Nutrient Metabolism in Quails Fed Diets Containing Noni (*Morinda citrifolia*) Shoot Leaf Meal and *Lemuru* Fish Oil

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ABSTRACT

Quail eggs are highly nutritious but limited by their cholesterol content. Noni (*Morinda citrifolia*) shoot leaf meal (NSLM) and *Lemuru* fish oil (LFO) are 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. A total of 200 laying quail were used in a completely randomized design (CRD) with five dietary treatments and four replications of 10 birds each. The treatments included: 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. Data were analyzed using ANOVA with a post-hoc test of Tukey's HSD test. The results showed that feeding 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 ($p<0.05$). The intestinal health of quail was not negatively affected, as indicated by a significant increase ($p<0.01$) in jejunal villus height-to-crypt depth ratio in quail in T2 and T3 treatments. All dietary treatments maintained the nutritional quality of quail eggs ($p>0.05$), particularly in protein and lipid contents. Feeding 5% NSLM and 2% LFO in T2 was identified as the most effective dietary formulation to optimize nutrient metabolism, improve intestinal morphology, and maintain nutrient-enriched quail eggs.

Keywords: Egg Quality; Lemuru Fish Oil; Metabolizable Energy; *Morinda citrifolia*; Quail.

1. Introduction

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

Feed modification can improve animal productivity and nutritional quality of animal products, particularly by increasing the content of omega-3 polyunsaturated fatty acids. The omega-3 concentration in animal products depends on fatty acid profile of the diet. *Lemuru* fish oil (LFO) is a rich source of omega-3 and has been shown to reduce fat and cholesterol formation [3]. However, high levels of 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 µg/mL that helps neutralize free radicals and improve intestinal villus growth. The shoot leaves contain higher protein content up to 20.64%, flavonoids, and zinc than the other leaf parts [2]. Phytochemicals of 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 that flavonoid-based extracts can enhance antioxidant status, immunity, nutrient digestibility and metabolism in quail [7]. The combination of antioxidants noni (*Morinda citrifolia*) shoot leaf meal (NSLM) and LFO are expected to maintain the stability of omega-3 in the diet for improving quail's antioxidant status and nutrient metabolism.

Improving feed quality using local feed ingredients directly influences quail productivity and health. Metabolizable energy (ME) and nutrient retention are key indicators of nutrient utilization efficiency, while intestinal histology reflects 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 Noni (*Morinda citrifolia*) Shoot Leaf Meal

The noni (*Morinda citrifolia*) shoot leaves used the top four leaves from each branch, harvested every two weeks. The leaves were aerated for one day, then oven-dried at 55°C for 24 hours [2]. 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. Nutrient and phytochemical content of NSLM are presented in Table 1.

Table 1 Phytochemical and nutrient content of noni (*Morinda citrifolia*) *shoot* leaf meal (NSLM)

Nutrient content[1]	**Composition**
Dry matter (%)	90.63
Gross energy (kcal/kg)	3596
Metabolizable energy (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

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

2.2 Animal, design, and diet

This study utilized 200 six-week-old Japanese quail (*Coturnix coturnix japonica*) obtained from Slamet Quail Farm, Sukabumi, Indonesia. The quail were randomly assigned to five dietary treatments with four replications per treatment of 10 birds each placed in multilevel colony cages. An adaptation period of one week was provided for environmental adjustment, followed by one week for dietary adaptation [8]. The birds were fed and watered *ad libitum* throughout the 12-week experimental period. The treatment diets were formulated based on Indonesian National Standard (SNI 01-3907-2006) for the nutritional requirements of laying-phase quail (Table 2). The experimental diets were prepared every two weeks to ensure freshness of the NSLM and to minimize lipid oxidation of the LFO. All ingredients were thoroughly mixed using a feed mixer (25 kg capacity) until a homogeneous mixture was obtained before feeding. The dietary treatments consisted of 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 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	0.0
***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^{-1})	4075	3958	3991	3952	3866
Crude protein (%)	17.31	17.10	17.10	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 (%)	0.50	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, Colin 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; and T4 = diet containing 10% NSLM and 2% LFO

