and Maggot Oil

The determination of the optimum formula modified in (11), namely formula optimization, was carried out using Design Expert software12 with the Simplex Lattice Design (SLD) method. The formula obtained from SLD consists of 3 formulas related to Table 2 with two component factors, namely noni leaf extract and maggot oil. The results of the formula optimization using the SLD method were statistically analyzed for each nano emulsion preparation response in terms of particle size, PI, zeta potential, and transmittance.

2.5. Antibacterial Test of Nano emulsion of Noni Leaf Extract and Maggot Oil

The antibacterial efficacy of nano emulsion preparations of noni leaf extract and maggot oil was tested using *E. coli* bacteria with the well diffusion method modified from (12). The test was performed by adding the nano emulsion preparation to each well with a sterile pipette and incubated for 24 hours. The inhibition ability was observed by measuring the clear zone around the resulting wells.

2.6. Experimental Design and Data Analysis

The research consisted of 3 treatments with 3 replicates. The treatments given were observed by comparing the concentration of each Nano emulsion formulation which was characterized by the nano emulsion preparation.

F1: Nano emulsion of noni leaf extract and maggot oil (1:5)
F2: Nano emulsion of noni leaf extract and maggot oil (3:3)
F3: Nano emulsion of noni leaf extract and maggot oil (5:1)

The research data from extraction of noni leaves and maggot oil were analyzed descriptively with qualitative and quantitative approaches. The data of nano emulsion characterization were analyzed by *analysis of variance* (ANOVA) and *Duncan's* test at 5% level.

3. Results

3.1. Noni Leaf Extraction and Phytochemical Compounds

Noni leaf extract has antibacterial compounds observed through the characteristics and results of phytochemical analysis. Phytochemical analysis of noni leaves yields groups of secondary metabolites presented in Table 3. Based on the results of the characteristics (Table 3), the color of the extract has changed in the form of simplia powder, which is green to blackish green, has changed due to oxidation reactions caused by heating during the evaporating the filtrate into extracts. The distinctive aroma of noni leaves in the form of bitterness is produced by the alkaloid compound group, which is alkaline and contains nitrogen elements (12). The extraction results in the form of a thick extract were obtained as much as 25.74 grams with an extract yield of 6.5% using the maceration method. Extracts that use maceration methods and solvents that are effective in attracting phytochemical compounds have not been able to produce high yields. This is suspected by the process of extraction of compounds that are less than optimal in different solvent concentrations, and maceration time. The correct extraction time can affect the results of high extract yields. Too short a time is not optimal in dissolving phytochemical compounds as a whole and too long a time can cause phytochemical compounds in plants to be damaged (13).

3.2. Characterization of Nano emulsion Preparation of Noni Leaf Extract and Maggot Oil

Nano emulsion preparations based on noni leaf extract and maggot oil were prepared to increase solubility and bioavailability and to keep the preparation of active compounds stable and optimal. The characterization of the nano emulsion was carried out by using high energy

through a mechanical device in the form of a high shear homogenizer stirrer Ultra Turrax which aims to reduce the particle size to nanodroplet size. The results of nano emulsion preparation characterization are presented in Table 4. Based on Table 4, it can be seen that different concentrations of noni leaf extract and maggot oil give different results. The difference in magnitude and characteristics is influenced by the type of the oil, herbal extracts and the type of emulsion as well as the concentration and ratio of the formula used (18).

3.3. Nano emulsion Formula Optimization of Noni Leaf Extract and Maggot Oil

Formula optimization was performed using the characterization data of nano emulsion preparation in Table 4. The experimental data were statistically analyzed using ANOVA to determine the best formula model. For statistical analysis, P Value and multiple correlation coefficient R^2 obtained from design expert 12 software are presented in Table 5.

Table 3. Characteristics and Phytochemical Compound of Noni Leaf Extracts

Characteristics	Result
Color	Blackish green
Texture	Thick liquid
Yield	6.5%
Metabolite Secondary	
Alkaloids	++
Flavonoids	++
Steroids	++++
Triterpenoidd	++
Tannins	++
Saponins	+

Notes: (+) indicates the presence and accumulation of metabolite secondary compounds.

