



Research Paper

Nutrient Metabolism in Quails Fed Diets Containing Noni
(*Morinda citrifolia*) Shoot Leaf Meal and Lemuru Fish OilAulia Rahmawati Purnawan¹ , Sumiati Sumiati¹ , Tuty Maria Wardiny^{2*}

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ABSTRACT

Introduction: Quail eggs are highly nutritious but their consumption is limited by their cholesterol content. Noni (*Morinda citrifolia*) shoot leaf meal (NSLM) and Lemuru fish oil (LFO) represent potential alternative feed ingredients. Yet, limited studies have evaluated the effects on nutrient metabolism and egg quality in quail. This research aimed to evaluate the effects of NSLM and LFO on nutrient metabolism, health status, and egg quality in quail.

Materials & Methods: A total of 200 laying quail were allocated to a completely randomized design (CRD) with five dietary treatments and four replications of 10 birds each. The treatments included: T0 (control)=diet with 0% NSLM and 2% palm oil (control); T1=diet with 2.5% NSLM and 2% LFO; T2=diet with 5% NSLM and 2% LFO; T3=diet with 7.5% NSLM and 2% LFO; and T4=diet with 10% NSLM and 2% LFO. Data were analyzed using ANOVA, followed by Tukey's HSD post-hoc test.

Results: The results showed that the diet supplemented with 5% NSLM and 2% LFO (T2) significantly increased ($P<0.05$) the metabolizable energy (ME) value to 2,992.66 kcal/kg, without reducing protein retention ($P>0.05$) or fat retention ($P<0.05$). Intestinal health of quail was not negatively affected, a significant increase ($P<0.01$) in jejunal villus height-to-crypt depth ratio was observed in quail in T2 and T3 treatments. All dietary treatments maintained the nutritional quality of quail eggs ($P>0.05$), particularly in terms of protein and lipid contents.

Conclusion: The T2 formulation (5% NSLM and 2% LFO) was identified as the most effective dietary formulation for optimizing nutrient metabolism, improving intestinal morphology, and producing nutrient-enriched quail eggs.

Keywords:

Egg quality, Lemuru fish oil, Metabolizable energy, *Morinda citrifolia*, Quail

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1. Introduction

The livestock sector is a vital component of agriculture in developing countries, including Indonesia. Eggs serve as an affordable source of animal protein, providing essential nutrients, including protein, minerals, and vitamins. Quail (*Coturnix coturnix japonica*) eggs offer a valuable alternative to chicken eggs, containing 13% protein, 11.1% fat, and 543 IU of vitamin A. However, the average cholesterol content of 844 mg per 100 g of yolk raises concerns regarding cardiovascular health risks [1, 2]. This challenge can be mitigated by producing nutrient-enriched eggs through dietary modification.

Feed modification strategies can improve animal productivity and nutritional quality of animal products, particularly by increasing the content of omega-3 polyunsaturated fatty acids. The concentration of omega-3 in animal products is directly influenced by the fatty acid profile of the diet. Lemuru fish oil (LFO) is rich in omega-3 and has been shown to reduce fat and cholesterol formation [3]. However, diets high in unsaturated fatty acids are prone to oxidation, which can trigger oxidative stress and cellular damage [4]. Natural antioxidants from phytobiotics in the diet may help reduce oxidative stress by inhibiting free radical formation and stimulating endogenous antioxidant enzyme activity [5].

Noni (*Morinda citrifolia*) leaves are rich in natural antioxidants, including β -carotene (161 ppm) and flavonoids (0.21-0.75%), with reported activity of IC_{50} 49.09 μ g/mL that helps neutralize free radicals and improves intestinal villus growth. The shoot leaves, in particular, contain higher protein content (up to 20.64%) along with flavonoids and zinc compared to other parts of the plant [2]. Phytochemicals found in noni shoot leaves, such as flavonoids, tannins, and saponins, can modulate physiological processes related to nutrient metabolism and gut health [6]. Previous research has shown that phytobiotics from antioxidant-rich plants, such as *Moringa oleifera* leaf powder, can enhance antioxidant status, immunity, and nutrient digestibility and metabolism in quail [7]. Therefore, the combination of antioxidants from Noni (*M. citrifolia*) shoot leaf meal (NSLM) and LFO is expected to stabilize omega-3 in the diet, thereby improving the antioxidant status and nutrient metabolism in quail.

Improving feed quality using locally sourced ingredients directly influences quail productivity and health. Metabolizable energy (ME) and nutrient retention are key indicators of nutrient utilization efficiency, while intestinal histology provides insights into gut health status. However, limited studies have examined the combined effect of NSLM and LFO in quail diets. Accordingly, this research aimed to

evaluate the effects of NSLM and LFO on nutrient metabolism, health status, and egg quality in quail.