2.3 Parameter Observed

2.3.1 Metabolizable Energy and Retention Nutrient Analysis

The digestibility analysis was conducted with slight modification of Farrell [9]. For each replicate, four quails from each treatment group were fed the experimental diets, while another four quails were fasted (not fed) to estimate endogenous energy losses (EE) that total used 84 quails. Before the experiment, all quails were fasted from feed for 24 hours, with drinking water was *ad libitum*. To measured endogenous energy (EE), the fasted group (4 quails) continued without feed for an additional 24 hours, water was given *ad libitum*. The fed group (80 quails) for metabolizable energy (ME) analysis received the experimental diets with five dietary treatments and four replications for three days (80 g/replicate/day). Excreta were collected over four days using trays and sprayed every two hours with 0.01% H_2SO_4 solution. Feed intake was measured. Excreta samples were weighed, frozen for 24 hours, thawed, oven-dried at 60°C for 72 hours, milled, cleaned from feather, and analyzed for dry matter, crude protein, ether extract, and gross energy following AOAC [10]. Retention of protein (nitrogen) and fat, as well as metabolizable energy were calculated according to Sibbald & Wolynetz [11].

2.3.2 Histopathology of Intestines

The jejunum of two quails per replicate (eight birds per treatment) aged 18-week-old quail was measured for villi height, basal width, apical width, crypt depth, and villi area. Intestinal histopathology necessitates histological preparations utilizing the hematoxylin-eosin preparations 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, then analyzed by proximate analysis according to AOAC [10] .

2.4 Data Analysis

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

3. Results

3.1 Metabolizable Energy and Nutrient Retention of Experimental Diet

Table 3 presents the metabolizable energy (ME) and nutrient retention of experimental diets for quail aged 18-weeks-old. The T3 and T4 groups significantly reduced ME of the diets in terms of apparent metabolizable energy (AME), true metabolizable energy (TME), nitrogen-corrected AME (AMEn), and nitrogen-corrected TME (TMEn). Nitrogen retention values were not be impacted by the treatments. Furthermore, dietary treatments significantly reduced fat retention in treatment T4.

Table 3 Metabolizable energy and nutrient retention of experimental diets of quail

Variables	Treatment					p-Value
	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

Note : ^{ab}Means in the same row without common letter are different at $p<0.05$; AME: apparent metabolizable energy; AMEn: nitrogen-corrected apparent metabolizable energy; TME: true metabolizable energy; TMEn: nitrogen-corrected true metabolizable energy; 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

3.2 Histopathology of Intestines

Table 4 presents the histopathological analysis of quail jejunum intestines. Feeding up to 10% NSLM and 2% LFO (T1-T4) for quail diets significantly reduced the value of histopathology of quail's intestine in terms of villus height, apical width, basal width, crypt depth, and jejunal villus surface area in quail. However, the villus height-to-crypt depth (V:C) ratio was significantly increased in T2 and T3 treatments.

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

Variables	Treatment					p-Value
	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 ± 92.00^{a}	408.60 ± 75.27^{a}	<0.01
Basal width (µm)	208.36 ± 60.61^{b}	190.48 ± 51.25^{b}	136.13 ± 33.51^{a}	115.30 ± 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 ± 30.30^{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 ± 0.60^{ab}	4.77 ± 0.81^{b}	3.73 ± 0.55^{a}	<0.01

Note: ^{ab}Means in the same row without common letter are different at $p<0.05$; 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

3.3 Nutrient Content of Egg Quail

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

Table 5 Nutrient content of research quail eggs with proximate analysis

Variables	**Treatment**					p-Value
	T0	**T1**	**T2**	**T3**	**T4**	
Moisture content (%)	72.88 ± 0.11	72.97 ± 0.20	73.16 ± 0.30	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	14.50 ± 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 Metabolizable Energy and Nutrient Retention of Experimental Diet

Metabolizable energy (ME) and feed digestibility are related; low digestibility causes more energy to be lost through excreta [13]. The T2 diet was optimal ME for quail. High-fiber dietary reduced energy metabolism in poultry, as 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. Reduced fat retention in T4 is affected by a bioactive chemical. Saponins affect lipid metabolism by increasing fatty acid oxidation and inhibiting lipid synthesis, leading to reduced fat retention [14]. Nevertheless, the AMEn values in this study still met the minimum ME requirement for laying quail diets (≥ 2700 kcal/kg).

