The Microencapsulation of Noni Fruit Extract (*Morinda Citrifolia* L.) with Maltodextrin and Its Implementation As Feed Additive on Carcass Quality and Histology of Intestinal Sentul Chicken

Tuti Widjastuti^{a,*}, Leni Nurlaeni^a, Abun Hasbuna^a, Iwan Setiawan^a, Indrawati Yudaasmara^a, Wiwin Tanwiriah^a

^a Faculty of Animal Husbandry, Universitas Padjadjaran, Jl Raya Bandung-Sumedang Km 21, Indonesia Corresponding author: ^{*}tuti.widjastuti@unpad.com

Abstract— Noni fruit contains photogenic compounds and active substances that function as *antibacterial, antioxidant, anthelmintics,* and *anti-cholesterol* and can increase the digestibility of feed. Active compounds with natural antioxidant activity have properties that are very unstable to heat and oxidation reactions. Microencapsulation is a technique to coat the components of bioactive compounds in extracts using a coating material. The coating material used is maltodextrin (70% extract:30% maltodextrin). Maltodextrin has high solubility and the ability to inhibit oxidation reactions. The research aims to get an optimal dose of the use of microcapsules product of noni extract (MPNE) on carcass quality and histology of intestinal Sentul chickens. The study used an experimental method with a Completely Randomized Design (CRD) to see the influential treatment using analysis of variance followed by Duncan's Multiple Range Test consisting of 4 replications and five treatments. So, the total number of chickens that had been reared was 100, and maintenance was carried out for 12 weeks. The feed treatments, namely P0=Basal ration, P1=50 mg/kg Zinc Bacitracin, P2=125 mg/kg MPNE, P3=250 mg/kg MPNE, P4=375 mg/kg MPNE. The results showed that MPNE with a dose of 250 mg/kg is the best treatment for producing live weight carcass, reducing meat cholesterol, dan improving villi height, width, surface area, and jejunum crypt depth. It was concluded that giving noni fruit extract microcapsules a dose of 125-250 mg/kg is recommended as a feed additive to replace AGP for Sentul chickens.

Keywords-Microencapsulation; maltodextrin; Sentul chicken; carcass quality; histology of intestinal.

Manuscript received 5 Oct. 2022; revised 27 Nov. 2022; accepted 5 Jan. 2023. Date of publication 28 Feb. 2023. IJASEIT is licensed under a Creative Commons Attribution-Share Alike 4.0 International License.

I. INTRODUCTION

Sentul chicken is a potential local chicken product to be developed as a dual-purpose type of chicken and has good performance in terms of productivity, both meat and eggs, and includes local chicken typical of the Ciamis area of West Java [1]. The productivity of Sentul chicken eggs is quite high, namely, 12-30 eggs in one laying period, and the body has dense muscles (compact) and good meat, can adapt to various environmental conditions and is accustomed to quality feed low [2]. Sentul chickens can be slaughtered at 8-10 weeks, while traditional broilers can be slaughtered at 12 weeks. Antibiotic growth promoters AGP are needed in feed to support livestock productivity by increasing body resistance and spurring livestock growth [3], [4]. Giving antibiotics continuously for a long time can cause livestock to become resistant to pathogenic bacteria, so animal products can leave antibiotic residues that can negatively impact human health [5]. Therefore, the Government prohibits the use of AGP in feed through Minister of Agriculture Regulation No. 14/2017 concerning the Classification of Veterinary Drugs, since January 1, 2018. The prohibition is based on the impact of the use of AGP, which causes resistance to microorganisms, and antibiotic residues will be carried in livestock products in the form of meat which will be harmful to humans who consume them.

