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Indigofera in Combination with *Spirulina* to Modulate the Gut Environment and Enhance Feed Efficiency in Local Meat-Type Muscovy Ducks (*Cairina moschata*)

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ABSTRACT

Despite the individual recognition of *Indigofera zollingeriana* and *Spirulina platensis* as functional poultry feed ingredients, their synergistic effects remain largely unexplored. In this study, the effects of *I. zollingeriana* and *S. platensis* on growth performance, carcass traits, blood biochemistry, and the intestinal environment were evaluated. In a randomized block design, five dietary treatments were administered to local-meat type Muscovy ducks, with five replicates of five birds per treatment. The diets that were tested included the following: T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); And T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%. Compared with the control diet, the combined inclusion of *Spirulina* and *Indigofera* significantly decreased the feed conversion ratio (p<0.05), while feed intake (FI) and body weight gain (BWG) remained unaffected. Carcass weight and percentage remained statistically comparable across treatments, with T3 yielding the highest numerical value. In summary, *I. zollingeriana* and *S. platensis* were combined to create a beneficial combination that improved feed efficiency and the gut environment without affecting carcass traits or organ development. Among the treatments, the combination consisting of 15% I. zollingeriana showed relatively favorable numerical responses in several measured variables, although these differences were not statistically significant for some variables.

Keywords: feed conversion ratio; Indigofera zollingeriana; local meat-type Muscovy ducks; Spirulina platensis; Waterfowl

INTRODUCTION

Over the past few decades, the global consumption of duck meat has been steadily increasing, mainly because of population growth, urbanization, and shifting dietary habits, which now include a wider variety of animal protein sources. The latter could not be satisfied only with the production of 7 million tonnes of duck meat, which was estimated to have occurred in 2023 (Food and Agriculture Organization, 2025). China, being the largest contributor, accounts for more than 80% of the total (Our World Data, 2025). Duck consumption has profound historical roots and remains a prevalent dietary practice throughout East and Southeast Asia, driven predominantly by China. Ducks are in the meals and the celebrations of the whole day. However, it is not just the Asian market. The rising income levels in other developing areas, along with the increasing awareness of nutritional benefits, continue to drive demand for it. While the poultry industry has grown at an exponential rate, the global supply of broiler chickens has not been able to keep up with the tremendous demand for alternative protein sources. Hence, alternative birds such as Muscovy ducks (*Cairina moschata*) have become potential sources for crop adaptation and the compatibility of certain meat traits, which could contribute to food security. In Indonesia, the massive introduction of indigenous Muscovy duck strains is a priority to not only broaden the range of domestic poultry production but also reduce the import of feedstuffs.

Nonetheless, feed cost is the main limiting factor in the production of Muscovy ducks, contributing between 65% and 75% of total operating costs (Rufino *et al.*, 2017). This excessive dependence on imported protein sources, such as soya bean meal, causes high and unstable production costs. To solve this problem, the processing of alternative plant protein components is a sustainable strategy. Among promising candidates are *Indigofera* (*Indigofera zollingeriana*) and *Spirulina* (*Spirulina platensis*), which not only provide high-quality protein (Tistiana *et al.*, 2025) but also contain functional bioactive compounds that can improve growth performance, nutrient utilization, and immune health in poultry (Tanjung *et al*., 2025). As previously reported, *Indigofera* is a leguminous shrub whose crude protein content (CP) ranges from 14%–31%, and it has a well-balanced amino acid composition and mineral profile (Snowball *et al*., 2021; Hassan *et al*., 2026). Its shade tolerance facilitates its establishment, and its relatively high biomass yield makes it a sustainable feed resource for Muscovy ducks. Moreover, *Spirulina* is known worldwide as a blue–green microalga that consists of essential fatty acids, vitamins, and pigments (Agusetyaningsih *et al*., 2025; AlFadhly *et al*., 2022). *Spirulina* contains phycocyanin, a potent antioxidant compound (Park *et al*., 2018). In poultry diets, it serves as a natural growth enhancer and immune enhancer with the potential to enhance gut health, antioxidant properties, and product quality (Agusetyaningsih *et al*., 2025; AlFadhly *et al*., 2022).