2. Materials and Methods

2.1. Preparation of NSLM

NSLM were harvested every two weeks, selecting the top four leaves from each branch. These leaves were aerated for one day, then oven-dried at 55 °C for 24 hours [2]. Subsequently, the dried noni leaves were ground gradually using a disk mill (100 kg/h capacity) into a fine powder (1 mm particle size) before feed mixing. The nutrient and phytochemical content of NSLM is presented in Table 1.

2.2. Animal, design, and diet

This study utilized 200 six-week-old Japanese quail (*C. coturnix japonica*) obtained from Slamet Quail Farm, Sukabumi, Indonesia. The birds were randomly assigned to five dietary treatments, with four replications per treatment and 10 birds per replication, housed in multilevel colony cages. A one-week adaptation period was provided for environmental acclimatization, followed by an additional one-week dietary adaptation [8]. The birds were fed and watered ad libitum throughout the 12-week experimental period. The experimental diets were formulated based on the Indonesian National Standard (SNI 01-3907-2006) to meet the nutritional requirements of laying-phase quail (Table 2). To maintain feed quality, the diets were prepared every two weeks to ensure freshness of NSLM and minimize lipid oxidation of LFO. All ingredients were thoroughly mixed using a feed mixer with a 25 kg capacity until a homogeneous mixture was obtained before feeding. The dietary treatments were as follows: T0=diet containing 0% NSLM and 2% palm oil (control), T1=diet containing 2.5% NSLM and 2% LFO, T2=diet containing 5% NSLM and 2% LFO, T3=diet containing 7.5% NSLM and 2% LFO, and T4=diet containing 10% NSLM and 2% LFO.

2.3. Parameter observed

2.3.1. ME and retention nutrient analysis

Digestibility analysis was conducted with slight modifications to the method of Farrell [9]. For each replicate, four quails from each treatment group were fed the experimental diets, while another four quails were fasted to estimate endogenous energy (EE) losses, resulting in a total of 84 quails used in this phase. Before the experiment, all quails were fasted from feed for 24 hours, with drinking water provided ad libitum. To determine EE, the fasted group (4 quails) remained without feed for an additional 24 hours,

Table 1. Phytochemical and nutrient content of NSLM

Nutrient content ¹	Composition
Dry matter (%)	90.63
Gross energy (kcal/kg)	3596
ME (kcal/kg) ³	1695.03
Crude protein (%)	28.43
Crude fat (%)	6.80
Crude fiber (%)	17.18
Calcium (%)	2.21
Total Phosphor (%)	1.84

Quantitative Phytochemistry (%) ²	Composition
β-carotene	7.04
Antioxidant IC ₅₀	0.14
Alkaloids (μg/g)	2.78
Saponins	0.66
Tannins	1.66
Flavonoids	1.61

¹Analysis results of the Laboratory of Feed Science and Technology, IPB University (2024), ²Analysis results of the Testing Laboratory of the Center for Standardization and Agro Industry Services (2024), ³Analysis results of the AMEn (nitrogen-corrected apparent metabolizable energy).

while water was still provided ad libitum. The fed group (80 quails) used for ME analysis received the experimental diets for three days across the five dietary treatments and four replications, at a rate of 80 g per replicate per day. Excreta were collected over four days using trays and sprayed every two hours with 0.01% H₂SO₄ solution. Feed intake was recorded. Excreta samples were weighed, frozen for 24 hours, thawed, oven-dried at 60 °C for 72 hours, milled, cleaned of feathers before analysis of dry matter, crude protein, ether extract, and gross energy according to the *Association of Official Analytical (AOAC)* [10]. Protein (nitrogen) and fat retention, as well as metabolizable energy, were calculated according to Sibbald & Wolynetz [11].

2.3.2. Histopathology of intestines

Jejunum samples from two quails per replicate, corresponding to eight birds per treatment aged 18-week-old, were measured for villi height, basal width, apical width, crypt depth, and villi area. Intestinal histopathology necessitates histological preparations utilizing the hematoxylin-eosin staining according to Wardiny et al. [12].

2.3.3. Egg nutrient analysis

Three eggs per replicate were collected as a composite sample during the last week of quail rearing for nutrient composition analysis. The samples were then subjected to proximate analysis according to AOAC [10].

2.4. Data analysis

Data were analyzed using a one-way analysis of variance (ANOVA) with a post-hoc test of the Tukey HSD test using IBM SPSS Statistics 25 software.

