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Blood profile, creatinine, and urea nitrogen levels in the blood of male duck fed noni leaf meal (*Morinda citrifolia* Linn)

Widya Hermana[1], *Asella*[1], *Dwi Margi* Suci[1*] and *Arif* Darmawan[1]
[1]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Indonesia

Abstract. The objective of this study was to assess the effect of noni leaf meal on the blood profile, creatinine, and urea nitrogen of male ducks at different levels of inclusion into the diet. The research involved 84 male local ducks that were reared for 6 weeks and fed diets containing crude protein at 18% and metabolizable energy at 3000 kcal/kg. The treatment diets in this study consisted of a control diet with no addition of noni-leaf meal and diets with the addition of noni-leaf meal at 2.5%, 5%, and 7.5%. The experimental design was a completely randomized design with four treatments and three replications. The results indicated that the noni leaf meal had no significant effect on the erythrocytes (2.71-2.88 million/mm³), haematocrit (32.80-37.20%), haemoglobin (12.85-13.88 g%), leukocytes (5.37-10.20 thousand/mm³), and creatine (0.08-0.12 mg/dl), but it decreased blood urea nitrogen. It was concluded that the dietary of up to 7.5% noni leaf meal did not adversely affect blood profile and creatine levels, but reduced blood urea nitrogen levels.

1. Introduction

Noni (*Morinda citrifolia* L.) belongs to the *Rubiacea* family and is an herbal plant that is often utilized in its leaves and fruit as a feed additive for livestock because it contains high bioactive compounds [1]. Noni leaves and fruits have many functions including antibacterial, anti-viral, anti-inflammatory, and antioxidant properties [2]. The content of bioactive compounds is proven to have a positive effect on the health and performance of poultry [3]. The content of anthraquinone, amino acids, glycosides, phenolic compounds, and ursolic acid is known to have antimicrobial, antibacterial, and anti-inflammatory activities [4].

Noni leaves contain 16.7% protein, 3.2% crude fat, 50.1% NDF, 45.8% ADF, and 15.7% lignin (% dry matter) [4]. Noni leaves also contain flavonoid phytochemicals (Quercetin-3-O-β-D-glucopyranoside, Quercetin-3-O-a- L- rhamnopyranosyl-(1-6)-β-D-glucopyranoside), triterpenoids, sterols, iridoids (Citrifolinoside B) [2]. The use of noni leaves as a feed additive needs to be evaluated for its effects on blood profile, urea nitrogen, and creatinine. Blood urea nitrogen levels are the main result of protein metabolism in the body and depend on the breakdown of protein in the liver, which is secreted into the kidneys and then excreted through urine. Creatinine is a muscle mass product that is produced from creatine and then passed through the blood circulation to the kidneys, most of which is filtered by the kidneys and excreted through urine [5]. The study aimed to evaluate the use of noni leaf meal in male local ducks on lipid profile, urea nitrogen, and blood creatinine as excessive phytochemical compounds can have negative effects.

*Corresponding author: dwi_margi@apps.ipb.ac.id

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2. Materials and Methods

2.1. Animal, Ration, and management

All procedures in this study were conducted under the supervision of the animal ethics committee of IPB University. This study used 84 local male ducks of 6 weeks of age and reared for 6 weeks. Ducks were placed in colony cages consisting of 12 plots, equipped with feed and drinking water containers, and given 60 watts of lighting. The study was carried out in the field laboratory of Poultry Nutrition Division, Faculty of Animal Science, IPB University. The ration provided was formulated to meet the nutrient requirements of male local ducks, containing 18% of crude protein and 3000 kcal/kg of metabolizable energy.

The treatments included control rations without noni meal and rations with 2.5%, 5%, and 7.5% noni leaf meal. A completely randomized design was applied with 4 treatments and 3 replications. Detailed composition of the ration and its nutrient content are presented in Table 1.

