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OPTIMATION OF NONI FRUIT EXTRACT USING ZINC OXIDE AND COPPER SULPHATE CATALYST AS AN ADDITIONAL FEED AND ITS EFFECT ON INTESTOLOGICAL HISTOLOGY OF SENTUL CHICKEN

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Abstract

The study was carried out experimentally, the first identified noni fruit extract on yield, and bacterial inhibition and the second was the application of noni fruit extract supplemented with Cu and Zn on intestinal histology. The first stage used a nested complete randomized design (CRD) and the second stage used RAL and was further tested with Duncan Multiple Range Test (DMRT). The livestock used were 100 DOC (Day Old Chicken) unsexing Sentul chickens, reared for 12 weeks. Data were analyzed using SAS JMP Pro version 14 software. The results showed that methanol solvent with a maceration time of 48 hours was the best treatment for producing yield, and inhibition of E. coli and S. aureus bacteria. The best treatment was the administration of 250 mg/kg of noni fruit extract supplemented with Cu and Zn. The conclusion is that the addition of noni fruit extract with Cu and Zn supplementation in the ration up to a level of 250 mg/kg can increase the height, width, and depth of the crypts and it is recommended to use it as a feed additive to replace Antibiotic Growth Promoter.

Key words*: antibacterial, intestinal villi, Noni fruit extract, Sentul chicken, yield.*

INTRODUCTION

Noni fruit is an herbal plant that has the potential to be used as additional feed to replace Antibiotic Growth Promoters (AGP). Noni fruit contains some phytogenic compounds and active substances that function as antibacterial, antioxidant, antihelmintic, and anti-cholesterol. The content of antibacterial compounds in noni fruit can suppress the activity of pathogenic microbes in the small intestine, so that it can increase the efficiency of the feed ingredients utilization. Evaluation of the efficiency of utilization of feed ingredients can be done by measuring the digestibility of animal feed ingredients. The higher the digestibility value of a feed ingredient, the more nutrients that are utilized by livestock. The surface area of the intestinal epithelium, the height of the villi, and the number of villi and microvilli will affect the absorption and digestibility of feed ingredients. The higher and wider the intestinal villi, the more nutrients are absorbed and digested. Anthraquinones are antibacterial and antioxidant compounds contained in noni fruit. Anthraquinones can also increase the absorption of ration protein by affecting the pH of the digestive tract to become more acidic. Noni fruit also contains scopoletin, which is a group of simple coumarin compounds that have antibacterial and immunomodulatory activity. The utilization of noni fruit as an additional feed ingredient for poultry must be processed first, because the active compound content of noni fruit is volatile due to improper treatment. Extraction is the main process that can extract antioxidant and bioactive phenolic compounds from a material. Extraction aims to maximize the number of target compounds withdrawn to obtain optimal biological activity in the livestock body. The resulting extraction results are not only influenced by the extraction technique, but also by the extraction solvent. Various kinds of solvents can be used in the extraction process, ranging from polar, semipolar, non-polar, or water solvents, which are usually adjusted to the solubility properties of each solvent according to the compound to be extracted. Some of the things, that affect the extraction of a material, include the type of solvent used and its concentration, solvent ratio, contact time, temperature, and the size of the solid particles to be extracted, besides that

the addition of a catalyst to an extract plays a role in influencing the effectiveness and efficiency of the target compound in the body. Copper sulphate ($CuSO_4$) and zinc oxide (ZnO) are catalysts that can be added to plant extracts. In addition to their role as catalysts, Cu and Zn minerals are also needed in the process of metabolizing food substances in the digestive tract and as metalloenzyme activators. Based on the above background, it is necessary to conduct a study on the utilization of noni fruit extract with ZnO and $CuSO_4$ catalysts as additional feed and its effect on the intestinal histology of Sentul chickens.