Table 4. Characterization of Nanoemulsion Preparations

Jenis	F1	F2	F3	Standar
Particle Size (nm)	405.93 ± 29.71^a	2541.63 ± 391.23^b	482.80 ± 13.31^a	20 – 500[34]
Polydispersity Index	0.455 ± 0.017^a	0.737 ± 0.039^b	0.627 ± 0.093^b	<0,5[35]
Zeta Potential (mV)	-38.63 ± 0.45^b	-48.20 ± 0.56^a	-23.03 ± 0.49^c	>±30[36]
%Transmittance	88.43 ± 1.77^a	64.10 ± 0.30^c	76.60 ± 0.46^b	>80[37]

Notes: F1 = Nanoemulsion of noni leaf extract and maggot oil (1:5); F2 = n Nanoemulsion of noni leaf extract and maggot oil (3:3); F3 = Nanoemulsion of noni leaf extract and maggot oil (5:1). Different superscripts on the same line with various letters indicate significant differences (P<0,05).

Table 5. Statistical Analysis of Nanoemulsion Formula Optimization

Response	*P Value*	R^2	Model
Particle Size (nm)	<0.0001	0.9662	*Quadratic*
Polydispersity Index	0.0032	0.8028	*Quadratic*
Zeta Potential (mV)	<0.0001	0.9984	*Quadratic*
Transmittance (%)	<0.0001	0.9923	*Quadratic*

Based on the results of statistical analysis of nano emulsion formulation optimization (Table 5), it shows that all responses are significant and valid values, namely $P < 0.05$ and supported by R^2 with values between 0.8 and 0.9, indicating that different concentrations of noni leaf extract and maggot oil affect the characterization results of nano emulsion preparations in the form of particle size, PI, zeta potential and transmittance with sequential percentages of 96.62%, 80.28%, 99.84% ,and 99.23%. The results of the Design Expert 12 recommended the quadratic model as a significant model for all responses. The results of statistical analysis followed by nano emulsion formula optimization with a criteria approach from each nano emulsion preparation characterization response. The best recommendation in formulation optimization is 2.125 g/100 mL noni leaf extract and 12.875 g/100 mL maggot oil with the optimization design shown in Table 6. Based on formula optimization using Simplex Lattice Design (Table 6), the best recommendation is characterized by a desirability of 1.000. The results of nano emulsion formula optimization showed an improvement in terms of quality of nano emulsion preparations compared to previous experimental data in the form of particle size, PI, and transmittance, namely 76.664 nm, 0.418, and 91.743%. As for the zeta potential of the formula optimization, it has lower results than in the F1 and F2 treatments, namely -36.516 mV, but all of the nano emulsion formula optimization designs have met the standards in Table 4.

3.4. Antibacterial Activity of Nano emulsion of Noni Leaf Extract and Maggot Oil

The results of the antibacterial activity test of nano emulsion of noni leaf extract and maggot oil against *E. coli* showed no antibacterial activity in all the formulations through the observation of clear zone diameter. Antibacterial activity was obtained only in the positive control, amoxicillin in each F1, F2, F3 respectively with an inhibition zone diameter of 14.50 ± 0.58 mm, 15.25 ± 0.50 mm and 14.75 ± 0.50 mm, as shown in Figure 1.

Table 6. Nanoemulsion Formula Optimization Results

Characteristics	FX Optimization Results
Particle Size	76,664 nm
Polydispersity Index	0,418
Zeta potential	-36,516 mV
Transmittance	91,743%

Notes: FX = nanoemulsion formula of noni leaf extract 2.125 g/100 mL and maggot oil 12.875 g/100 mL.