The addition of *Indigofera* and *Spirulina* to Muscovy duck diets is a strategy for the production of functional animal feeds designed to increase productivity and physiological robustness. The blend is expected to be synergistic. On the other hand, *Indigofera* offers a sustainable protein source, and *Spirulina* has bioactive and immune-modulating potential. However, the findings remain inconsistent. For instance, Maura *et al.* (2025) reported that the inclusion of spirulina powder significantly improved the villus height and duodenal morphology in Peking ducks. Conversely, a significant difference was observed in erythrocytes (Agusetyaningsih *et al*., 2025). In spite of this, earlier investigations focused mainly on *Indigofera* and *Spirulina*, with limited information available about their use together, particularly in Muscovy ducks. The potential synergistic effects and optimal inclusion levels of these two functional feed ingredients on growth performance, intestinal health, and physiological responses are still unclear. Therefore, the novelty of this study lies in the evaluation of a combined *Indigofera*–*Spirulina* dietary strategy as a functional protein source in Muscovy ducks, with a focus on identifying optimal inclusion levels and assessing their synergistic effects on growth performance, gut morphology, and hematological variables. Accordingly, the objective of this study was to evaluate the effects of different inclusion levels of *Indigofera* and *Spirulina* in the diets of meat-type Muscovy ducks and to determine the most effective combination for improving productivity and physiological health.

MATERIALS AND METHODS

Study Area and Ethical Approval

After an ethical review was conducted, the Research and Innovation Ethics Committee of Universitas Brawijaya granted ethical approval for the experimental procedures, with ethical approval no. 184/EC/KEPK/07/2025.

Study Period and Location

The study was conducted from June 5 to August 28, 2025, at the Muscovy duck Mr. Prayitno, Rambanan hamlet, Randugading village, Tajinan sub district, Malang Regency, East Java. Serum biochemical analysis was conducted at the Clinical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Additionally, intestinal histopathology was performed concurrently at Satwa Sehat Laboratory, Malang City, East Java Province, Indonesia (latitude 7.9549° S and longitude 112.5947° E).

Ducks, Diets, and Experimental Design

A total of 125 day-old local meat-type Muscovy ducks, with initial body weights ranging from 40 to 64 g/bird, were stratified into five blocks based on their body weight. The birds were ranked from lowest to highest weight, and dietary treatments were randomly assigned within each block. Each treatment comprised five replicates with five ducks per pen (two males and three females). The ducks were reared and evaluated over an 84-day (12-week) experimental period. The ducks were raised in an open-space facility, using colony cages that measured 2.5 × 2 m^2 each. Muscovy ducks were vaccinated against Newcastle disease and infectious bronchitis (ND-IB) via eye drops at 3 and 45 days of age. The 24-hour illumination of the Muscovy duck was also included. The incandescent light bulbs were utilized as a heat source to control the temperature of the houses. During the initial 3 days, the brooding temperature was maintained at 30–34 °C and then gradually decreased each week to 28–30 °C for fourteen days. In strict compliance with Indonesian government regulations (PERMENTAN/14/16/2017), the ducks were not given antibiotic growth promoters (AGPs) at any point during the study. The composition of the experimental diets, which included yellow maize, rice bran, commercial concentrate, and premix components, is shown in Table 1. The dietary treatments were as follows: T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%. The feeding experiment utilized a randomized block design (RBD).

Data Collection and Analysis

Growth performance, carcass quality, and relative organ weight. During the first day of the experiment, the initial body weights were examined on a pen basis and recorded to ensure uniformity in the replicates and treatments. In addition, the final body weight (FBW), FI, BWG, and FCR were examined at weekly intervals for 12 weeks (Adli *et al*., 2025). Feed intake was calculated as the total amount of feed offered minus the weight of the residual unconsumed feed. Every day, the ducks were observed for mortality or abnormal health conditions, and the number of dead

Table 1. Nutrient composition of diets provided to Muscovy ducks under different *Spirulina* and *Indigofera* inclusion levels