MATERIALS AND METHODS

Study area

This study was divided into two stages. Stage 1 was the determination of the solvent and maceration time of noni fruit extract, which produced the greatest yield and inhibition of bacteria. Phase 2 of the study was to determine the best dosage of adding noni fruit extract with ZnO and $CuSO_4$ catalysts into the ration and its effect on the histology of the jejunum of Sentul chickens. The materials used in this study were ripe noni fruit, 96% ethanol, methanol, n-Hexane, chloroform p.a, $CuSO_4$, ZnO, filter paper, methanol p.a, DPPH, ascorbic acid, distilled water, Muller Hinton Agar (MHA), amoxicillin, physiological NaCl, 70% alcohol, sulfuric acid, $BaCl_2$, blank disk, *Escherichia coli* and *Staphylococcus aureus* bacterial cultures, Sentul chicken and Bouin solution. Research Procedure Phase 1 (Sogandi & Rabima, 2019 with modifications) Noni fruit powder is dissolved with several solutions including ethanol, methanol, chloroform, and n-hexane with a ratio of 1: 3. The noni fruit solution was macerated for 24 and 48 hours. After that, the noni fruit solution was filtered using filter paper and concentrated using a rotary evaporator until it became a solid extract, then analysis was carried out on the inhibition of *E. coli* and *S. aureus* bacteria. Data were analyzed using a nested completely randomized design (CRD). The real effect on the measurement parameters was further tested with the Duncan Multiple Range Test (DMRT). The treatment applied in phase 1 research can be seen in Table 1.

Table 1. Noni fruit extraction treatment

Treatment	W1 (24 Hours)	W2 (48 Hours)
P0 (Ethanol)	P0W1	P0W2
P1 (Methanol)	P1W1	P1W2
P2 (Chloroform)	P2W1	P2W2
P3 (*n*-Hexana)	P3W1	P3W2

Phase II Research Procedures

The experimental design used in this phase 2 study was a Completely Randomized Design (CRD). A total of 100 DOC Sentul chickens (unsexing) were grouped into 5 feed treatment groups. The feed treatment applied to each experiment was repeated 4 times. Maintenance is carried out for 12 weeks. Treatment feed was given to livestock in the 2nd to 12th week. The feed treatment applied is P0 = 100% Basal ration (RB), P1 = 100% RB + 50 mg/kg Zinc bacitracin, P2 = 100% RB + 125 mg/kg Noni extract with ZnO and $CuSO_4$ (EBMM), P3 = 100% RB + 250 mg/kg EBMm, P4 = 100% RB + 375 mg/kg EBMm. After 12 weeks of rearing, the chickens were slaughtered and prepared for histological analysis of the jejunum (Iji et al., 2011). The Sentul chicken was dissected from the chest to the neck and its digestive tract was removed. The intestine is cut in the duodenum to the ileocaecocolic junction and the large intestine, then cleaned of fat and mesentery using NaCl. The posterior jejunum of the small intestine is cut 2 cm long and put into a bottle to be fixed in a Bouin solution, then closed tightly and stored for 24-48 hours. After that, remove the small intestine sample from the bottle and put it in another container to be dehydrated with alcohol with graded concentrations (70%, 80%, 90%, and 100%). After that, the sample was cleaned using xylol and blocked into paraffin. Then the sample in the paraffin block container is soaked in cold water to dry the paraffin wax. Furthermore, the intestinal sample was removed and cut with a microtome 5 µm thick and transferred to a warm water bath. Samples in a floating state are taken and attached to the object glass. Then, the sample on the glass object was dried at 37°C, after drying it was painted by immersing the glass object in a container containing haematoxylin solvent for 10 minutes, then rinsed with running water and dipped in alcohol, then dipped in eosin-solvent for 10 minutes. Take measurements using a

microscope with the help of a computer on intestinal histology preparations that are ready in glass objects.

RESULTS AND DISCUSSIONS

Yield and Inhibitory Power of E. coli and S. aureus Bacteria

Noni (*Morinda citrifolia* L.) is a tropical plant that has been used as a food ingredient, spice, and traditional medicine in Southeast Asia for more than 2000 years (Motshakeri & Ghazali, 2015; Yang et al., 2010). Noni fruit contains active components of phenolic compounds, especially coumarins, flavonoids, and iridoid compounds which are useful as antioxidants, antibacterial and antiallergic (Saraphanchotiwitthaya & Sripalakit, 2015). In its ability as an antibacterial, noni fruit can inhibit the growth of several types of bacteria, both Gram-negative and Gram-positive (Azizah & Widjastuti, 2021). Based on the results of statistical analysis, the type of solvent, different solvents, and maceration time had a significant effect (P<0.05) on the yield and inhibition of the bacteria produced. The average yield and inhibition of gram-positive and negative bacteria for each treatment can be seen in Table 2 below.