One of the potential substitutes for AGP that can be used as a feed additive is the noni fruit (*Morinda citrifolia Linn*). Noni fruit contains some *phytogenic* compounds and active substances that function as *antibacterial*, *antioxidant*, *antihelmintic*, and *anticholesterol* and can increase the digestibility of food substances [6]. These compounds are *anthraquinone*, *scopoletin*, *proxeronin*, and *xeronin*. Noni fruit contains *flavonoid* and *antimicrobial phenolic* compounds [7] that can be used as natural antibiotics in poultry. Noni fruit used for feed additives in poultry must be processed first because it is constrained to contain high levels of crude fiber and anti-nutritional substances, which can increase the rate of digestion, resulting in stunted digestion and decreased absorption of nutrients. Therefore, to extract bioactive compounds and reduce the content of antinutrients, an extraction process is necessary. In general, extraction is separating the active substance from a solid or liquid using a solvent. A simple extraction that can be used is the maceration method. Antioxidant compounds contained in noni fruit extract have unstable characteristics, are sensitive to heat, react easily, and are easily oxidized. Therefore, efforts need to be made to protect these compounds.

Microencapsulation can protect bioactive compounds from various environmental influences to avoid damage, increase compound stability, and are safe during storage [8]. In addition, microencapsulation is the process of encapsulating these natural bioactive compounds to protect them from degradation under various processing and storage conditions [9]. The coating or encapsulation material greatly influences the coating of the core material. The encapsulation process must use a kind of coating that is non-toxic, non-reactive with core materials, and can be tailored to the desired end product [10]. The coatings often used are maltodextrin, Arabic gum, gelatin, and carboxymethyl cellulose [10], [11]. Maltodextrin was chosen as a coating material because of its advantages; it is easily soluble in water, colorless, has an excellent ability to form amorphous solids at low cost, improves water solubility of encapsulated materials, and has low viscosity at high concentrations [12]. Maltodextrin has the potential to be used as a coating material because it is cheap, does not have a strong taste, so it does not affect the core components, has high solubility, and protects the core components from oxidation [13]. The use of maltodextrin in amounts up to 70% as a coating material exhibits thin and resilient film-forming properties, the ability to bind aromas and product fats, and the excellent ability to lessen the oxygen permeability of the wall matrix [14].

The application of microcapsules in this study was in dry form using the dry oven method. These microencapsulations make it easy to use in the field and extend shelf life. The provision of microcapsules to Sentul chickens is expected to increase livestock productivity. It is suspected that anthraquinone compounds from noni can be utilized more effectively and efficiently in the digestive tract of Sentul chickens because they have undergone a microencapsulation process. The addition of MPNE will degrade the coating material in the stomach so that anthraquinone compounds are readily available in the small intestine. The small intestine is responsible for absorbing nutrients from the feed so that anthraquinone compounds will be digested and absorbed optimally. Indicators of proper use of anthraquinone compounds in chickens are reflected in the increase in body weight which ultimately increases the weight of the chicken carcass. The main absorption process of Anthraquinone compounds occurs in the intestines, and others are transported through the bloodstream to both body tissues and organs [15]. This condition expands the surface of the intestinal villi, thus improving the process of absorbing nutrients. Antioxidant compounds can restore free radicals, slowing the oxidation process [16]. Based on phytochemical screening, noni fruit ethanol extract contains active compounds, namely alkaloids, flavonoids, saponins, tannins, steroids, and phenols [17]. The active substance of flavonoids in noni fruit extract can protect arteries from damage and reduce cholesterol deposits on the endothelial surface of arterial blood [18]. Alkaloids can reduce blood cholesterol levels in the cholesterol synthesis process. Alkaloids have been suggested to provide better inhibition of cholesterol synthesis and promote cholesterol catabolism and excretion [19].

The research results on microcapsules using maltodextrin coating have the highest yield [20]. Then, the balanced use of mangosteen peel extract and maltodextrin (1:1) resulted in beneficial microcapsule properties [21]. Giving a mixture of noni fruit extract in the treated birds has better body weight, weight gain, and carcass weight [22]. Adding 133 ml/kg of mineral-free noni fruit extract feed positively affected the egg production of Sentul chickens [1]. Another study [23] revealed that the addition of mineralized mangosteen extract up to 180 mg MPEm/kg ration positively impacted the performance of Sentul chickens. A mixture of 70%:30% mangosteen rind extract and maltodextrin produced a fairly good particle size (<1.00 m) [24]. Based on this description, this study aimed to investigate the effects of maltodextrin microencapsulated noni fruit extract as a feed additive on carcass quality and gut histology in Sentul chickens.