Results

3.1. ME and nutrient retention of experimental diet

Table 3 presents the ME and nutrient retention values for the experimental diets fed to 18-weeks-old quail. The T3 and T4 groups significantly reduced ME of the diets in terms of apparent ME (AME), true ME (TME),

Table 2. Treatment feed composition and nutrient content of laying-phase quail

Feed Ingredients	Composition (%)				
	T0	T1	T2	T3	T4
Yellow corn	52.5	52.5	52.5	51.5	52.5
Rice bran	10	10	10	10	7.9
Soybean meal	22.5	20	17.5	15	12.5
Fish meal	5	5	5	6	7
Noni shoot leaf meal	0.0	2.5	5	7.5	10
Palm oil	2	0	0.0	0.0	0.0
Lemuru fish oil	0.0	2	2	2	2
CaCO ₃	6.7	6.7	6.7	6.7	6.7
DCP	0.5	0.5	0.5	0.5	0.5
Premix**	0.5	0.5	0.5	0.5	0.5
NaCl	0.2	0.2	0.2	0.2	0.2
DL-methionine	0.1	0.1	0.1	0.1	0.1
L-lysine	0.0	0.0	0.0	0.0	0.1
Nutrient Content*					
Dry matter (%)	88.85	88.81	89.14	88.93	89.21
Gross energy (kcal/kg)	4075	3958	3991	3952	3866
Crude protein (%)	17.31	17.1	17.1	17.08	17.69
Crude fat (%)	5.61	4.77	4.18	5.58	3.61
Crude fiber (%)	7.11	7.01	7.09	7.43	7.49
Calcium (%)	3.01	3.19	2.99	2.97	3.14
Available phosphor (%)	0.5	0.51	0.51	0.56	0.59
Lysine (%)	1.15	1.02	0.96	1.06	1.05
Methionine (%)	0.59	0.44	0.40	0.41	0.40

*Analysis results of the Laboratory of Feed Science and Technology (IPB), **Premix composition (per kg premix): Vitamin A 1.200.000 IU, Vitamin D3 200.000, Vitamin E 800 IU, Vitamin K3 200 mg, Vitamin B2 500 mg, Vitamin B6 50 mg, Vitamin B12 1.200, Vitamin C 2.500, Calcium-D-pantothenate 600 mg, Niacin 400 mg, Colicin chloride 1.000 mg, Methionine 3.000 mg, Lysine 3.000, Manganese 1.200, Iron 2.000 mg, Iodine 20 mg, Zinc 10.000 mg, Cobalt 20 mg, Copper 400 mg, Antioxidant 1.000 mg; T0=Diet containing 0% NSLM and 2% palm oil (control); T1=Diet containing 2.5% NSLM and 2% LFO; T2=Diet containing 5% NSLM and 2% LFO; T3=Diet containing 7.5% NSLM and 2% LFO; T4=Diet containing 10% NSLM and 2% LFO.

Table 3. ME and nutrient retention of experimental diets of quail

Variables	Mean±SD					P
	Treatment					
	T0	T1	T2	T3	T4	
AME (kcal/kg)	3031.28±20.77 ^b	2952.65±35.13 ^b	2998.92±46.96 ^b	2716.94±98.04 ^a	2707.17±17.86 ^a	0.001
TME (kcal/kg)	3067.75±20.96 ^b	2989.57±34.93 ^b	3036.09±45.53 ^b	2754.00±97.62 ^a	2744.02±17.61 ^a	0.001
AMEn (kcal/kg)	3024.95±19.23 ^b	2945.26±35.46 ^b	2992.66±46.52 ^b	2711.45±97.18 ^a	2700.10±17.69 ^a	0.001
TMEn (kcal/kg)	3061.43±19.44 ^b	2982.18±35.30 ^b	3029.83±45.10 ^b	2748.51±96.76 ^a	2736.95±17.44 ^a	0.001
Nitrogen retention (%)	88.37±2.25	87.53±4.31	87.82±1.61	86.72±3.12	85.02±3.78	0.622
Fat retention (%)	81.11±6.69 ^b	79.83±4.15 ^b	75.28±3.97 ^b	79.53±4.53 ^b	63.83±1.29 ^a	0.001

Abbreviations: AME: Apparent metabolizable energy; AMEn: Nitrogen-corrected apparent metabolizable energy; TME: True metabolizable energy; TMEn: Nitrogen-corrected true metabolizable energy.