Based on the results of statistical analysis, the difference in the type of solvent had a significant effect (P<0.05) on the percentage of extract yield, but maceration time had no significant effect (P>0.05). Duncan's test showed that the yields produced from polar solvents (ethanol and methanol) were not significantly different from each other, but significantly different from non-polar solvents (chloroform and n-hexane). The amount of yield produced depends on the solubility of the bioactive components of the sample extracted. The solvent will diffuse into components that have the same polarity level so that the solvent which has the same polarity level as the extracted bioactive compounds will produce a greater yield. The order of the largest yield based on treatment was P1, P2, P3, and P4 namely 21.50%, 20.72%, 4.97%, and 3.81%. Based on this, it is suspected that the active compounds contained in noni fruit mostly consist of polar compounds. The large value of the yield of methanol extract is caused by its polar nature, so it can dissolve almost all organic compounds present in the sample, both polar and non-polar compounds. Research Jacobeb et al (2011) on api-api leaves (*Avicenia marina*) showed that more extract content was obtained using methanol compared to other solvents. The results of this yield are in line with the inhibition of *E. coli* and *S. aureus* bacteria, the average noni fruit extract extracted with polar solvents is higher than non-polar solvents.

Table 2. Average yield and inhibition of noni fruit extract bacteria

Variabel		Treatments							
		P1		P2		P3		P4	
		W1	W2	W1	W2	W1	W2	W1	W2
Yield	Average P(W) (%)	21.65± 2.98	21.35± 3.32	21.67± 10.81	19.77± 5.28	4.91± 1.00	5.03± 1.33	4.31± 2.38	3.3± 1.34
	Average P (%)	21.50 ± 2.83^{b}		20.72 ± 7.68^{b}		4.97 ± 1.05^{a}		3.81 ± 1.81^{a}	
Inhibition *E. coli*	Average P(W) (mm)	6.70± 0.37^{a}	12.17± 0.29^{d}	6.56± 0.64^{a}	12.96± 0.59^{d}	7.91± 0.36^{bc}	8.65± 0.21^{c}	7.62± 0.39^{b}	6.73 ±0.20^{a}
	Average P (mm)	9.48±2.96		9.76±3.55		8.28±0.48		7.18±0.56	
Inhibition *S. aureus*	Average P(W) (mm)	15.47± 0.46	15.50± 1.25	14.27± 0.95	16.35± 1.43	11.29 ±1.05	11.63± 0.06	7.93 ±0.08	7.51 ±0.68
	Average P (mm)	15.48 ± 0.84^{c}		15.31 ± 1.57^{c}		11.46 ± 0.69^{b}		7.75 ± 0.75^{a}	

Different superscripts on the same line show significant differences (P<0.05)
P1 = Ethanol, P2 = Methanol, P3 = Chloroform, P4 = n-Hexane, W1 = 24 hours, W2 =48 hours

Table 3. Average histology of the small intestine of Sentul chickens fed AGP and noni fruit extract at various levels

Variable	Treatment				
	P0	P1	P2	P3	P4
Villi Height (µm)	692.479±29.980[a]	810.51±35.252[c]	735.408±86.065[b]	833.863±13.731[d]	747.555±42.843[b]
Villi Width (µm)	133.563± 3.756[c]	109.437±4.692[a]	109.358 ± 4.189[a]	141.995 ± 6.707[d]	125.998 ± 1.031[b]
Crypte Depth (µm)	51.947 ± 1.449[b]	45.633 ± 2.334[a]	57.031 ± 1.805[b]	66.295 ± 3.105[c]	57.328 ± 8.537[b]

Different superscripts on the same line show significant differences (P<0.05)
P0 = basal diet, P1 = basal diet + 50 mg/kg zinc bacitracin, P2 = basal diet + 125 mg/kg noni fruit extract, P3 = basal diet + 250 mg/kg noni fruit extract, P4 = basal diet + 375 mg/kg noni fruit extract

