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The effect of Noni (*Morinda citrifolia*) leaf shoot meal as an antioxidant and antibacterial against *Salmonella sp.* and *Escherichia coli*

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Abstract. Indonesia as a tropical country is rich in biological resources, among which are known as herbal plants. One of the herbs that are often used is Noni (*Morinda citrifolia*), which has been reported to possess antioxidant and antimicrobial activities. The objective of this study was to evaluate the antioxidant and antibacterial effects of Noni Leaf Shoot Meal (NLSM) to inhibit *Salmonella sp.* and *Escherichia coli* as poultry ration. Two hundred and forty kampung chickens of 4 weeks old were reared until 12 weeks old. The diet treatments were R0= 0% NLSM (control), R1= 0.5% NLSM, R2= 1% NLSM, R3= 1.5% NLSM, R4= 2% NLSM, and R5= 2.5% NLSM. Six treatments and four replications are used with ten kampung chickens per replicate. Data were analyzed using descriptive statistics. The parameters measured were phytochemical analysis, vitamin A, beta carotene, Malondialdehyde (MDA) blood, inhibition zone diameter, and Clearance test. Results showed that NLSM could reduce MDA blood and bacterial concentration. It is concluded that lower MDA concentration in kampung chicken blood indicates that NLSM contains high antioxidants. NLSM can reduced bacterial concentration in chicken blood so it can be used as a natural antibacterial to improve kampung chickens production and immune system.

1 Introduction

Indonesia as a tropical country, is rich in biological resources, among which are known as herbal plants. One of the herbs that is often used is Noni (*Morinda citrifolia*), contain bioactive substances that function as antibacterial, antioxidant or antifungal [1-4]. This plant has the potential to replace antibiotics or antibiotic growth promoters (AGP) which function to fight pathogenic bacteria and increase livestock production. And it does not leave residue in livestock products produced. The use of AGP has been banned in many countries, including Indonesia. The prohibition on the use of AGP in Indonesia has been regulated in Law No. 18/2009 juncto Law No. 14/2014 concerning Animal Husbandry and Health which states the prohibition on the use of feed mixed with certain hormones and /or feed additive antibiotics. Updated with Permentan No. 14/2017 concerning Veterinary Medicines Classification, the government has banned the use of AGP in feed since 1 January 2018. This prohibition was also strengthened by Permentan No. 22/2017 concerning Registration and Distribution of Feed,



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which requires a statement not to use AGP in the feed formula produced for producers who will register feed.

Some parts of the plants can be used as a natural antibacterial source. Leaf is especially containing more antibacterial substances [5]. So far there has been no study of the separation between plant parts in rations. The shoot leaf usually has a better nutrient content than the other parts, so it can be used as poultry feed. However, research on noni leaf shoot meal has not been much, especially its antioxidant and antibacterial effects. So this research aims to evaluate the antioxidant and antibacterial effects of Noni (*Morinda citrifolia*) leaf shoot meal (NLSM) to inhibit *Salmonella sp.* and *Escherichia coli* as poultry ration.

2. Materials and method

This research was carried out in Department Nutrition and Food Technology, Faculty of Animal Husbandry, IPB University. Vitamin A and β -carotene content was analyzed at the Bogor Agro Industrial Center. Phytochemical analysis at Analytical Chemistry Laboratory, blood MDA analysis at the Physiology Laboratory and Bacterial Inhibition and Clearance Test was carried out at the Bacteriology Laboratory of the Faculty of Veterinary Medicine, IPB University.

2.1. Noni leaf shoot meal preparation

Select noni leaf shoots that have 3–4 young leaves. Then thinly sliced and aerated for one day. After that, it was dried in the oven for one day at 55°C. The dried noni leaf shoots were ground to flour.

2.2. Animal and rations

A completely randomized design with six treatments and four replications with ten kampung chickens (Indonesian native chicken) in each replicate was carried out in this research. Two hundred and forty local/native chickens of 4 weeks old were reared until 12 weeks old. The rations treatments were R0: 0% NLSM (control), R1: 0.5% NLSM, R2: 1% NLSM, R3: 1.5% NLSM, R4: 2% NLSM, and R5: 2.5% NLSM. The formulated rations were isocaloric and isoprotein with 17% protein and 2800 kcal kg⁻¹ according to the recommendation of Leeson and Summers [6].

At the end of the experiment 48 native chickens were taken 5 cc of the chicken blood sample was taken from the Axillaris vein (on the wing) using a syringe and then inserted into a vacutainer tube containing EDTA anticoagulant to obtain whole blood. Blood tests were carried out to measure the antioxidant activity of Malondialdehyde (MDA) according to the method of Rice-Evans *et al.* [7] and bacterial concentration by Clearance Test.