Table 1 Ration composition of 12-week-old male local ducks and their nutrient content

Feed ingredients	Noni leaf meal			
	(0%)	(2.5%)	(5%)	(7.5%)
Corn	55.00	55.00	55.00	55.00
Rice bran	11.90	9.20	6.60	3.80
Soybean meal	23.30	23.30	23.30	23.30
Fish meal	5.00	5.00	5.00	5.00
Noni leaf meal	0.00	2.50	5.00	7.50
Crude palm oil	2.60	2.80	2.90	3.20
Dicalcium Phosphate	0.75	0.75	0.75	0.75
$CaCO_3$	0.75	0.75	0.75	0.75
NaCl	0.10	0.10	0.10	0.10
Premix	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient content(*as fed* basis)				
Ash (%)	30.52	32.92	31.94	26.82
Crude protein (%)	18.07	18.2	18.4	18.5
Crude fat (%)	3.95	4.52	5.94	5.71
Crude fiber (%)	3.44	3.83	4.22	4.59
Metabolizable energy (kcal/kg)	3002.45	3004	3000	3008
Calcium (%)	0.82	0.87	0.93	0.98
Phosphorus (%)	0.58	0.72	0.78	0.96
Lysine (%)	1.02	1.01	0.99	0.98
Methionine (%)	0.42	0.42	0.41	0.40

Ducks were reared from 6 weeks to 12 weeks of age. Feed was given twice a day at 6:00 am and 5:00 pm at 120-180 g/head/day and drinking water was provided ad libitum. The cage was cleaned daily and wet litter was changed regularly. Body weight was weighed when arrived, and every week until 12 weeks of age. Ambient temperature was recorded daily and is presented in Table 2.

Table 2 Average ambient temperature for 6 weeks (°C)

Week	Morning	Afternoon	Evening
1	28.83	32.67	30.29
2	26.71	35.29	30.00
3	27.43	35.14	31.14
4	27.43	31.14	28.57
5	27.43	33.29	29.57
6	27.14	32.43	28.86

2.2. Noni Leaf Meal Preparation

Fresh noni leaves were wilted in an open room for 1 day and then dried in the sun for 2-3 days. The dried noni leaves were ground into flour and then analysed for nutrient and phytochemical compounds. The nutrient content and qualitative phytochemicals of noni leaves meal are presented in Table 3 and Table 4.

2.3. Blood Profile Measurement

Measurement of blood profile variables, urea nitrogen, and creatinine was conducted on ducks at the age of 12 weeks. Blood samples were collected in the morning from 2 ducks per replicate via the pectoralis vein (vein at the bottom of the wing) as much as 3 ml, then put into an EDTA tube and stored temporarily in a coolbox, then taken to the laboratory for analysis.

Table 3 Noni leaf meal nutrient content

Nutrient	(%)
Dry matter	92.92
Ash	9.72
Crude protein	21.63
Crude fiber	29.38
Crude fat	3.06
Nitrogen free extract	29.13
Calcium	2.28
Phosphor	0.28

Table 4 Bioactive compound of noni leaf meal

Bioactive compound	Results
Phenol	++
Tannin	+
Saponins	+
Flavonoids	++
Steroids	+++
Triterpenoids	+++

+ (weak positive), ++ (positive), +++ (strong positive), ++++ (very strong positive)

2.4. Erythrocyte Count Calculation

Blood samples were sucked using an erythrocyte pipette with the help of an aspirator to the limit of the number 0.5 on the pipette, the tip of the pipette was then cleaned using a tissue. Then Hayem solution was sucked until mark 101 indicated on the pipette quickly, if there were bubbles then the procedure must be repeated. The aspirator was removed from the red blood pipette using the right thumb and forefinger, and the contents of the pipette were shaken in a figure 8 motion pattern. The part that was not shaken should be discarded by attaching the pipette to a tissue paper. A drop of blood was then inserted into the counting chamber by placing the tip of the pipette at the junction between the base of the counting chamber and the cover glass. The blood grains were allowed to settle for approximately one minute. Then it was counted under a 100 times magnification microscope.

The counting of red blood cells was done using a hand counter and was calculated using the formula according to [6].

2.5. Hematocrit Value Calculation

Haematocrit determination was conducted by filling a haematocrit tube with blood and an anticoagulant. The blood mixture was then centrifuged until the blood cells were collected at the bottom. Filling the microcapillary tube was carried out by tilting the tube containing the blood sample by placing the red marked end of the microcapillary. The microcapillary tube was filled with blood until it reached 4/5 part and then the end of the tube was plugged with crest seal, the microcapillary

tube was gentrified for 3 minutes at 2,500-4,000 rpm. The haematocrit value was determined by measuring the red blood cell volume using a microcapillary haematocrit reader [6].