Note: Means in the same row with different superscripts differ significantly ($P < 0.05$). T0=Diet containing 0% NSLM and 2% palm oil (control); T1=Diet containing 2.5% NSLM and 2% LFO; T2=Diet containing 5% NSLM and 2% LFO; T3=Diet containing 7.5% NSLM and 2% LFO; and T4=Diet containing 10% NSLM and 2% LFO

nitrogen-corrected AME (AMEn), and nitrogen-corrected TME (TMEn). Nitrogen retention values were not affected by any of the dietary treatments. Furthermore, dietary treatments significantly reduced fat retention in treatment T4.

3.2. Histopathology of intestines

Table 4 presents the histopathological analysis of quail jejunum intestines. Feeding diets containing up to 10% NSLM and 2% LFO (T1-T4) significantly reduced intestinal histopathology values in quail, as indicated by

decreases in villus height, apical width, basal width, crypt depth, and jejunal villus surface area. However, the villus height-to-crypt depth (V:C) ratio was significantly increased in T2 and T3 treatments.

3.3. Nutrient content of egg quail

Table 5 shows the proximate nutrient composition of quail eggs at the 18th week of the experiment. The results showed that the experimental diets had no significant effect on egg nutrient composition, including moisture, ash, fat, and protein contents.

Table 4. Jejunal histopathology of quail aged 18-weeks-old

Variables	Mean±SD					P
	Treatment					
	T0	T1	T2	T3	T4	
Villus height (µm)	590.11±85.95 ^b	451.75±61.85 ^a	459.20±84.42 ^a	472.28±92 ^a	408.6±75.27 ^a	<0.01
Basal width (µm)	208.36±60.61 ^b	190.48±51.25 ^b	136.13±33.51 ^a	115.3±18.54 ^a	127.56±24.41 ^a	<0.01
Apical width (µm)	156.51±38.85 ^b	160.59±45.04 ^b	111.14±30.3 ^a	84.38±14.29 ^a	94.10±24.65 ^a	<0.01
Crypt depth (µm)	124.05±14.98 ^b	120.26±14.01 ^a	118.36±21.56 ^a	90.73±8.85 ^a	121.58±32.22 ^a	<0.01
Villus area (10 ³ µm ²)	114.38±26.15 ^b	90.05±45.36 ^a	49.02±11.86 ^a	45.36±18.25 ^a	42.21±16.88 ^a	<0.01
V:C ratio	4.82±0.88 ^b	3.84±0.54 ^a	4.30±0.6 ^{ab}	4.77±0.81 ^b	3.73±0.55 ^a	<0.01

Note: Means in the same row with different superscripts differ significantly ($P < 0.05$); V:C ratio: Villus height-to-crypt depth; T0=Diet containing 0% NSLM and 2% palm oil (control); T1=Diet containing 2.5% NSLM and 2% LFO; T2=Diet containing 5% NSLM and 2% LFO; T3=Diet containing 7.5% NSLM and 2% LFO; and T4=Diet containing 10% NSLM and 2% LFO

Table 5. Nutrient content of research quail eggs with proximate analysis

Variables	Mean±SD					P
	Treatment					
	T0	T1	T2	T3	T4	
Moisture content (%)	72.88±0.11	72.97±0.2	73.16±0.3	72.79±0.01	73.42±0.06	0.104
Ash (%)	1.04±0.06	1.07±0.04	1.03±0.08	1.09±0.07	1.04±0.03	0.074
Fat (%)	9.47±0.08	10.37±0.04	10.18±0.21	10.22±0.21	10.83±0.02	0.100
Protein (%)	12.41±1.06	14.47±0.24	14.5±0.01	14.74±0.13	13.86±0.09	0.113

Note: T0=Diet containing 0% NSLM and 2% palm oil (control); T1=Diet containing 2.5% NSLM and 2% LFO; T2=Diet containing 5% NSLM and 2% LFO; T3=Diet containing 7.5% NSLM and 2% LFO; and T4=Diet containing 10% NSLM and 2% LFO.

4. Discussion

4.1. ME and nutrient retention of experimental diet

ME and feed digestibility are closely related; as low digestibility leads to greater energy loss through excreta [13]. In this study, the T2 diet produced the optimal ME for quail. High-fiber diets generally reduced energy metabolism in poultry because the absence of cellulase limits fiber degradation and decreases nutrient digestibility. The inclusion of 2% LFO in all dietary treatments as an additional energy source was insufficient to counteract the adverse effects of NSLM at 7.5% and 10% addition levels. Nitrogen retention remained relatively stable across treatments, indicating efficient protein digestion and absorption. The reduced fat retention in T4 have been influenced by bioactive compounds in NSLM. Saponins affect lipid metabolism by increasing fatty acid oxidation and inhibiting lipid synthesis, thereby reducing fat retention [14]. Nevertheless, the AMEn values in this study still met the minimum ME requirement for laying quail diets (≥ 2700 kcal/kg).