2.3. Data analysis

Data analysis using descriptive statistics.

3. Results and discussion

3.1. Noni shoot leaf meal bioactive compound contents

To determine the types of bioactive compounds contained in the material being tested, a phytochemical analysis is carried out. The phytochemical analysis of noni leaf shoot meals is shown in table 1. It appears that noni leaf shoot meal contains alkaloids, flavonoids, tannins, saponins, and steroids. Noni leaf is reported to contain the main components of alkaloids, flavonoids, glycosides, saponins, steroids, tannins, triterpenoids, and amino acids [8,9]. Bioactive compounds that have been identified and isolated from noni plants have been collected by Assi [10] showed that the leaf parts of compounds that were found to include amino acids, flavonoids, triterpenoids, sterols, iridoids, kaempferol, scopoletin.

Table 1. Bioactive compounds of noni leaf shoot meal.

Component	Concentration
Alkaloid (%) ¹	0.23
Flavonoid (%) ¹	5.36
Tanin (%) ²	0.03
Saponin (%) ²	0.26
Steroid (mg 100g ⁻¹) ¹	14.58

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Noni leaf contains protein, lime, iron, carotene, ascorbine, and is known to have antimicrobial, antifungal, antiprotozoa, antidiabetic, antioxidant, antihypertensive, antidiarrheal, and can accelerate wound healing [11]. Groups of steroids and triterpenoids have high anti-microbial activity, both killing pathogenic bacteria by attacking or slowing down the growth of these bacteria [12].

Flavonoids have an important role in human health because of their strong antioxidant activity. It has been widely proven that flavonoids can scavenge free radicals and have good antioxidant activity in vitro and in vivo [13].

The tannin content of NLSM is 0.03% and this finding is lower than that of Suparno (2017) is 0.36% [9]. It could be caused by the difference in the form of noni leaf. If extracts noni leaf used, the secondary metabolites obtained will be higher. Tannins are powerful antioxidants that protect against oxidative tissue damage [14]; they protect animal cells against damage-induced UV radiation [15]. Noni leaf shoot meal tannin content is 0.03% and this yield is lower than that of Suparno [9] is 0.36%. This can be caused by differences in the use of noni leaf forms, where if it is in the form of extracts the secondary metabolites obtained will be higher.

Saponins from *Gypsophila* have antioxidant activity based on the activity of capturing free radicals. In addition, saponins also exhibit antibacterial activity [16]. Mandey (2013) stated that steroid and triterpenoid compounds have a high anti-microbial activity, both killing pathogenic bacteria by attacking and slowing the growth of these bacteria [17]. Likewise and Hassan (2010) reported that *Staphylococcus aureus*, *Salmonella thypimurium* and *Escherichia coli* can be inhibited with saponin in a fraction of 100% methanol from guar flour [18].

Deng (2011) states that noni plants that grow in parts of the world show variations in the type and number of phytochemical compounds [19]. Variation of phytochemical compounds related to differences in environmental conditions during growth (temperature, lighting and soil), level of maturity and activity during harvest and post-harvest (harvest age, maturation, harvesting, storage, transportation and processing).

3.2. MDA Blood

The use of NLSM from 0.5% to 2.5% can reduce the concentration of Malondialdehyde (MDA) kampong chicken blood. The greatest reduction in MDA content was achieved by administering 2.5% NLSM (R5), ie 42.93% compared to controls (1.84 $\mu\text{g/g}$ to 1.05 $\mu\text{g/g}$).

In table 2, it can be seen that a decrease of 29.58% from 0.71 (R0) to 0.50 (R2) occurred at the MDA concentration. This proves that noni leaf shoot flour has potential as a natural antioxidant because it contains vitamins A and β -carotene as well as the presence of flavonoids, tannins, saponins.

Table 2. MDA blood concentration.

Treatments	MDA blood	Decrease (%)
R0	0.71	-
R1	0.50	29.58
R2	0.63	11.27
R3	0.65	8.45
R4	0.59	16.90
R5	0.59	16.90

Note: R0 = control ration; R1 = 0.5% NLSM in ration; R2 = 1% NLSM in ration; R3 = 1.5% NLSM in ration; R4 = 2% NLSM in ration, and R5 = 2.5% NLSM in ration

One of the most commonly used peroxidation indicators associated with oxidative stress is malondialdehyde (MDA) [20]. According to Chen (2009) MDA can be used as an indicator of antioxidant activity [21]. Antioxidants act as a deterrent to lipid peroxidation because antioxidants stabilize free radicals so that they are not harmful to the body [22]. Lower MDA concentrations in kampong chicken blood indicate that kampong chicken contains high antioxidants.

