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Research Article

Impact of *Morinda citrifolia* Leaf Shoot Meal on Gastrointestinal Traits, Organ Histology and Blood Profiles in Quail

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Abstract

Background and Objective: Digestive function and health status can impact the productivity of quail. This study evaluated the effects of Noni (*Morinda citrifolia*) leaf shoot meal (NLSM) supplementation on the gastrointestinal morphology, jejunal histopathology, visceral organ characteristics and hematological profile of quails. **Materials and Methods:** A total of 300 quails (aged 4-14 weeks) were randomly assigned to five dietary treatments: T0 (0% NLSM), T1 (2%), T2 (4%), T3 (6%) and T4 (8%) with four replicates of 10 birds each. A completely randomized design was applied. Data were analyzed using ANOVA followed by Duncan's multiple range test with a 0.05 level of significance. **Results:** About 8% NLSM significantly ($p < 0.05$) increased the percentage of duodenum and relative colon length, while 2-6% NLSM highly significantly ($p < 0.01$) increased the percentage and relative length of jejunum, ileum and colon. Villi area was significantly increased ($p < 0.01$) by 8% NLSM, villus height by 2% and villus height to crypt depth (H:D) ratio by 4%. No significant effects were observed on abdominal fat or major visceral organs (liver, heart, kidney). Hematological analysis revealed that 8% NLSM significantly ($p < 0.05$) increased erythrocyte counts and decreased leukocyte percentages; it also significantly ($p < 0.01$) reduced lymphocyte and eosinophil counts. **Conclusion:** Dietary supplementation with 2-8% NLSM improves nutrient absorption by enhancing gastrointestinal morphology without adverse effects. Moreover, NLSM positively influences immune function, as evidenced by improved hematological parameters and reduced thymus and bile sizes, indicating better physiological and immunological status in quails.

Key words: Antioxidant, hematology profile, gastrointestinal tract, noni leaf shoot meal, quail

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Quail productivity is closely linked to the efficiency of digestive systems¹. According to Syamsuryadi and Khaeruddin², the digestive system plays a fundamental role in the early growth stages of poultry. A well-functioning digestive tract is characterized by optimal organ weight and length development, as well as the proper growth of intestinal villi, which maximize nutrient absorption³.

Blood profile is important for assessing the health status of quail. The blood profile of quails is influenced by various dietary supplements and environmental factors, such as heat stress⁴. Blood has an essential function that supplies oxygen and nutrition, metabolic substances and materials that organize the body's immune system throughout the whole body⁵. Impaired digestive function and compromised health status can negatively impact the productivity of quail. One effort to improve the quail's production and digestive tract quality is by providing herbal plants used in animal feed that are useful as antioxidants and antibacterials.

Noni (*Morinda citrifolia*) has been used as an herbal plant in animal feed because it has a phytobiotic content and is a natural antioxidant. Besides that, noni leaves contain 49.09 µg/mL IC₅₀ of antioxidants, 180 mg/kg of β-carotene⁶ and 0.21 to 0.75% w/w of flavonoids⁷, which can inhibit free radical reactions in the body that cause vitamin deficiencies that impact to poultries growth performance of intestinal villi.

The use of noni leaves as an antioxidant is widely recognized for improving the quality of animal products such as meat and eggs, particularly in lowering cholesterol levels. However, limited studies have been conducted on their potential to enhance digestive tract health in livestock. Therefore, this study aimed to evaluate the effect of noni (*Morinda citrifolia*) leaf shoot meal (NLSM) on the gastrointestinal tract, histopathology of jejunal, visceral organs and hematology profile of quails.

MATERIALS AND METHODS

Ethical clearance: The procedures used in this study were under the rules of the Animal Ethics Commission of SKHB IPB (No. 039/KEH/SKE/IV/2023).

Study area: This study was carried out between March to August 2023 in Field Laboratory C, Poultry Nutrition, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Indonesia.

Preparation of noni leaf shoot meal (NLSM): Noni leaves were sourced from Bogor. According to Wardiny *et al.*⁶. Three to four young shoots were selected for use. The leaves were chopped and left to air-dry for one day to ensure uniform moisture reduction before undergoing oven drying at 55°C for 24 hrs. After drying, the leaves were finely ground. The weight loss of the NLSM during processing was recorded at 16.32%. The nutritional composition of NLSM is presented in Table 1.

Animal and experimental treatments: The experiment used a Completely Randomized Design (CRD) consisting of five treatments and four replications with 15 quails for each replicate. The animals used in this research were 300 laying quails (*Coturnix japonica*) aged 4 to 14 weeks. The diet treatments were T0: 0% NLSM (control), T1: 2% NLSM, T2: 4% NLSM, T3: 6% NLSM and T4: 8% NLSM. Diets were given *ad libitum* at 7:00 and 16:00 during the experiment. Diets are prepared based on the nutrient requirements of the laying quail's production phase by SNI 3907:2023⁸ presented in Table 2.