Based on the results of statistical analysis, the time treatment and the type of solvent used for noni fruit extraction had a significant effect on the inhibition of *E. coli* and *S. aureus* bacteria. Time nested in the solvent showed a significant difference (P<0.05) in the inhibition of *E. coli* bacteria. Polar solvents (ethanol and methanol) with a maceration time of 48 hours have a greater inhibition of *E. coli* bacterial activity than the same solvents with a maceration time of 24 hours, as well as other treatments. The inhibition power of *E. coli* bacteria in noni fruit extract was P2W2, P1W2, P4W2, P3W2, P4W1, and P4W2 respectively with the largest inhibition power of *E. coli* 12.96 mm. Longer extraction times can extract greater antibacterial compounds compared to extracts with shorter maceration times. Extracts with a maceration duration of 48 hours had an average inhibition of *E. coli* bacteria that was greater than extracts macerated for 24 hours. The use of the type of solvent alone had a significant effect (P<0.05) on the inhibition of *S. aureus* bacteria. This shows that both solvent and maceration time have a significant effect on the inhibition of Gram-negative and Gram-positive bacteria. The ability of noni fruit extract to inhibit these bacteria is due to the presence of iridoids (deacetylasperulosidic and asperulosidic acids) (Deng et al., 2011), secondary metabolites such as phenols, steroids, terpenoids, alkaloids, tannins, flavonoids, saponins, glycosides, reducing sugars, and acid compounds (Anugweje, 2015). Wall et al. (2015) reported that organic acids in noni fruit juice include acetic, ascorbic, butyric, citric, dehydroascorbic, galacturonic, malonic, succinic, shikimic, and tartaric acids.

The formation of the inhibition zone area for gram-positive bacteria is generally larger than the inhibition zone formed for Gram-negative bacteria. Noni fruit extract was able to inhibit the activity of *S. aureus* bacteria by 16.35 mm, while the inhibition of *E. coli* bacteria was 12.96 mm. This is due to the characteristics of each bacteria that are different from one another. According to Sudewi & Widya (2016), the difference in the bacterial inhibition of noni fruit extract occurs due to the structure of the bacterial cell wall which affects the sensitivity of the bacteria. Gram-positive bacteria tend to be more sensitive to antibacterial compounds, making it easier for antibacterial compounds to enter gram-positive bacterial cells. The structure of the cell wall of Gram-positive bacteria is simpler than the structure of the cell wall of Gram-negative bacteria, so the penetration of antibacterial compounds in Gram-positive bacteria is easier than that of Gram-negative bacteria.

Based on the research results on the yield value and inhibition of *E. coli* and *S. aureus* bacteria produced, the ethanol and methanol extracts of noni fruit with a maceration time of 48 hours had a higher average than other treatments. The ethanol extract of noni fruit with a maceration duration of 48 hours had a yield of 21.35%, the inhibition of *E. coli* and *S. aureus* bacteria were 12.17 mm and 15.50 mm respectively, while the methanol extract of noni fruit was 19.77% respectively, 12.96 mm and 16.35 mm. The results of these two treatments were not significantly different in each measurement parameter, meaning that both have the same potential to be applied in the second stage of the study.

Histology of intestines

The digestive tract has an important function in absorbing nutrients. In animal production systems, a healthy gut is required to achieve the best performance results, and the concept of gut health can be summarized as a state of gut homeostasis. The results of observations of the histology of the Sentul chicken's jejunum can be seen in Table 3.