Noni leaf contains bioactive compounds such as tannins and saponins, with estimated intakes during research up to 10% NSLM addition was 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 broiler diets aged 42-days-old are 30.99 mg/100 g of broiler's body weight for tannins [15] and 10.80 mg/100 g of broiler's body weight for saponins [16]. However, quail appear more sensitive to these compounds than broiler, as evidenced by changes in physiological and metabolic responses. Although tannins are known to bind proteins and digestive enzymes, which can reduce protein availability [17], feeding with NSLM in this study did not appear to exert a negative impact on protein utilization efficiency.

4.2 Histopathology of Intestines

Jejunal histopathology is a sensitive parameter due to its significant role in nutrient absorption and reliably indicates structural changes in intestinal tissues [18] . Feeding with NSLM provided flavonoids, tannins and saponins with antioxidant and antibacterial activity that may influence intestinal villi growth [2]. In this study, a reduction of villus height and villus area reflects decreased absorptive surface area, possibly due to mild antinutritional effects of tannins that reduce epithelial proliferation [19]. Furthermore, shallower crypt depth suggests improved gut health and enhanced nutrient absorption capacity, which implies slower cell turnover and stable intestinal lining that indicates better mucosal integrity [20,21]. Crypt depth also reflects the regenerative capacity of villi in response to toxin inflammation [22]. The highest villus height-to-crypt depth (V:C) ratio in T3 reflects optimal absorptive capacity and intestinal integrity, which are key indicators of healthy gut morphology [12]. This improvement is attributed to the moderate level of bioactive compounds in 5% - 7,5% NSLM without the antinutritional effects observed at higher levels. Flavonoids as antioxidant properties that stimulating villus growth and improving epithelial integrity [23]. Collectively, feeding NSLM 2,5% - 10% not negatively affect intestinal health, as supported by preserved jejunal morphology. The ME reduction at 10% NSLM (T4) was more likely caused by limited fiber digestibility rather than intestinal mucosal damage.

4.3 Nutrient Content of Egg Quail

The moisture content of quail eggs across all treatments has standard range that is essential for maintaining the freshness and quality of eggs. The ash content of quail eggs may reflect the uniform addition of LFO as a macro-mineral source and the inclusion of NSLM rich in flavonoids that enhance mineral absorption [24] . The protein and lipid contents of quail eggs showed an increasing trend with the composition of NSLM up to 10%, ranging from 9.47–10.83% for fat and 12.41–14.74% for protein. This study shows egg lipid levels decrease while protein levels increase compared to the USDA standard of 11.1% fat and 13.1% protein [1]. The stability of nitrogen retention across treatments corresponds with the consistent protein content in 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 maintaining balanced yolk lipid levels [25]. This improvement may be the synergistic effect of LFO as an omega-3 source and NSLM as a flavonoids and saponin source that supports lipid stability and improve nutrient utilization, leading to nutrient-enriched quail eggs.

Conclusion

Feeding 5% noni (*Morinda citrifolia*) shoot leaf meal (NSLM) and 2% *Lemuru* fish oil (LFO) in quail diets improved nutrient metabolism and efficient nutrient retention without adverse effects on intestinal health, as indicated by the improved villus height-to-crypt depth ratio. This dietary combination also maintained the nutritional quality of quail eggs supporting to production of nutrient-enriched quail eggs.

Acknowledgment

The authors would like to thank the Poultry Nutrition Division team (IPB) and Universitas Terbuka for collaborating in this project.

The authors acknowledge the use of generative AI, specifically ChatGPT by OpenAI, in the preparation of this manuscript. The AI was used to translate and improve overall language clarity. It did not contribute to the study design, data collection, data analysis, or interpretation of results. All AI-assisted text was carefully edited by the author to ensure accuracy in line with the research objective.

Funding/Support

Institute for Research and Community Service (LPPM) Universitas Terbuka funded this study (Fund number: B/73/UN31.LPPM/PT.01.03/2024).

Author’s Contribution

Study concept and design: A.R.P.; S; and T.M.W.

Acquisition of data: A.R.P.

Analysis and interpretation of data: A.R.P.; S; and T.M.W.

Drafting of the manuscript: A.R.P.

Study supervision and critical revision on the manuscript: S; and T.M.W.

Statistical analysis: A.R.P.

Administrative, technical, material support: A.R.P; S; and T.M.W.

Ethics

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

Conflict of interest

The authors declare that they have no conflict of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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