In addition, noni leaf shoot meal contains vitamin A of 405 IU 100 g⁻¹ and the active ingredient in the form of β -carotene is 180 mg kg⁻¹ and secondary metabolite which has the potential as an antioxidant for livestock. β -carotene is a natural antioxidant that plays an important role in reducing free radical chain reactions in tissues, and inhibits lipid oxidation [23–26].

3.3 Antibacterial activity of noni shoots

The concentration of antibacterial activity can be identified by using in vitro Clearance Test. The results of the challenge tests on kampong chicken blood in this study are presented in table 3 and table 4. The results of the study showed that blood samples treated with noni leaf shoot meal affected the endurance of kampong chicken. This is indicated by the results of the final concentration of *Salmonella typhimurium* bacteria and *Escherichia coli* bacteria which are decreasing.

Table 3. Clearene Test results Kampung of chicken blood samples against the population *Salmonella thypimurium*.

Treatment	Final concentration bacteria (cfu ml ⁻¹)	Mortality (%)
R0	2.1 x 10 ¹²	0
R1	3.1 x 10 ⁹	98.52 %
R2	1.2 x 10 ⁹	99.42 %
R3	2.0 x 10 ⁷	99.99 %
R4	1.1 x 10 ⁷	99.99 %
R5	1 x 10 ⁶	99.99 %

Note: R0 = control ration; R1 = 0.5% NLSM in ration; R2 = 1% NLSM in ration; R3 = 1.5% NLSM in ration; R4 = 2% NLSM in ration, and R5 = 2.5% NLSM in ration

Tabel 4. Clearene test of kampung chicken blood samples against the population *Escherichia coli*.

Treatment	Final concentration bacteria (cfu ml ⁻¹)	Death (%)
R0	5.7 x 10 ¹¹	0
R1	3.5 x 10 ⁹	99.38 %
R2	3.8 x 10 ⁸	99.93 %
R3	2.1 x 10 ⁷	99.9 %
R4	2.6 x 10 ⁶	99.99 %
R5	4.6 x 10 ⁵	99.99 %

R0 = control ration; R1 = 0.5% NLSM in ration; R2 = 1% NLSM in ration; R3 = 1.5% NLSM in ration; R4 = 2% NLSM in ration, and R5 = 2.5% NLSM in ration

The potential antimicrobial activity of noni flour is indicated because there are phenol and flavonoid compounds. Oliver et al (2001) stated that phenol compounds have been reported to have antibacterial activity against gram-positive and gram-negative bacteria. Phenol compounds in high concentrations can damage bacterial cell walls and in low concentrations, phenols can interfere with the work of important enzyme systems in bacterial cells [27]. Antimicrobial compounds in noni flour can inhibit the growth of gram-negative bacteria (*Escherichia coli*) while the inhibition of gram-positive bacteria (*BAL*) is relatively weak. The difference in inhibition is caused by differences in bacterial cell walls. Pelczar and Chan (1988) state that differences in cell wall thickness of gram-positive and gram-negative bacteria produce different reactions to phenolic compounds [28].

Damayanti and Suparjana (2007) explain the mechanism of phenol in inhibiting microbes by damaging cell membranes, activating enzymes and denaturing proteins so that permeability is decreased [29]. Changes in permeability will disrupt the transportation of organic ions into cells which results in stunted microbial growth or even microbial death. Flavonoid compounds are compounds that have antibacterial activity by disrupting the function of cell walls so that lysis occurs in bacterial cells. Flavonoids have greater inhibition in gram-positive bacteria than gram-negative bacteria because they are polar so that they penetrate the polar peptidoglycan layer rather than the non-polar lipid layer [30].

Steroids and triterpenoids in noni leaf meal were detected positively as an antibacterial. The mechanism of action of steroids in inhibiting microbes is by destroying the plasma membrane of microbial cells, thus causing cytoplasmic leakage out of the cell which subsequently causes cell death. This is because steroid molecules have non-polar (hydrophobic) and polar (hydrophilic) groups so they have a surfactant effect that can dissolve the phospholipid component of the plasma membrane [31]. The mechanism of triterpenoids as an antibacterial is reacting with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymeric bonds that cause damage to the porin. Damage to the porin which is the entrance and exit of the compound will reduce the permeability of the bacterial cell wall and cause bacterial cells will lack nutrients so that bacterial growth is inhibited or dead.

Saponin is a substance that can increase membrane permeability so that cell hemolysis occurs when interacting with bacterial cells the bacterial cell wall will break or lysis, while tannin compounds can inhibit bacterial growth by coagulating bacterial protoplasm because it forms stable bonds with bacterial proteins [32].

4. Conclusion

Noni Shoot Leaf Meal can decrease MDA concentration and **reduced bacterial concentration in chicken blood so it can be used as natural** antioxidant and **antibacterial to improve** kampung chicken's immune system.

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