Ingredients (%)	Treatments				
	T0	T1	T2	T3	T4
Starter periods (1-21 d)					
Yellow maize	45.00	40.50	40.50	38.50	38.00
Rice bran	14.50	14.50	14.50	14.50	5.00
Commercial concentrate	40.00	34.00	33.50	31.00	40.50
Premix	0.50	0.50	0.50	0.50	0.50
Spirulina powder	0.00	0.50	1.00	0.50	1.00
Indigofera powder	0.00	10.00	10.00	15.00	15.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition (%)					
Dry matter	87.50	87.45	87.44	87.42	87.66
Crude protein	20.05	20.06	20.07	20.04	21.48
Fat	5.00	5.14	5.15	5.24	4.06
Crude fibre	4.82	4.91	4.91	4.97	4.09
Metabolizable energy (Kcal/kg)	2,952.00	2,960.00	2,960.51	2,966.69	2,985.00
Lysine	0.88	0.84	0.84	0.82	0.95
Methionine	1.06	1.05	1.05	1.05	1.22
Methionine + cysteine	1.12	1.13	1.13	1.13	1.24
Proximate composition (%)					
Moisture	12.50	12.90	12.10	13.10	13.40
Crude protein	20.04	20.73	20.84	20.97	21.03
Fat	3.67	3.67	3.69	3.65	3.66
Crude fibre	5.33	6.27	6.25	6.74	6.71
Ash	8.64	9.06	9.01	9.20	9.20
Mineral composition					
Calcium	0.95	0.99	0.98	1.01	1.26
Total phosphorus	0.61	0.58	0.5	0.57	0.65
Energy value					
Metabolizable energy (Kcal/kg)	2,872.00	2,934.00	2,934.00	2,965.00	2,951.00
Grower – Finisher periods (22-84 d)					
Yellow maize	45.00	40.50	40.50	38.50	38.50
Rice bran	14.50	14.50	14.50	14.50	14.50
Commercial concentrate	40.00	34.00	33.50	31.00	30.50
Premix	0.50	0.50	0.50	0.50	0.50
Spirulina powder	0.00	0.50	1.00	0.50	1.00
Indigofera powder	0.00	10.00	10.00	15.00	15.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition (%)					
Dry matter	87.50	87.45	87.44	87.44	87.42
Crude protein	19.55	19.06	18.97	19.10	19.00
Fat	4.96	5.14	5.15	5.19	5.20
Crude fibre	4.78	4.91	4.91	4.92	4.92
Metabolizable energy (Kcal/kg)	2,842.00	2,906.00	2,960.00	2,966.00	2,971.00
Proximate composition (%)					
Moisture	12.50	12.80	12.90	12.90	12.70
Crude protein	18.27	18.48	18.59	18.47	18.58
Fat	4.16	4.16	4.19	4.16	4.18
Crude fibre	5.95	6.81	6.79	7.24	7.22
Ash	7.52	7.70	7.66	7.79	7.74
Mineral composition					
Calcium	0.82	0.82	0.81	0.82	0.81
Total phosphorus	0.56	0.52	0.52	0.50	0.50
Energy value					
Metabolizable energy (Kcal/kg)	2,827.00	2,908.00	2,911.00	2,950.00	2,953.00

Note: d – days, kcal – kilocalorie, kg – Kilogram; % – Percentage; T0 (Control): Basal diet without the addition of *Spirulina or Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%. Nutrient requirements were formulated based on SNI 8508:2018 standards for duck feed.

local meat-type Muscovy ducks with any condition other than normal, such as lameness, ruffled feathers, lethargy, broken wings, diarrhea, or injury, was recorded, and the affected ducks were removed. This was carried out to calculate the FCR on the basis of the actual number of birds for that day. At the end of the feeding trial, one local meat-type Muscovy duck from each replicate (totalling 25 birds) was randomly selected and slaughtered by slitting the jugular vein. The relative organ weight and body mass and different intestinal compartments of the birds were recorded on day 84. After the live weight of Muscovy ducks was determined, the jugular vein, trachea, and carotid artery were cut. They also opened their abdominal cavity and performed thorough surgery on all visceral organ(s) to extract the carcass. Analysis of individual organs, including the heart, liver, spleen, proventriculus, and ventriculus, as well as other organ components, such as the duodenum, jejunum, and ileum, for the determination of intestinal weight and length or the cecum using an analytical balance. To determine their proportional yields, the breast, wings, and legs were separated and weighed as major components of commercial carcasses.