II. MATERIALS AND METHOD

100 Sentul chickens that were fed until the age of 12 weeks were used as test material. The 20 cages used were 1.55m x 0.9m x 0.85m. Completely Randomized Design (CRD) is the type of research used. The study consisted of 5 types of treatment and four replications. The feed components consisted of yellow corn, fine bran, yellow corn, fish, soybean, bone, and CaCO3. Then added noni fruit extract microcapsules for feed additive. Broiler chicken ration contains 17% protein and 2,850 kcal/kg metabolic energy. MPNE is mixed into the ration in the pelleting process. The basic ratio formula and nutritional content in experimental comparisons can be observed in Table 1. and Table 2.

TABLE I BASAL RATIO FORMULATION

Feed Ingredients	Ratio Composition (%)		
Fine brand	15		
Yellow corn meal	59		
Fish meal	9.5		
Soybean meal	15		
Bone Meal	0.5		
CaCo ₃	0.5		
Topmix	0.5		

Source: Calculation Results Based on Afos Application

TABLE II

CONTENT OF NUTRITION AND METABOLIC ENERGY OF USED BASIC RATIONS

Nutrient	Contents	
EM (Kkal/kg)	2781	
Crude Protein (%)	16.57	
Crude Fat (%)	9.59	
Crude Fibre (%)	8.65	
Calcium (%)	1.03	
Phosphor (%)	0.82	
Lysine (%)	1.31	
Methionine (%)	0.57	

A. Manufacture of noni fruit extract microcapsules (Modification of Hardi et al. [25]])

The harvested noni fruit is yellow-white, washed with running water, cut into thin strips ± 0.5 cm thick, and dried in the sun. Sprinkle dry noni with flour and sieve [26]. Noni fruit that has been in the form of flour is done extraction by maceration method using methanol solvent with a ratio of 1:3 and the maceration time is 48 hours. Then the filtrate was filtered and concentrated with a rotary evaporator at a temperature of 60°C and a speed of 40 rpm. Mix the extract and aquadest in a ratio of 1:1. The maltodextrin coating is also mixed with distilled water in the same ratio. Then stirred using a homogenizer for 30 minutes at a speed of 800 rpm. Furthermore, the thick extract was mixed with maltodextrin adjusted to the research formulation, namely 30: 70% until completely mixed. Furthermore, drying using the dry oven method with a temperature of 60°C.

B. Statistical Analysis

The study used an experimental method with a completely randomized design (CRD). The study used five treatments, P0 and P1 were used as controls. The treatment in question is as follows. P0 = Basal ration (RB), P1 = RB + 50 mg/kg ZincBacitracin, P2 = RB + 125 mg/kg MPNE, P3 = RB + 250mg/kg MPNE and P4 = RB + 375 mg/kg MPNE. Duncan's Multiple Test analyzed the data in the study, namely, the Analysis of Variance and differences between treatments. The research was carried out in cages, and the analysis was carried out in the poultry laboratory, Faculty of Animal Husbandry, Universitas Padjadjaran, Jatinangor.

C. The Measured Parameters

1) Carcass Quality: final body weight= live preslaughtering weight (g), Carcass yield = empty carcass weight (g), dressing Percentage= $\frac{empty \ carcass \ weight}{live \ pre-slaughtering \ weight} 100\%,$ of abdominal fat = is found around surrounding the gizzard, cloaca, and adjacent abdominal muscles [27]. The CHOD-PAP (Cholesterol Oxidase Phenylperoxidase Amino Phenozonphenol) method is a method that has been used to determine meat cholesterol levels [28][29]. Meat samples were taken from the breast and thigh. According to the following calculation: level cholesterol $\frac{Sample \ Absorbance}{Sample \ Absorbance} x \ standar \ concentration.$

Standard Absorbance

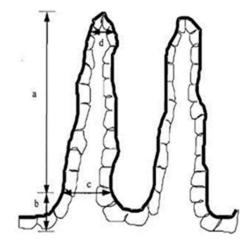


Fig. 1 The histological components of the small intestine: a= height of the villi, b= crypt depth, c= width of basal villi, d= width of apical villi

III. RESULTS AND DISCUSSION

A. Carcass Quality

The effect of MPNE as a feed additive on the quality parameters Sentul is shown in Table 3 and Fig. 2.