Sample collection: Samples were collected when the quails were 14 weeks old. Two birds were randomly selected from each replicate following the random sampling method described by Farzin and Seraj⁹. Samples are weighed before and after surgery and then immediately weighed in the area of the organ to be observed.

Gastrointestinal organs and visceral organ analysis: The gastrointestinal tract consists of the proventriculus, ventriculus, duodenum, jejunum, ileum, cecum and colon. Also, visceral organs consist of the liver, heart, kidneys, bile, spleen, thymus, abdominal fat and pancreas. Weight measurements were converted into percentage units (%) by dividing organ weight by cutting weight multiplied by 100% and the intestinal tract was calculated into the relative length (cm/100 g) by dividing organ length by cutting weight multiplied by 100¹⁰.

Histopathology of jejunal analysis: Used the jejunum as a sample for histopathology analysis. The jejunum is often chosen for villi analysis due to its significant role in digestion and absorption¹¹. In this study, the jejunum of quails was examined for villi height, crypt depth and villi area, as well as for the villi height-to-crypt depth ratio, in response to the inclusion of NLSM in the diet. Based on Harimurti and Rahayu¹² method, intestinal histopathology requires making histology preparations using hematoxylin-eosin preparations. The samples were fixed with 10% neutral buffered formalin, left for

Table 1: Nutrient content and active substance of NLSM

Nutrient	Content	Nutrient	Content
Dry matter (%) ¹	88.99	Sodium chloride (%) ¹	0.31
Ash (%) ¹	10.02	Antioxidant IC ₅₀ (%) ²	0.12
Crude protein (%) ¹	21.10	β-carotene (%) ²	3.59
Crude fiber (%) ¹	17.05	Alkaloid (μg/g (%) ³	2.78
Crude fat (%) ¹	6.11	Saponin (w/w (%) ³	1.14
Calcium (%) ¹	1.04	Tannin (w/w (%) ³	5.26
Phosphorus (%) ¹	1.86	Flavonoid (w/w (%) ³	1.46

¹ITP Laboratory (Animal Science Faculty, IPB University), ²Research Institute for Spices and Medical Plants (Bogor, Indonesia), ³Integrated Research and Testing Laboratory (UGM, Yogyakarta, Indonesia) in 2023 and NLSM: Noni leaf shoot meal

Table 2: Composition and nutrient content of the experimental diets

Feed ingredients (%)	Treatments (%)				
	T0	T1	T2	T3	T4
Corn	52.20	52.20	51.50	51.00	51.00
Rice bran	14.00	12.50	11.70	10.70	9.20
Soybean meal	20.00	20.00	20.00	19.50	19.00
Fish meal	5.00	4.50	4.00	4.00	4.00
Crude palm oil	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate (DCP)	0.50	0.50	0.50	0.50	0.50
Calcium carbonate (CaCO ₃)	6.50	6.50	6.50	6.50	6.50
Sodium chloride (NaCl)	0.20	0.20	0.20	0.20	0.20
Premix	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.10	0.10	0.10	0.10	0.10
Noni leaf shoot meal	0.00	2.00	4.00	6.00	8.00
Total	100	100	100	100	100
Nutrient composition					
Dry matter (%)	87.30	87.14	87.33	87.49	85.78
Metabolism energy (kcal/kg)	3,289	3,318	3,228	3,296	3,187
Ash (%)	10.06	10.34	11.46	11.82	11.40
Crude protein (%)	21.31	20.99	19.68	19.82	20.70
Crude fat (%)	4.86	3.73	3.83	3.04	3.34
Crude fiber (%)	4.94	5.23	5.81	5.99	6.12

T0: Control diet, T1: 2% NLSM in the diet, T2: 4% NLSM in the diet, T3: 6% NLSM in the diet and T4 8% NLSM in the diet

24-48 hrs. The tissue was dehydrated with alcohol, placed in xylitol and dipped in paraffin. The samples were sectioned using a microtome and stained using haematoxylin-eosin. The preparations were observed using a microscope and a computer system took measurements three times per slide for each parameter. Random samples of jejunal quails per treatment were divided into eight slides. The surface area of villi was measured by the sum of the base width and apical width, divided by the base width, then multiplied by the villi height¹³. The ratio of villi height to crypt depth was calculated by dividing villi height by crypt depth¹³.