2.6. Hemoglobin Level Calculation

Sahli method was used to measure haemoglobin levels. HCL 0.01 N solution was dripped on the Sahli tube until the 0.1 mark then the blood sample was sucked using a pipette until it reached the top mark. The blood sample was inserted into the Sahli tube and placed between the two parts of the colour standard in the hemoglobinometer and waited for 3 minutes until the colour turned blackish brown due to the reaction of HCl with haemoglobin to form hematin acid. After that, the solution was dripped with distilled water little by little while stirring, until the colour of the solution was the same as the colour of the hemoglobinometer standard. The haemoglobin value was seen in the "gram%" column on the haemoglobin tube, which means the amount of haemoglobin in grams per 100 ml of blood [6].

2.7. Leukocyte Count Calculation

Calculation of leukocyte counts was carried out using a leukocyte pipette with the help of an aspirator to a limit of 0.5 and then the tip of the pipette was cleaned with a tissue. After that, Hayem's modified solution was sucked up to mark11 on the leukocyte pipette, then homogenized with an 8 pattern of hand movement, the unshaken liquid was then discarded. After that, the blood sample was dripped in the hemacytometer and left for a while until the liquid settled and then the number of leukocytes was counted under a microscope at 100 times magnification. To count the number of white blood cells in the hemacytometer, four boxes located at the four corners of the counting chamber were used, each consisting of 16 boxes with an area of 1/16 mm2. The number of leukocytes was calculated by the formula according to [6].

2.8. Leukocyte Differentiation

Blood was dripped and made a review on a glass slide. The review preparations were fixed with 75% methanol for 5 minutes and then removed to air dry. Blood reviews were soaked with Giemsa for 30 minutes, removed and washed using running water to remove excessive dye, and then dried with suction paper. The review preparations were placed under a 1000 times magnification microscope and immersion oil was added to count lymphocytes, heterophils, monocytes, basophils, and eosinophils in a zigzag manner. The number of leukocyte differentiation (%) was calculated from each 100 leukocyte.

2.9. Creatinine

Blood samples were first centrifuged to extract the serum. The centrifuge was done for 15 minutes, and then 0.5 ml of serum was taken into a volumetric flask. 10 ml picric acid solution was added and then added 10% NaOH (0.75 ml) and H_2O (50 ml) into the volumetric flask and then homogenized. The sample was inserted into the quartet observed on a spectrophotometer with a wavelength of 520 nm to obtain the absorbance value.

2.10. Blood Nitrogen Urea

The analysis was performed using a DiaSys KIT with register number (AKL20101804026). The solution used was R1a solution with 1000µ reagent blank and 1000µ sample or standard solution. The solution was mixed and incubated for 5 minutes at 20-25°C. The solution was added and mixed with R2 solution with the help of a vortex, then incubated for 10 minutes at 20-25°C. Then, the samples and standards were measured for absorbance and compared with the blank.

Blood urea nitrogen level= $\frac{Absorban\ Sampel}{Absorban\ Standard}$ x (37.28 mg dl)-1

2.11. Data Analysis

Data were analysed for variance (Analysis of Variance/ANOVA) then Duncan's test was conducted if it showed significantly different results from the analysis of variance.

3. Results

The use of different levels of noni leaf meal in the ration of male local ducks did not significantly affect ($P>0.05$) the blood profile of ducks. The values of lymphocytes, heterophils, and monocytes were not affected ($P>0.05$) by the feeding of noni leaf meal. Feeding noni leaf meal did not affect ($P>0.05$) leukocyte differentiation and H/L ratio. Creatinine levels were still within the normal range and not affected by the use of noni leaf meal in the ration but blood urea nitrogen levels decreased ($P<0.05$) at the level of 2.5% compared to the control treatment.

4. Discussion

The use of different levels of noni leaf meal in the ration of male local ducks did not significantly affect the blood profile of ducks (Table 5).