TABLE III
CARCASS QUALITY PARAMETERS IN THE TREATMENT OF ADDITION OF MPNE

Variables	P0	P1	P2	P3	P4
Final Body Weight (g)	757.75 ^a	865.67 ^b	892.25 ^b	905.75 ^b	937.75 ^b
Carcass Weight (g)	474.65ª	550.25 ^b	575.35 ^b	583.35 ^b	599.41 ^b
Abdomen fat (g)	5.00 ^a	4.75 ^a	4.75 ^a	5.50 ^a	5.00 ^a
Dressing Percentage (%)	62.64ª	62.56ª	64.48 ^b	64.40 ^b	63.92 ^b
Meat Cholesterol (mg/100g)	114.71ª	102.36 ^a	94.65 ^b	92.12 ^b	91.16 ^b

Note: the same letters a, b, on the same line indicate a non-significant difference (P>0.05)

P0 (Only basal diet), P1 (basal diet +50 mg/kg Zinc Bacitracin), P2 (basal diet + 125 mg/kg MPNE), P3 (basal diet + 250 mg/kg MPNE, P4 (basal diet + 375 mg/kg MPNE)

This means that rations containing MPNE can be digested equally well with ration control and ratio containing Zn Bacitracin. This means that MPNE products range from 125 mg/kg to 375 mg/kg, which does not affect the palatability and appetite of native chickens as the final weight gain increases. It is suspected that the noni fruit is rich in substances that can meet the needs for growth and the necessities of life for chickens, such as carbohydrates, protein, amino acids, and vitamins. So, consuming a lower ratio in the P4 treatment can produce a greater average body weight. Antibacterials contained in alkaloids enhance nutrient absorption by inhibiting bacteria's growth in the digestive tract. The role of alkaloids is under the mechanism of action of antibiotics.

Also, the anthraquinone content in the noni fruit is antifungal and antiseptic to kill or prevent the growth of microorganisms. The protein breaker works optimally. This shows that anthraquinone at the level of administration of 375 mg/kg microcapsules of noni fruit extract works according to its function as a growth promoter to produce higher body weight than the negative control treatment.

²⁾ Intestinal histology: the sample used to count the number of villi was taken from the ileal section 4 -5 cm from the anterior part of the ileum. The preparations were observed with a digital microscope with a magnification of 40 times equipped with a camera and a micrometer and then counted the number, height, width, area, and number of villi and crypts. Measurements of the histological components of the small intestine are shown below in Fig.1.