Hematology profile analysis: Blood was collected from 2 quails, each replication, through the brachial vein on the wings using a syringe with a 1-2 mL volume. The blood samples were stored in Vacutainer tubes containing the anticoagulant Ethylene Diamine Tetra Acetic Acid (EDTA).

Statistical analysis: Data on the gastrointestinal tract, visceral organs, hematology profile and jejunal histopathology were

analyzed for variance analysis (ANOVA). Statistical significance was determined at $p < 0.05$. Furthermore, Duncan's Multiple Distance Test was used to determine if significant differences were made using SPSS 25 software.

RESULTS AND DISCUSSION

Gastrointestinal organs and histopathology of the jejunal

quail: Table 3 presents the gastrointestinal organs of quail-fed experimental diets. Using 8% NLSM in diets significantly ($p < 0.05$) increased the percentage of duodenum and relative length of the colon. The addition of NLSM in diets significantly ($p < 0.01$) increased the percentage of jejunum, ileum and colon, as well as the relative length of jejunum, ileum and cecum compared to the control diet.

Table 3 presents the effects of different dietary NLSM (Noni Leaf Shoot Meal) levels on the weight percentage and relative length of various segments of the quail intestinal tract. Body weight gradually increased from 171.11 g (T0) to

Table 3: Weight percentage and relative length of the quail intestinal tract

Organs	Unit	Treatments ($\bar{x} \pm \text{SD}$)				
		T0	T1	T2	T3	T4
Body weight	g	171.11 $\pm$ 17.65	171.20 $\pm$ 14.65	173.54 $\pm$ 26.04	181.24 $\pm$ 16.42	191.52 $\pm$ 31.08
Proventriculus	g	0.78 $\pm$ 0.12	0.90 $\pm$ 0.19	0.87 $\pm$ 0.32	0.88 $\pm$ 0.24	0.83 $\pm$ 0.18
	%	0.46 $\pm$ 0.06	0.53 $\pm$ 0.15	0.44 $\pm$ 0.10	0.49 $\pm$ 0.15	0.43 $\pm$ 0.07
Ventriculus	g	2.95 $\pm$ 0.29	3.64 $\pm$ 0.43	4.05 $\pm$ 0.55	3.85 $\pm$ 0.63	3.93 $\pm$ 1.11
	%	1.74 $\pm$ 0.23	2.13 $\pm$ 0.21	2.36 $\pm$ 0.34	1.84 $\pm$ 0.81	2.05 $\pm$ 0.41
Duodenum	g	0.88 $\pm$ 0.15	0.83 $\pm$ 0.13	1.44 $\pm$ 0.45	1.12 $\pm$ 0.52	1.76 $\pm$ 0.08
	%	0.52 $\pm$ 0.07 ^{bc}	0.48 $\pm$ 0.05 ^c	0.83 $\pm$ 0.27 ^{ab}	0.65 $\pm$ 0.35 ^{ac}	0.96 $\pm$ 0.05 ^a
	cm	11.25 $\pm$ 1.50	13.50 $\pm$ 1.52	14.58 $\pm$ 2.18	12.65 $\pm$ 1.91	14.88 $\pm$ 1.03
	cm/100 g	6.51 $\pm$ 0.74	8.09 $\pm$ 0.68	8.08 $\pm$ 1.33	7.45 $\pm$ 1.75	7.77 $\pm$ 0.32
Jejunum	g	0.78 $\pm$ 0.25	1.54 $\pm$ 0.34	1.54 $\pm$ 0.21	1.47 $\pm$ 0.29	1.75 $\pm$ 0.38
	%	0.48 $\pm$ 0.15 ^b	0.88 $\pm$ 0.18 ^a	0.89 $\pm$ 0.11 ^a	0.85 $\pm$ 0.23 ^a	0.92 $\pm$ 0.04 ^a
	cm	15.63 $\pm$ 4.03	25.00 $\pm$ 2.65	24.67 $\pm$ 5.95	28.70 $\pm$ 2.89	30.17 $\pm$ 2.87
	cm/100 g	9.22 $\pm$ 1.98 ^b	14.73 $\pm$ 1.51 ^a	13.71 $\pm$ 2.72 ^a	16.33 $\pm$ 3.05 ^a	16.30 $\pm$ 1.93 ^a
Ileum	g	0.50 $\pm$ 0.18	0.98 $\pm$ 0.25	1.28 $\pm$ 0.40	0.91 $\pm$ 0.45	1.48 $\pm$ 0.39
	%	0.29 $\pm$ 0.08 ^{bc}	0.60 $\pm$ 0.16 ^a	0.74 $\pm$ 0.17 ^a	0.51 $\pm$ 0.23 ^{ab}	0.73 $\pm$ 0.05 ^a
	cm	15.00 $\pm$ 3.56	22.69 $\pm$ 1.71	23.66 $\pm$ 4.03	25.25 $\pm$ 6.01	25.19 $\pm$ 4.61
	cm/100 g	8.52 $\pm$ 1.14 ^b	13.31 $\pm$ 1.12 ^a	14.24 $\pm$ 3.59 ^a	13.66 $\pm$ 3.64 ^a	13.10 $\pm$ 0.71 ^a
Cecum	g	0.55 $\pm$ 0.15	0.52 $\pm$ 0.25	0.60 $\pm$ 0.30	0.36 $\pm$ 0.21	0.74 $\pm$ 0.17
	%	0.32 $\pm$ 0.08	0.30 $\pm$ 0.14	0.35 $\pm$ 0.17	0.20 $\pm$ 0.11	0.39 $\pm$ 0.09
	cm	12.63 $\pm$ 2.20	12.00 $\pm$ 3.12	15.79 $\pm$ 2.31	12.00 $\pm$ 4.00	17.13 $\pm$ 2.63
	cm/100 g	7.45 $\pm$ 1.56 ^{ab}	7.00 $\pm$ 1.60 ^{ab}	9.27 $\pm$ 1.91 ^a	6.82 $\pm$ 0.04 ^b	9.18 $\pm$ 0.13 ^a
Colon	g	0.25 $\pm$ 0.09	0.34 $\pm$ 0.05	0.37 $\pm$ 0.12	0.18 $\pm$ 0.08	0.55 $\pm$ 0.31
	%	0.15 $\pm$ 0.06 ^{bc}	0.20 $\pm$ 0.04 ^{ac}	0.22 $\pm$ 0.09 ^{ab}	0.10 $\pm$ 0.04 ^c	0.29 $\pm$ 0.13 ^a
	cm	4.56 $\pm$ 1.72	3.94 $\pm$ 1.78	4.63 $\pm$ 1.70	5.90 $\pm$ 1.68	7.71 $\pm$ 2.12
	cm/100 g	2.71 $\pm$ 1.04 ^{ab}	2.30 $\pm$ 1.03 ^b	2.78 $\pm$ 1.31 ^{ab}	3.29 $\pm$ 0.75 ^{ab}	4.05 $\pm$ 0.90 ^a