Noni leaves contain bioactive compounds such as tannins and saponins. In this study, estimated intakes at up to 10% NSLM inclusion were 19.12 mg/100 g quail's body weight and 7.60 mg/100 g quail's body weight, respectively. These levels are below the tolerance thresholds reported for 42-day-old broiler diets, which are 30.99 mg/100 g body weight for tannins [15] and 10.80 mg/100 g of broiler's body weight for saponins [16]. However, quail appear to be more sensitive to these compounds than broiler, as evidenced by physiological and metabolic responses. Although tannins are known to bind proteins and digestive enzymes, potentially reducing protein availability [17], feeding with NSLM in this

study did not appear to negatively affect protein utilization efficiency.

4.2. Histopathology of intestines

Jejunal histopathology is a sensitive parameter due to its significant role in nutrient absorption and its usefulness in detecting structural changes in intestinal tissues [18]. NSLM contains flavonoids, tannins, and saponins, which possess antioxidant and antibacterial activity that may influence intestinal villi growth [2]. In this study, reduced villus height and villus area reflects a decrease in absorptive surface area, possibly due to mild antinutritional effects of tannins that reduce epithelial proliferation [19]. In contrast, shallower crypt depth suggests improved gut health and enhanced nutrient absorption capacity, reflecting slower cell turnover and a more stable intestinal lining, which are signs of better mucosal integrity [20, 21]. Crypt depth also reflects the regenerative capacity of villi in response to toxins or inflammation [22]. The highest villus height-to-crypt depth (V:C) ratio observed in T3 reflects optimal absorptive capacity and intestinal integrity, both of which are key indicators of healthy gut morphology [12]. This improvement is attributed to the moderate level of bioactive compounds at 5-7.5% NSLM, without the stronger anti nutritional effects observed at higher levels. Flavonoids may also contribute through antioxidant properties that stimulate villus growth and improve epithelial integrity [23]. Collectively, feeding NSLM at 2.5-10% did not negatively affect intestinal health, as supported by preserved jejunal morphology. The reduction in ME at 10% NSLM (T4) was more likely due to limited fiber digestibility than to intestinal mucosal damage.

4.3. Nutrient content of egg quail

The moisture content of quail eggs across all treatments remained within the standard range, which is essential for maintaining egg freshness and quality of. The ash content of quail eggs may reflect the uniform addition of LFO as a macro-mineral source, as well as the inclusion of NSLM, which is rich in flavonoids that may enhance mineral absorption [24]. The protein and lipid contents of quail eggs showed an increasing trend with NSLM inclusion up to 10%, ranging from 9.47–10.83% for fat and 12.41–14.74% for protein. Compared with the U.S. Department of Agriculture (USDA) standard values of 11.1% fat and 13.1% protein [1], this study suggests lower egg lipid levels and higher protein levels. The stability of nitrogen retention across treatments aligns with the consistent protein content of the eggs, indicating efficient protein utilization to egg synthesis.

Meanwhile, the reduction in fat retention at higher NSLM levels (T4) suggests that bioactive compounds such as saponins may enhance lipid catabolism and reduce lipid deposition in eggs, thereby helping maintain balanced yolk lipid levels [25]. This improvement may also be the synergistic interaction between LFO as an omega-3 source and NSLM as a source of flavonoids and saponin, supporting lipid stability and improving nutrient utilization, which may lead to nutrient-enriched quail eggs.

5. Conclusion

The inclusion of 5% NSLM and 2% LFO in quail diets significantly improved nutrient metabolism and enhanced nutrient retention efficiency without adverse effects on intestinal health, as indicated by an improved villus height-to-crypt depth ratio. Furthermore, this dietary combination also maintained the nutritional quality of quail eggs, supporting the production of nutrient-enriched quail eggs.

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Compliance with ethical guidelines

All research methodologies were approved by the Animals Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia (Code: 039/KEH/SKE/IV/2023).

Data availability

The data supporting the findings of this study are available upon request from the corresponding author.

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Authors' contributions

Conceptualization, study design, analysis, data interpretation, administrative, technical, and material support: All authors; Data acquisition, statistical analysis, and writing the original draft: Aulia Rahmawati Purnawan; Supervision, review and editing: Sumiati Sumiati and Tuty Maria Wardiny.

Conflict of interest

The authors declared no conflict of interest.

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