Based on Table 3, it is known that the supplementary feeding of noni fruit extract with the supplementation of Cu and Zn minerals had a significant effect (P<0.05) on the height of the small intestinal villi of Sentul chickens. P0 is significantly different from P1, P2, P3, and P4, but P2 and P4 are not significantly different from each other. P3 has a higher average villous height compared to P0, P1, P2, and P4. The order of the largest intestinal villi height was P3 = 833.863 µm, P1 = 810.51 µm, P4 = 747.555 µm, P2 = 735.408 µm and P0 = 692.479 µm. The average height of small intestinal villi of Sentul chickens given a control diet and with the addition of AGP was smaller than the average height of intestinal villi given an additional 250 mg/kg of fruit extract with Cu and Zn supplementation. This shows that the level of addition of 250 mg/kg of noni fruit extract in the ration is the optimal level in producing the best intestinal villi height. Singh et al. (2012) stated that *Morinda citrifolia* has bioactive compounds that function as antimicrobials that can suppress *E. coli* in the small intestine, so the antimicrobial activity of noni fruit extract added to the ration may be the cause of the increase in villi height compared to controls. Satimah et al. (2019) added that increasing the length of the small intestinal villi causes a wider surface area for absorption, so that nutrient absorption becomes more optimal.

The results showed that the provision of additional feed in the ration had a significant effect (P<0.05) on the villi width of the Sentul chicken's small intestine. P0 is significantly different from P1, P2, P3, and P4, but P1 and P2 are not significantly different. P3 had the largest average villi width of 141.995 µm, followed by P0 = 133.563 µm, P4 = 125.998 µm, P1 = 109.437 µm and P2 = 109.358 µm. The addition of 250 mg/kg of noni fruit extract with Cu and Zn supplementation in the ration was the best treatment in producing the largest width of the villi compared to the positive and negative control and other treatments, this is due to the presence of an active compound in noni fruit, namely xeronine, which can help dilate the small intestine and make the intestinal villi widen. The higher and wider the intestinal villi will further expand the surface of the intestinal villi, so that the absorption of nutrients will also increase. emphasized by Asmawati (2014) that the wider the villi, the more nutrients will be absorbed, so that it can have an impact on organ growth, and the carcass will increase. In addition, the anthraquinone active substances contained in noni fruit can stimulate the growth process of the intestinal villi and affect the absorption of feed and digestive activity in the intestine (Widjastuti et al., 2023).

Based on the results of the study in Table 3, the administration of noni fruit extract into the ration had a significant effect (P <0.05) on the depth of the crypts. P0 has no significant effect on P2 and P4 but has no significant effect on P1 and P3. The highest mean jejunum crypt depth was in treatment P3, which was 66,295 µm and the lowest was in treatment P1, which was 45,633 µm. The depth of the crypts of the small intestine of Sentul chickens fed with feed additive treatment of 250 mg/kg of noni fruit extract with Cu and Zn supplementation was much greater than that of the treatment without administration of noni fruit extract and with the provision of AGP feed additives, this was due to the dose of 250 mg/kg Noni fruit extract is the most optimal dose in impacting the growth of small intestinal crypts, in contrast to the smaller intestinal crypt depth when given noni fruit extract at doses of 125 mg/kg and 375 mg/kg in Sentul chicken rations. The crypt depth of the small intestine of Sentul chickens, which was higher compared to other treatments, was in line with the results obtained from observations of villi height, villi width, and villi surface area which was larger than the other treatments. This indicates that the treatment of noni fruit extract feed additive with Cu and Zn supplementation at a dose of 250 mg/kg is the optimal dose for administration in the ration.

Increasing the height, width, surface area of the villi, and depth of the crypts of the small intestine will affect the increase in nutrient digestibility of feed ingredients. Nutrient absorption can be affected by the surface area of the intestinal epithelium, the number of folds in it, the height of the villi, and the number of villi and microvilli which expand the area of absorption (Ruttanavut et al., 2009). Awad et al. (2009) stated that an increase in the height and width of the villi in the chicken intestine is

closely related to an increase in digestive function and absorption function due to the wider area of absorption of nutrients throughout the body's tissues. One of the parameters that can be used for growth performance is the length and morphological structure of the intestine (Fitasari, 2012).

CONCLUSIONS

The best solvent and maceration time of noni fruit in producing yield, and inhibition of *E. coli* and *S. aureus* bacteria is a methanol solvent with a long maceration time of 48 hours. The optimal dose of adding noni fruit extract with Cu and Zn supplementation in producing villi height, villi width, and crypt depth is 250 mg/kg

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TECHNOLOGIES OF ANIMAL HUSBANDRY