Table 5 Blood profile of 12-week-old male local ducks fed noni leaf meal

Parameters	Noni leaf meal			
	0%	**2.5%**	**5%**	**7.5%**
Erythrocytes(10^6/mm^3)	2.71±0.44	2.75±0.36	2.88±0.48	2.82±0.30
Hematocrit(%)	35.82±4.24	36.98±2.72	37.20±4.68	32.80±1.84
Hemoglobin(g%)	13.67±1.34	13.10±0.97	13.88±1.61	12.85±1.74
Leukocytes (10^3/mm^3)	9.37±5.70	9.60±6.56	5.37±1.16	10.20±7.01

Dietary noni leaf meal from 2.5% to 7.5% was not significantly different from the control treatment (Table 4). Erythrocyte, haematocrit, haemoglobin, and leukocyte levels in this study were still within the normal range compared to the values reported by [7]. This indicates that the metabolic process in the body takes place normally where phytochemical compounds in noni leaf meal such as tannins and saponins which are known as anti-nutrients do not interfere with the metabolic process and the formation of blood profile components. Indeed, tannin can bind to proteins that can affect the formation of red blood cells as well as it can bind with Fe ions to form complex compounds. Fe ion is presumed to be bound to the hydroxyl group of tannin where Fe^{3+} is reduced to Fe^{2+} when it binds to tannin, then a tannin chelate complex compound is formed. This results in the decreasing availability of Fe^{2+} [8].

The leukocyte differentiation of male ducks fed noni leaf meal is shown in Table 6. The values of lymphocytes, heterophils, and monocytes were not affected ($P>0.05$) by the feeding of noni leaf meal and were in the normal range based on [7]. The results of the study by [9] showed that the use of herbal pulp meal had no negative effect on leukocytes and leukocyte differentials in 32-week-old Mojosari layer ducks. According to [9], the percentage of lymphocytes, heterophils, monocytes, eosinophils, and basophils of Mojosari layer ducks was 86.45 - 88.35%, 8.83-10.48%, 2.73 -3.88%, 2.08 - 3.21%, and 0.47- 1.28%, respectively. However, in this study, the value of eosinophils and basophils was not detected which indicates that ducks do not experience allergies and inflammation [9].

Table 6 Leukocyte differentiation of 12-week-old male local ducks fed noni leaf meal

Parameters (%)	Noni leaf meal			
	0%	**2.5%**	**5%**	**7.5%**
Lymphocytes	54.67±9.56	55.67±27.30	53.67±12.36	45.00±15.40
Heterophiles	41.67±9.52	40.00±28.80	40.17±12.29	51.00±16.00
Monocytes	3.67±1.63	4.17±2.32	5.17±2.79	4.00±1.79
Eosinophils	un	0.17±0.41	un	un
Basophils	un	un	un	un
H /L	0.82±0.42	1.39±1.79	0.88±0.61	1.66±1.88

Note, un: undetected

Feeding noni leaf meal did not affect (P>0.05) leukocyte differentiation and H/L ratio. However, both control and noni-leaf meal treatments showed H/L ratios above normal. The H/L ratio can be used to evaluate the level of stress in livestock [10]. Increased H/L ratio above normal indicates increased stress in ducks, thus spurring increased production of glucocorticoid hormones [11]. Stress conditions may be due to the high environment temperature in this study reaching 35.29°C (Table 2). This assumption is supported by [12] who stated that quail suffer from heat stress at ambient temperatures above 30°C.

Creatinine levels were still within the normal range and not affected by the use of noni leaf meal in the ration but blood urea nitrogen levels decreased (P<0.05) at the level of 2.5% compared to the control treatment (Table 7). This indicates that phytochemical compounds in noni leaf meal in rations up to 7.5% did not interfere with the kidney. Meanwhile, urea nitrogen is an indicator that shows the concentration of urea in serum or plasma. Urea is formed in the liver from amino acid catabolism and is a product of protein metabolism derived from feed.

Table 7 Blood creatinine and urea nitrogen levels of 12-week-old male local ducks fed noni leaf meal.

Parameters	**Noni leaf meal**			
	0%	**2.5%**	**5%**	**7.5%**
Creatinine(mg/dl)	0.12±0.07	0.10±0.04	0.11±0.09	0.08±0.06
Urea nitrogen (mg/dl)	12.35±5.11a	6.07±1.69b	8.52±3.00ab	8.34±1.87ab

5. Conclusions

Feeding noni (*Morinda citrifolia* Linn) leaf meal up to 7.5% did not negatively affect blood profile and keratin levels, but reduced blood urea nitrogen levels.

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