Means in the same line with different superscripts differ significantly ($p < 0.05$) or ($p < 0.01$), T0: Control diet, T1: 2% NLSM in the diet, T2: 4% NLSM in the diet, T3: 6% NLSM in the diet and T4: 8% NLSM in the diet

191.52 g (T4). The relative weight percentage of the duodenum was significantly higher in T4 (0.96%) compared to T1 (0.48%), with a similar trend observed for duodenal length (14.88 cm) and length per 100 g body weight (7.77 cm/100 g). The jejunum also showed increased weight and length with supplementation, particularly in T4, where the weight percentage (0.92%), length (30.17 cm) and length per 100 g (16.30 cm/100 g) were the highest, showing significant differences compared to T0.

The ileum followed a similar trend, with T2 and T4 treatments showing significantly higher values in both weight percentage (0.74 and 0.73%) and length per 100 g body weight (14.24 and 13.10 cm/100 g) compared to the control. In the cecum, the length per 100 g was significantly increased in T2 and T4 (9.27 and 9.18 cm/100 g, respectively). The colon weight percentage and relative length were also highest in T4 (0.29% and 4.05 cm /00 g), significantly differing from control and lower treatment levels.

Based on this study, NLSM enhanced the duodenum, jejunum, ileum and colon weight percentages. This is related to the antioxidant content of NLSM, antioxidants neutralize ROS generated during metabolic processes, protecting intestinal cells from oxidative damage¹⁴. This impacts the optimal absorption of nutrients, particularly protein, which

works to repair dead cells and create body tissue in the small intestine¹⁵. Furthermore, Che *et al.*¹⁶ found that flavonoids, as antioxidants, can promote gastrointestinal tract development by increasing microflora quality and preventing pathogenic disorders. Kudukhova *et al.*¹⁷ stated that antioxidants can significantly increase the activity of digestive enzymes, improving nutrient breakdown and absorption.

Diet with the addition of NLSM also had a higher crude fiber content. In Table 1, it can be seen that NLSM contains 17.05% crude fiber; however, the crude fiber content in this study is lower compared to the study by Nguru *et al.*¹⁸, which reported that noni leaf meal contains up to 24.99% crude fiber. The crude fiber content in the feed can increase gastrointestinal weight and length¹⁹. Fiber enhances the development of the gastrointestinal tract by stimulating enzyme production and improving the mucosal structure, which supports better nutrient digestion and absorption²⁰. This efficient nutrient absorption is expected to improve the productivity and health of quails.