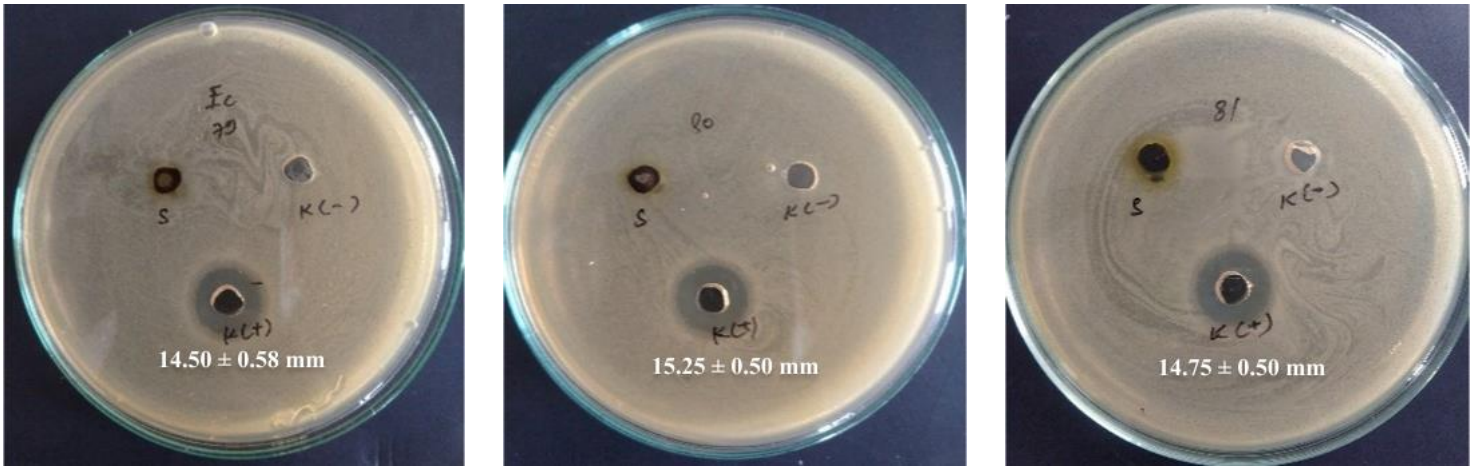

Figure 1. Zone of inhibition of nano emulsion of noni leaf extract and maggot oil against *E. coli.* (F1) nano emulsion of noni leaf extract and maggot oil (1:5); (F2) nano emulsion of noni leaf extract and maggot oil (3:3); (F3) nano emulsion of noni leaf extract and maggot oil (5:1).

4. Discussion

4.1. Phytochemical compounds of Noni Leaves Extract

Based on Table 2 shows that the extraction results have all classes of secondary metabolites including alkaloids, flavonoids, steroids, triterpenoids, tannins, and saponins. This is due to the use of the maceration method is able to minimize the occurrence of cell damage phytochemical compounds because the extraction process is carried out without heating so that there is no increase in free radicals in the form of reactive oxygen species (ROS) in reactive plants and produce phytochemical compounds that can be optimally dissolved (19). The results of the phytochemical analysis were dominated by the group of steroid compounds. The steroid content in noni leaves shows as anti-inflammatory and antibacterial potential. The antibacterial potential is also obtained by the presence of compounds in the form of alkaloids as activators of immune cells in damaging bacterial cells and flavonoids that cause damage to the protein structure contained in the cytoplasmic wall of bacteria through the process of denaturation of the bacterial cell wall (20). Based on (21), saponins and triterpenoids may also act as antibacterial agents by disrupting the permeability of bacterial cell membranes, resulting in inhibited bacterial cell growth. Compounds in noni leaves have a diversity of active compounds against certain biological activities.