Blood collection and biochemical analysis. Before the blood sample was taken, the ducks were fasted for 8–12 hours with access to water. Blood samples were collected from the brachial vein (*Vena brachialis*) into plain vacutainer tubes without any anticoagulant. After the clotting process at room temperature, the samples were centrifuged at 704.34 × g for 10–15 minutes (Hettich® Universal 320/320R centrifuge, Andreas Hattic GmbH, Germany) to obtain the serum, following the steps by Sjofjan *et al.* (2025). The serum lipid profile variables, such as total cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein levels, were subsequently determined using diagnostic kits according to the manufacturer's instructions. Total cholesterol and triglycerides were analyzed using the CHOD-PAP and GPO-PAP (glycerol-3-phosphate oxidase–phenol amino phenazone) methods, respectively. HDL concentration was measured following precipitation of non-HDL lipoproteins, whereas LDL concentration was calculated using Friedewald's equation:

$$LDL\left(\frac{mg}{dL}\right) = Total\ cholesterol - \left(HDL + \frac{triglycerides}{5}\right) \quad (1)$$

The manufacturer's instructions were followed for all reactions, with incubation at 37 °C if needed. A UV–Vis spectrophotometer was used to measure the absorbance, which was subsequently compared to that of standard solutions before the concentrations were computed.

Measurement of small intestinal digesta pH. Immediately after slaughter, digesta samples were collected from the duodenum, jejunum, and ileum. Approximately 5 g of digesta from each intestinal segment was placed in marked film containers and stored at 4°C before the analysis. Digesta pH was examined using a calibrated digital pH meter following the steps described by Sjofjan *et al.* (2025).

Measurement of ileal digesta viscosity. Ileal digesta samples were collected immediately after intestinal dissection and stored at a low temperature until analyzed. Viscosity was determined without dilution using a digital viscometer. Measurements were performed at 60 rpm for 10 minutes, and the results were expressed in mPas, referring to the steps provided by Sjofjan *et al.* (2025).

Measurement of small intestinal length and weight. At 12 weeks (84 days) of age, ducks were fasted for 8 h with free access to water prior to slaughter. The small intestine was excised, cleaned of adhering mesenteric tissues, and measured without stretching. Intestinal length was determined using a measuring tape, whereas intestinal weight was recorded using a digital balance according to Sjofjan *et al.* (2025).

Measurement of intestinal histomorphology. At 84 days of age, ileal samples were collected from two birds per replicate following slaughter, resulting in a total of 10 samples. Ileum segments were excised approximately 10 cm proximal to the cecum, rinsed with physiological saline solution, and fixed in 10% formalin solution for 48 h. Samples were subsequently dehydrated through a graded ethanol series, cleared in xylene, embedded in paraffin wax, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E) following the procedures outlined by Sjofjan *et al.* (2025).

Histopathological variables, including the number of villi, height and width of the villi, surface area of the villi, villus-to-crypt ratio, and crypt depth, were evaluated under a light microscope (Nikon Eclipse Ei) equipped with an Optilab microscope camera. Measurements were obtained from five randomly selected fields per sample at 100× magnification and analyzed using ImageRaster software. The average value from the five fields was used for statistical analysis (Sjofjan *et al*., 2025).

Statistical Analysis

All statistical analyses and graphical visualizations were conducted using Python (version 3.10), employing Pandas, SciPy, Matplotlib, and Seaborn, imitating data visualization referring to Adli *et al.* (2025). Statistical significance was defined as $p<0.05$, in which $0.05 \leq p < 0.10$ was considered to indicate statistical significance, and was determined using Duncan's multiple range test.

The model is written as follows:

$$Y_i = \beta_0 + \beta_i X_i + \varepsilon_i \quad (2)$$

where: Y_i represents the observed variable; β_i represents the effect of *Indigofera zollingeriana* and *Spirulina platensis* inclusion levels; X_i is the level of *I. zollingeriana* and *S. platensis* in the diet (%); ε_i is the random error; $\beta_i > 0$ if the responses decrease with supplementation; and $\beta_i < 0$ if the responses decrease with supplementation.