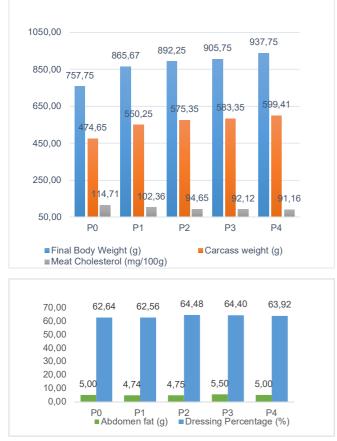


Fig. 2 Average carcass quality graph

Based on the research results, the meat's cholesterol content is 91.16 - 114.71 mg/100g, and the protein content is around 21.50-22.30%. Chicken cholesterol ranges from 100-120 mg [30], and the cholesterol content in the blood of small animals has an average chicken blood cholesterol level of about 52-148 mg/dL [31]. According to the results of this study, mean cholesterol levels for all treatments were within normal limits. Based on the averages in Table 3, it can be seen that treatment with MPNE administration up to a level of 375 mg/kg resulted in slightly lower meat cholesterol levels compared to controls. This is because the noni fruit extract contains active flavonoids and alkaloids. Cholesterol levels can be lowered by flavonoids, namely by inhibiting the performance of the activity of *Hydroxymethylglutaryl-CoA* (HMG-CoA) reductase, flavonoids show great potential to improve blood HDL cholesterol levels due to their antioxidant and antiinflammatory properties [32]. This also follows that HMG-CoA reductase plays a role in converting acetyl-CoA into mevalonate, which causes a reduction in cholesterol synthesis products that occurs when the liver transports cholesterol from the intestine [33]. Flavonoids are secondary plant metabolites that have high inhibitory activity against pancreatic lipase, where this enzyme plays a key role in fat absorption [34] [35]. Giving flavonoids in sufficient quantities will be protective, but if given more than the average, they will become hepatocytes (toxic to the liver). Blood cholesterol levels can decrease in the presence of alkaloids, which inhibit the activity of pancreatic lipase enzymes, which can increase fat secretion through feces so that the inhibition of fat absorption in the liver is disrupted and consequently is not converted into cholesterol. This is indicated by the results [36] that the flavonoid content of noni fruit extract was 5.69 ± 0.21 mg RE/gram extract. In this study, the treatment of noni fruit extract was 87.5 mg/kg ratio, 175 mg/kg ration, and 262.5 mg/kg ration. Thus, the range of flavonoids contained in each treatment of microcapsules of noni fruit extract was 0.5 mg RE; 1 mg RE; and 1.5 mg RE. This shows that flavonoid levels of 0.5-1.5 mg RE can replace the role of AGP Zinc Bacitracin as much as 50 mg/kg in lowering cholesterol levels in Sentul chicken meat.

B. Small Intestine Histology

The following is data for measuring the number of villi, height, width, area, and depth of villous crypts, and the surface area of the small intestine with antibiotics and MPNE at certain levels, which are presented in Table 4 and Fig. 3.

HISTOLOGICAL PARAMETERS OF THE SMALL INTESTINE ON THE ADDITION OF MPNE							
Variable	PO	P1	P2	P3	P4		
Number of Villi	$35 \pm 2,63$ a	$42 \pm 2,63$ b	$45 \pm 3,41 \text{ b}$	$47\pm3,\!59~b$	$44 \pm 4,83$ b		
Villi Width	147,27 ± 3,61 a	$155,\!67 \pm 2,\!92$ b	$155,93 \pm 2,93$ b	$166,34 \pm 3,16$ c	$157,37 \pm 2,93$ b		
Villi height	$628,05 \pm 3,38$ a	$644,02 \pm 7,07$ ab	655,93 ± 5,71 ab	$780,42 \pm 4,17$ c	$668,18 \pm 6,00$ b		
Depth of Crypta	$92,35 \pm 1,46$ a	$101,26 \pm 1,11$ b	$110,97 \pm 6,79$ c	$115,50 \pm 2,67$ cd	117,33 ± 1,99 d		
Surface area	1732,82 ±37,9 a	1744,54±69,54 b	$1743,32 \pm 42,61$ b	1786,93 ± 41,54 c	$1745,76 \pm 58,84$ b		

TABLE IV

Note: the same letters a, b, c, d on the same line indicate a non-significant difference (P>0.05)

P0 (Only basal diet), P1 (basal diet +50 mg/kg Zinc Bacitracin), P2 (basal diet + 125 mg/kg MPNE), P3 (basal diet + 250 mg/kg MPNE, P4 (basal diet + 375 mg/kg MPNE)

The results showed a significant effect on the number of intestinal villi. Treatments P2, P3, and P4, compared with controls, had the highest number of villi. This indicates that small intestinal villi tend to perform more vigorously. That is, by enlarging the basal villus and the villus apical width, which was found in the treatment group that received 125 - 375 mg/kg MPNE. This condition was caused by the anthraquinones contained in the noni fruit which were encapsulated in treatments P2 to P4, which stimulated the growth process of intestinal villi and affected the absorption

of feed and digestive activity in the intestine. In this case, the role of anthraquinones is to stimulate the secretion of digestive enzymes such as amylase, protease, and lipase in the small intestine, resulting in better and optimal nutrient digestion. Several in vivo experiments have shown that encapsulated herbal supplements are superior to non-encapsulated herbs regarding broiler productivity and health. [37]. This condition indicates that the encapsulation process in feed containing herbs can increase the number of villi in the small intestine of poultry.