Noni leaves contain tannins and saponins, which have antioxidant and antibacterial properties²¹. Table 4 shows histopathology parameters of quail. Using 8% NLSM significantly ($p < 0.01$) increased vill area. This effect is likely associated with the presence of a specific metabolite

Table 4: Histopathology of jejunal quail

Variable	Unit	Treatments ($\bar{x}$)				
		T0	T1	T2	T3	T4
Villi height (H)	μm	323.93 ^a	344.26 ^a	333.33 ^a	320.79 ^a	201.62 ^b
Crypt depth (D)	μm	85.54 ^b	127.37 ^a	96.42 ^b	69.66 ^b	87.00 ^b
Apical wide	μm	39.97 ^a	17.54 ^b	33.04 ^a	36.89 ^a	18.23 ^a
Base wide	μm	132.19	121.00	145.79	120.43	153.97
Villi area	$10^{-3} \mu\text{m}^3$	14.10 ^b	21.40 ^b	17.70 ^b	15.00 ^b	52.40 ^a
H:D ratio		3.28 ^b	2.88 ^{bc}	3.46 ^a	3.39 ^a	2.36 ^c

Means in the same line with different superscripts differ significantly ($p < 0.05$) or ($p < 0.01$), T0: Control diet, T1: 2% NLSM in the diet, T2: 4% NLSM in the diet, T3: 6% NLSM in the diet and T4: 8% NLSM in the diet

compound, proxeronase, an enzyme that can convert proxeronine into xeronine, which contributes to increasing villi by maintaining cellular structure and function, to enhance nutrient absorption in the small intestine²². The increase in villi area observed with the inclusion of 8% NLSM in the diet is anticipated to enhance protein absorption in the jejunum.

Table 4 summarizes the histopathological changes in the jejunum. Villus height (VH) was significantly reduced in T4 (201.62 μm) compared to all other groups, while crypt depth (CD) was highest in T1 (127.37 μm). The villus area dramatically increased in T4 ($52.40 \times 10^{-3} \mu\text{m}^3$), suggesting morphological alteration despite shortened height. The height-to-depth (H:D) ratio was highest in T2 and T3 (3.46 and 3.39), indicating optimal mucosal structure, while the lowest ratio was observed in T4 (2.36), suggesting potential villus atrophy or compromised intestinal architecture.

Table 4 shows that using 2% NLSM in diets significantly ($p < 0.01$) increased villi height and using 4% NLSM highly significantly ($p < 0.01$) increased H:D ratio compared to treatment using 8% NLSM. The antioxidant content in NLSM is suspected to improve jejunal morphology, particularly increasing villi height and H:D ratio. Higher villi height is associated with an increased absorptive surface area, which facilitates greater nutrient uptake in the intestines²³. The increased villus height, combined with reduced crypt depth observed with 4% NLSM supplementation in the diet, suggests a lower energy requirement for tissue regeneration, thereby allowing more energy to be allocated to growth and nutrient absorption²⁴.

It is possible that the addition of NLSM to diets can improve jejunal morphology, thereby enhancing nutrient absorption. Although the use of 8% NLSM reduced the H:D ratio, it did not negatively affect jejunal morphology, as it increased the villus area, which also contributes to absorption in the small intestine.

The following is an explanation of the histopathology image of quail jejunum based on image observation. For more details, refer to Fig. 1a-e. Villi of quail-fed NLSM treatments.

Long, slender and well-organized villi structures were seen leading to the lumen. The jejunum mucosal structure is healthy and efficient in nutrient absorption, with no visible tissue damage or villi atrophy. The physiological activity of the mucosa was normal, with no burden or stress from supplementation. There was no indication of edema, inflammatory cell infiltration, or tissue damage.

There was an improvement in the health of the intestinal mucosa, with villi appearing long, erect and tight and more structured than in the control. The T1 had longer villi and a larger area, indicating improved absorption ability. Deeper crypts indicated active epithelial regeneration activity, visible as depressions at the base of the mucosa due to stimulation from 2% NLSM. The tissue structure showed high absorption efficiency, along with an increase in villi area. No pathological signs such as atrophy, necrosis, or edema were found.

Villi appear functionally and morphologically healthy, long, erect and slender with sharp peaks. The mucosal structure is regular, with no tissue damage. Crypts are visible, but not as deep as T1. This is the ideal jejunum structure based on the balance between villi height and crypt depth, which reflects the optimization of the absorption and regeneration process. The dose of 4% NLSM (T2) was the most efficient in maintaining the health of the quail jejunum mucosa. There were no signs of inflammation, degeneration, or atrophy in the tissue. It can be said to be the treatment with the best structural balance among all groups (T0-T4).