4.2. Characterization of Nanoemulsion of Noni Leaf Extract and Maggot Oil

Based on Table 4 shows the significance value ($P < 0.05$) with formulas F1 and F3 give the same effect on the value of particle size, which is 405.93 ± 29.71 nm and 482.80 ± 13.31 nm. The high concentration of noni leaf extract with the same concentration of maggot oil in formula F2 has a particle size of 2541.63 ± 391.23 nm which is caused by the agglomeration process or an increase in particle size due to the tendency of particles to clump or form larger particles (22). Based on the results of Table 4, formulas F1 and F2 are in accordance with the nano emulsion particle size standard of 20-500 nm (14). The particle size <500 nm can help improve absorption, especially in the performance of the gastrointestinal system in poultry and nutrient digestibility caused by extract ingredients with small particle sizes that can dissolve in the intestine (6). The polydispersity index obtained in Table 4 shows the effect ($P<0.05$) with formula F1 having a uniform particle size compared to F2 and F3. The difference in the Polydispersity index value is due to the agglomeration process of the concentration difference factor in the formula (22). F2, which experienced agglomeration, showed a tendency of non-uniform particle size, resulting in a high polydispersity index of 0.737 ± 0.039. The polydispersity index in F3 has the same effect as F2, which may be caused by other factors such as zeta potential. Based on (16) state that a good polydispersity index is in the range of polydispersity index (PI) <0.5 so that only the F1 formula of 0.455 ± 0.017 has a uniform particle size and the potential to maintain the stability of the long-term emulsion system (8). The Zeta potential is a parameter that indicates the degree of repulsion between adjacent and similarly charged particles in a dispersion system (15). Based on Table 4, it can be seen that the different concentrations of each formula cause significant differences ($P < 0.05$) in zeta potential. Formula F2 has the most stable zeta potential value compared to F1 and F3 with a value of -48.20 ± 0.56 mV. The stable zeta potential of F2 is based on (23) who reported that high fatty acid accumulation causes a negative charge on the droplet surface and the extract can be negatively charged by the hydroxyl group of polyphenolic compounds. The unstable formula F3 correlates with a high polydispersity index. A non-uniform dispersion system is associated with an unstable zeta potential, which increases the attractive force between particles and results in flocculation or merging of colloids from small to large (15). Based on (15), formulations with stable zeta potential values greater than ± 30 mV can prevent the potential for particle aggregation due to repulsive forces. Based on the characterization results of nano emulsion preparations, formulas F1 and F2 have zeta potentials of -38.63 ± 0.45 mV and -48.20 ± 0.56 mV, respectively, which may maintain the physical stability of the emulsion during storage (8). The F2 formula with a stable zeta potential value may still experience flocculation or agglomeration caused by the interaction of the concentration and particle size of the extract compound (22). This is supported by (8) who reported that the zeta potential value is not able to visually represent stability and some studies show visually stable emulsions but have unstable absolute zeta potential values. This indicates that the zeta potential is not the main indicator that affects emulsion stability as shown by the results of the study that the optimum potential zeta value in F2 was found to be large or unstable in particle size and polydispersity index. Characterization of nanoemulsion preparations refers to transmittance measurements to verify the clarity of the dispersion system based on the absorbance of distilled water solution at a wavelength of 650 nm (15). Based on the transmittance measurement results, the significance ($P<0.05$) was obtained by formula F1 with the largest transmittance value of 88.43% compared to formula F2 and F3. The difference in the transmittance value is influenced by the nano- size of the formula with the indication of transmittance as a clear visual standard of nano emulsion preparation. This is indicated by the transmittance value in F1 which is >80%, indicating contencysis with a smaller particle size compared to formulas F2 and F3, which have a transmittance value of 64.10 ± 0.30% and 76.60 ± 0.46%. Nano emulsions that have a small particle size are able to transmit light , so the light beam will be transmitted and the color of the solution appear transparent and the resulting transmittance is greater (17).

4.3. Antibacterial Activity

Antibacterial activity, which is not formed in nano emulsion preparations is caused by the structure and nature of bacteria as gram-negative bacteria, which have a three-

layered cell wall in the form of lipoproteins, outer membranes, phospholipids, lipopolysaccharides, and lipid content in the cell wall ranging from 11-22% and are surrounded by a thick capsule, which makes it difficult to penetrate the bacterial cell wall (24). Due to the structure and properties of the bacteria, the nano emulsion formulation has a low extract yield of 6.5% and many mixed ingredients may result in the the extract not to be pure (25). Low extract yields are thought to indicate low quantification of phytochemical compounds and qualitative screening results show low antibacterial potential of phenolic compounds. Based on (21), phenolic compounds play an important role in inhibiting bacterial growth and development in a bacteriostatic manner by damaging the bacterial cell membrane. Phenolic compounds in high concentrations are able to penetrate and disrupt thick bacterial cell walls in gram- negative bacteria. It is believed that the concentration of extracts mixed in the nano emulsion formula is thought to be unable to optimize the antibacterial efficacy, so an increase in extract concentration in the formula is needed.

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Authors' Contribution
Study concept and design: M. S. D.
Data Acquisition: M. S. D.
Analysis and interpreatation of data: M. S. D.
Drafting of the manuscript: M. S. D.
Critical revision of the manuscript for important intellectual content: I.W. and Y. R.
Statistical analysis: M. S. D.
Administrative, technical, and material support: I.W. and Y. R.

Ethics
The current study was approved by and carried out under the protocol of the Scientific Committee of the Department of Nutrition Science and Feed Technology, IPB University (Bogor, Indonesia).

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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