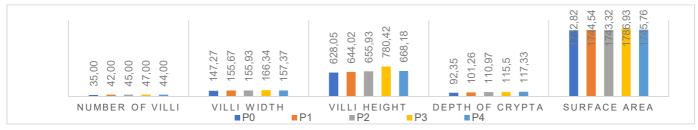


Fig. 3 Average graph of Histological parameters of the small intestine on the addition MPNE

Based on Table 4, the provision of MPNE in the diet also significantly affected villus height and width and small intestinal surface area in Sentul chickens (P < 0.05). P0 was significantly different from P1, P2, P3, and P4, but P1, P2, P3, and P4 were not significantly different. The addition of MPNE 250 mg/kg (P3) in the ratio was the best treatment in producing the widest villi height and width compared to positive (P1) and negative (P0) controls and other treatments. This may be due to the active compound *xeronine*, which helps expand the intestinal opening smoothly and make the intestinal villi taller and wider. The height and width of the intestinal villi will further expand the surface of the intestinal villi so that the absorption of food substances will also increase. The organic growth and carcass increase were caused by the wider villi so that more nutrients were absorbed. [38] In addition, higher villi enlarge the absorptive surface of the luminal capillaries, producing sufficient digestive enzymes and their nutrient transport at the surface of the villus absorption, making nutrient absorption more optimal. Increased villus height in P3 and P4 treatments with a wider range of nutrient intake not only results in smoother nutrient transport systems throughout the poultry system, improved digestive function, and better absorption of nutrients but also improves poultry health, as indicated by increased villi was shown to be healthier at height. Obj., 10)

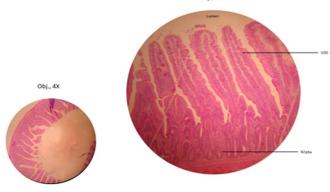


Fig. 4 One sample of treatment intestine histology

Table 4 shows that the crypt depth of the small intestine of Sentul chickens fed with feed additive treatment of 250 mg/kg MPNE was much greater than the treatment without MPNE and with *Zinc Bacitracin*. Factors that can enhance nutrient absorption Include intestinal epithelial surface area, number of folds, villus height, and villus number. The increased height and width of the chicken intestine due to the increased villus area is closely related to increased digestive and absorptive functions of nutrient absorption throughout the body tissue. The surface area of the small intestine can affect its ability to digest and absorb nutrients [38]. So, increased growth capacity in broilers may be associated with increased absorptive activity in the small intestine.

These results are consistent with previous research [39] that supplementation of herbal mixtures (turmeric and bioflavonoid) because broiler diet admixture may increase villus height and width, crypt depth, and surface area compared to controls. More surface area means more surface area to absorb nutrients through the walls of the small intestine, leading to better growth. Feeding can promote intestinal development by increasing intestinal weight, improving intestinal mucosal morphometric properties, increasing regulation of mRNA, and protein expression of tight junctions [40].

IV. CONCLUSION

Administration of noni fruit extract microcapsules (MPNE) at a dose of 250 mg/kg decreased live weight and carcass, reduced meat cholesterol, and increased villi height, width, surface area, and jejunum crypt depth. Giving microcapsules of noni fruit extract at a dose of 125 to 250 mg/kg is recommended as a feed additive to replace AGP for Sentul chickens.

ACKNOWLEDGMENT

This study was funded by the Directorate of Research and Community Service and Innovation, Padjadjaran University, with contract number: 2203/UN6.3.1/PT.00/2022. The authors thank the Chancellor, Director of Research and Community Service and Innovation, and Dean of Animal Husbandry for supporting this research.

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