The villi appeared quite long and regular but began to show a slight change in shape, with the tip not as sharp as the control group, indicating the ability to absorb nutrients is still optimal. The depth of the crypts appeared normal, although shallower than T1 (2%), suggesting that epithelial regenerative activity was not excessive. The mucosa was still quite thick and there was no major structural damage such as ulceration or desquamation. The intestinal mucosal structure remains healthy and functional, exhibiting no signs of stress or severe damage. This indicates that the administration of 6% NLSM is still histologically safe and does not cause significant damage to intestinal tissue.

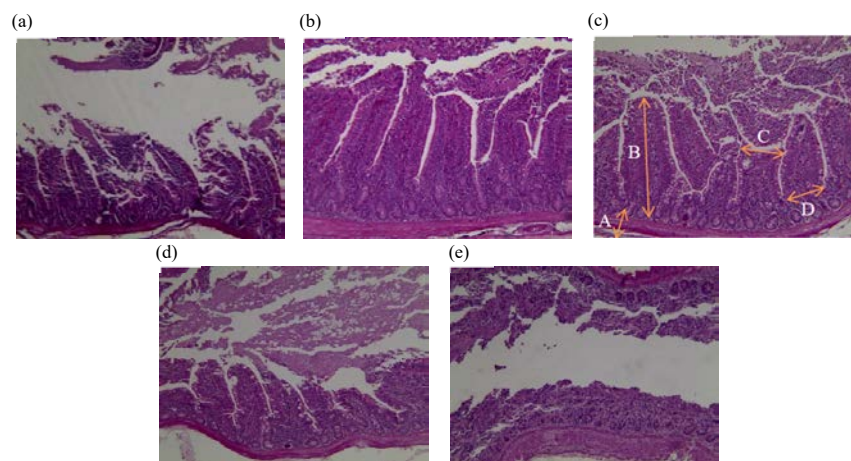


Fig. 1(a-e): Villi of quail-fed NLSM treatments, (a) T0 = control diet, (b) T1 = diet contains 2% NLSM, (c) T2 = diet contains 4% NLSM, (d) T3 = diet contains 6% NLSM and (e) T4 = diet contains 8% NLSM
A: Crypt depth, B: Villi height, C: Apical wide and D: base wide

Table 5: Weight percentage of quail visceral organs

Organs	Unit	Treatments ($\bar{x} \pm SD$)				
		T0	T1	T2	T3	T4
Liver	g	4.57 $\pm$ 0.26	4.11 $\pm$ 0.47	4.40 $\pm$ 0.91	4.09 $\pm$ 0.42	5.21 $\pm$ 1.36
	%	2.70 $\pm$ 0.31	2.42 $\pm$ 0.37	2.58 $\pm$ 0.68	2.26 $\pm$ 0.21	2.75 $\pm$ 0.72
Heart	g	1.18 $\pm$ 0.26	1.28 $\pm$ 0.15	1.19 $\pm$ 0.51	1.23 $\pm$ 0.42	1.29 $\pm$ 0.16
	%	0.70 $\pm$ 0.17	0.76 $\pm$ 0.11	0.70 $\pm$ 0.27	0.68 $\pm$ 0.24	0.68 $\pm$ 0.07
Bile	g	0.14 $\pm$ 0.05	0.19 $\pm$ 0.05	0.24 $\pm$ 0.07	0.28 $\pm$ 0.15	0.16 $\pm$ 0.07
	%	0.08 $\pm$ 0.03 ^b	0.11 $\pm$ 0.03 ^{ab}	0.14 $\pm$ 0.05 ^a	0.16 $\pm$ 0.08 ^a	0.09 $\pm$ 0.04 ^b
Kidney	g	0.56 $\pm$ 0.27	0.76 $\pm$ 0.46	0.85 $\pm$ 0.17	0.80 $\pm$ 0.22	0.89 $\pm$ 0.40
	%	0.32 $\pm$ 0.14	0.46 $\pm$ 0.29	0.49 $\pm$ 0.09	0.45 $\pm$ 0.11	0.45 $\pm$ 0.13
Pancreas	g	0.32 $\pm$ 0.10	0.36 $\pm$ 0.10	0.35 $\pm$ 0.10	0.44 $\pm$ 0.24	0.37 $\pm$ 0.14
	%	0.18 $\pm$ 0.06	0.20 $\pm$ 0.04	0.20 $\pm$ 0.07	0.19 $\pm$ 0.16	0.21 $\pm$ 0.07
Abdominal fat	g	0.65 $\pm$ 0.56	1.22 $\pm$ 0.98	0.61 $\pm$ 0.49	0.65 $\pm$ 0.46	0.82 $\pm$ 0.56
	%	0.36 $\pm$ 0.30	0.67 $\pm$ 0.54	0.33 $\pm$ 0.27	0.36 $\pm$ 0.25	0.45 $\pm$ 0.31
Thymus	g	0.39 $\pm$ 0.05	0.25 $\pm$ 0.07	0.21 $\pm$ 0.06	0.23 $\pm$ 0.12	0.17 $\pm$ 0.11
	%	0.23 $\pm$ 0.04 ^a	0.14 $\pm$ 0.03 ^b	0.13 $\pm$ 0.04 ^b	0.12 $\pm$ 0.06 ^{bc}	0.08 $\pm$ 0.04 ^c
Spleen	g	0.27 $\pm$ 0.29	0.05 $\pm$ 0.02	0.08 $\pm$ 0.06	0.08 $\pm$ 0.04	0.09 $\pm$ 0.03
	%	0.16 $\pm$ 0.27	0.02 $\pm$ 0.05	0.04 $\pm$ 0.08	0.05 $\pm$ 0.08	0.04 $\pm$ 0.09

Means in the same line with different superscripts differ significantly ($p < 0.05$) or ($p < 0.01$), T0: Control diet, T1: 2% NLSM in the diet, T2: 4% NLSM in the diet, T3: 6% NLSM in the diet and T4: 8% NLSM in the diet

Villi appear short, thickened, and irregular, with narrow apical surfaces. The space between villi is widened, showing a decrease in mucosal density. The crypta structure is less deep and indistinct, indicating decreased mucosal regeneration activity. The jejunum of quail receiving 8% NLSM showed adverse histopathologic changes, including villi atrophy, shape changes, and potential mucosal irritation.

Visceral organs of quail: Table 5 presents the weight (g) and percentage (%) of various visceral organs of quail under five different treatment groups (T0-T4). Liver weight percentage ranged from 2.26 to 2.75%, with the highest value observed in T4. The heart percentages were relatively stable across all treatments (0.68-0.76%). Bile percentage increased

significantly in T2 and T3 (0.14 and 0.16%) compared to T0 and T4 (0.08 and 0.09%), showing treatment-related variation (a,b superscripts indicate significance). Kidney, pancreas and abdominal fat showed no marked percentage differences among treatments. Thymus weight percentage significantly decreased from T0 (0.23%) to T4 (0.08%) with distinct groupings (a-c superscripts), indicating a treatment effect. The spleen showed a sharp drop in percentage from T0 (0.16%) to T1-T4 (0.02-0.05%), though variation was high in T0.

The NLSM contains antioxidants that can help the liver work without increasing its size. Animal activities and feed fiber content influence heart size²⁵. The kidneys have more catalase antioxidant activity, indicating that more hydrogen peroxide is broken down in the kidneys²⁶. The

Table 6: Hematology profile of quail blood

Organs	Unit	Treatments ($\bar{x} \pm \text{SD}$)				
		T0	T1	T2	T3	T4
Erythrocytes	item/mm ³	2.98 $\pm$ 0.23 ^b	3.62 $\pm$ 0.21 ^a	3.44 $\pm$ 0.20 ^{ab}	3.67 $\pm$ 0.39 ^a	3.73 $\pm$ 0.58 ^a
Leukocytes	item/mm ³	16.13 $\pm$ 3.49 ^a	12.53 $\pm$ 2.71 ^{ab}	13.93 $\pm$ 6.32 ^{ab}	10.96 $\pm$ 0.69 ^b	11.00 $\pm$ 1.99 ^b
Haemoglobin	g%	11.93 $\pm$ 1.53	12.05 $\pm$ 1.43	11.40 $\pm$ 1.67	11.53 $\pm$ 1.26	12.00 $\pm$ 0.88
PCV	%	37.00 $\pm$ 1.69	37.13 $\pm$ 3.87	35.63 $\pm$ 2.56	34.75 $\pm$ 3.49	37.00 $\pm$ 2.22
Lymphocytes	%	60.02 $\pm$ 1.41 ^a	56.80 $\pm$ 1.58 ^b	57.12 $\pm$ 2.27 ^b	56.68 $\pm$ 1.16 ^b	53.72 $\pm$ 2.78 ^c
Neutrophil	%	36.07 $\pm$ 4.17	28.12 $\pm$ 2.86	29.06 $\pm$ 3.24	28.49 $\pm$ 2.59	29.39 $\pm$ 3.74
Eusinophil	%	11.48 $\pm$ 0.64 ^a	8.05 $\pm$ 0.99 ^b	7.53 $\pm$ 1.41 ^b	7.93 $\pm$ 1.31 ^b	7.76 $\pm$ 1.91 ^b
Monocytes	%	3.77 $\pm$ 0.61	3.64 $\pm$ 0.58	3.74 $\pm$ 0.67	3.92 $\pm$ 0.79	3.67 $\pm$ 0.56
Basophil	%	0.89 $\pm$ 0.04	0.88 $\pm$ 0.06	0.91 $\pm$ 0.05	0.88 $\pm$ 0.06	0.90 $\pm$ 0.06

Means in the same line with different superscripts differ significantly ($p < 0.05$) or ($p < 0.01$), T0: Control diet, T1: 2% NLSM in the diet, T2: 4% NLSM in the diet, T3: 6% NLSM in the diet and T4: 8% NLSM in the diet

pancreas can be affected by thickening of the duodenal wall because the enzyme amylase is secreted in ingested crude fiber. The absence of significant differences in these organs indicates that there is no swelling of the organs due to excessive work. Diets with high crude fiber cause the organs to work more, but are balanced with antioxidants, which help reduce organ work. The larger organ size indicates high detoxification due to tannin and saponin compounds²⁷.

The liver produces more bile salts, which are stored in the gallbladder, increasing its capacity; one factor comes from NLSM saponins²⁸. Using 8% NLSM also reduced the thymus weight to 0.08%. As an immune organ, the thymus produces T-lymphocyte cells, which help fight against pathogens or infections in the body²⁹. The decrease in organs indicates the antioxidant ability of NLSM, which can reduce free radicals that are excessively produced in the quail's body and cannot be balanced with endogenous antioxidants. The more efficiently the thymus works, the more accessible it is to reduce the production of T-lymphocyte cells and the size of the thymus.

Hematology profile of quail: Table 6 shows the results of the blood profile that gives NLSM in quail diets. 8% of NLSM in diets significantly ($p < 0.05$) increased erythrocytes and decreased the percentage of leukocytes; in addition, highly significantly ($p < 0.01$) decreased the percentage of lymphocytes and eosinophils.

Diet with 8% of NLSM in the diets increases erythrocyte levels (3.73 ± 0.58 items per mm³) within the standard range of 2.30-3.86 items per mm³ and reduces leukocyte levels from 11.00 ± 1.99 items per mm³ to 20-40 grains per mm³, which occurs due to temperature conditions in the cage that reach 30-36 °C, making quail stressed³⁰. The increase in erythrocyte levels occurs due to the high demand for oxygen and the antioxidants that help supply it and facilitate circulation properly³¹.

A decrease in leukocyte percentage on a diet with 8% NLSM compared to control suggests the presence of antioxidants that help overcome inflammation due to environmental stress so that lymphocytes are not produced in excess. This is supported by a significant decrease in lymphocytes and eosinophils, reaching 53.72 and 7.76%. Lymphocytes in this study are still within the normal range of 30-60%³⁰. Exposure to pathogenic agents in poultry triggers the proliferation of lymphocyte cells³². Lymphocyte production in controls classified as high in standards occurs due to high environmental temperatures. However, it can be supplied with exogenous antioxidants from NLSM so that the treatment decreases in lymphocytes.

The percentage of eosinophils in this study is lower compared to the study by Nadia *et al.*³², which involved vitamin E supplementation in the feed and resulted in an eosinophil percentage of 10-11%. The higher eosinophils indicate that the body's defenses in preventing infection are pretty good³³. The condition causes a decrease in eosinophils in the diet with 8% NLSM due to the content of the antioxidant NLSM, which helps the spleen work to reduce the production of white blood cells, including eosinophils, when inflammation or oxidative stress.

CONCLUSION

In summary, supplementation of NLSM at 2, 4, 6 and 8% in the diet effectively enhances nutrient absorption within the gastrointestinal tract of quails without causing any adverse effects. Moreover, the inclusion of NLSM positively modulates the immune response, as indicated by elevated erythrocyte counts, reduced leukocyte levels and decreased sizes of the thymus and bile, suggesting improved physiological and immunological status.

SIGNIFICANCE STATEMENT

The digestive tract is vital for nutrient absorption and health in quail, yet the use of natural feed additives for gut enhancement remains underexplored. This study evaluates the effects of *Morinda citrifolia* leaf shoot meal (NLSM) supplementation (2-8%) in quail diets. NLSM significantly improved intestinal morphology, particularly jejunal villous structure and intestinal length, enhancing nutrient absorption. At 8%, NLSM also boosted immune response by increasing erythrocyte count while reducing lymphocyte and eosinophil levels, without causing visceral organ hypertrophy. These findings highlight the potential of NLSM as a phytobiotic feed additive that enhances digestive health and physiological balance. This supports the sustainable integration of herbal, antioxidant-rich ingredients in poultry diets as alternatives to synthetic additives.

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