RESULTS

The growth performance of Muscovy ducks is depicted in Table 2. Across all the treatments, the FCR significantly decreased ($p<0.05$) (3.4 ± 0.13) compared with that of the control (4.2 ± 0.49). Although neither feed intake nor body weight gain (BWG) differed significantly among the groups, birds in the second treatment (T2) exhibited numerically optimal growth variables. As shown in Table 3, carcass traits ranged from 61.27% to 63.17% across treatments, representing a range of 1.9%. Compared with the control treatment, T3 was slightly better (+0.88%) and T2 (+0.13%). Moreover, the differences in carcass weight improved to 26% throughout the entire treatment period. The highest carcass weight was observed in the T3 treatment group, which represented an increase of approximately 25.8% compared with that in the control group (Table 3). Additionally, the effects of the treatments on relative organ weights are summarized in Table 4. Taken together, these findings revealed no marked differences in relative organ weight among the treatments. The intestinal environmental variables are listed in Table 5. Compared with the other treatments, the different treatments resulted in significant differences in viscosity, with the T4 treatment having the greatest difference ($p<0.05$) (5.4 ± 0.83). Interestingly, the intestinal weight was also markedly different ($p<0.05$) among these experiments. Similarly, the *E. coli* population was significantly lower in both T3 and T4 (6.226 ± 0.05; 6.292 ± 0.15) (Table 6).

Additionally, as shown in Table 7, the biochemical variables of Muscovy ducks were significantly affected by dietary treatment. Total cholesterol levels were significantly different, with T0 presenting higher levels (169.8 mg/dL) compared to T1 (148.0 mg/dL) ($p<0.05$). However, compared with the other treatments, T2, T3, and T4 presented significantly higher levels (lower limits). T4 had the lowest cholesterol value (133.4 mg/dL), despite being statistically similar to T2 and T3. The pattern observed for the LDL concentration was similar. LDL levels in ducks were significantly higher during treatment with T0 (78.4 mg/dL vs. T3) and T4 ($p<0.05$) than they were during the other treatments. Despite the lack of significant differences between T2, T3, and T4, T3 had the lowest recorded LDL level (65.8 mg/dL). In comparison, the HDL concentration increased in response to specific treatments. In terms

Table 2. Growth performance variables of Muscovy ducks fed diets containing *Spirulina* and *Indigofera*

Variables	Treatments				
	T0	T1	T2	T3	T4
FBW (g)	2926.20 ± 379.78	3291.00 ± 322.93	3580.20 ± 262.16	3704.60 ± 253.79	3324.60 ± 534.69
Feed Intake (g)	11925.00 ± 271.64	11814.40 ± 433.99	11907.80 ± 834.84	12782.20 ± 400.83	12416.00 ± 939.69
BWG (g)	2875.00 ± 384.55	3240.00 ± 320.51	3470.00 ± 219.09	3161.00 ± 211.67	3233.00 ± 487.67
FCR	4.20 ± 0.499^{b}	3.669 ± 0.31^{a}	3.432 ± 0.134^{a}	4.057 ± 0.276^{a}	3.882 ± 0.40^{a}

Note: T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%. Means within a single measured variables with different superscripts differ significantly ($p<0.05$). BWG = body weight gain; FBW = final body weight; FCR = feed conversion ratio.

Table 3. Carcass quality variables of Muscovy ducks fed diets supplemented with *Spirulina* and *Indigofera*

Carcass quality	Treatments				
	T0	T1	T2	T3	T4
Carcass yield	62.29 ± 5.53	62.13 ± 2.27	62.42 ± 3.57	63.17 ± 2.94	61.27 ± 8.52
Carcass weight (g)	1864.00 ± 529.87	2037.00 ± 156.03	2229.00 ± 122.55	2344.80 ± 248.96	2014.00 ± 611.26
Wing meat (g)	1157.00 ± 376.10	1244.40 ± 69.28	1337.40 ± 61.24	1471.20 ± 201.7	1257.20 ± 419.16
Breast meat (%)	61.46 ± 2.78	61.17 ± 1.46	60.11 ± 3.70	62.60 ± 3.17	62.11 ± 3.04
Leg quarter (g)	605.40 ± 149.78	673.60 ± 72.54	766.60 ± 42.68	763.40 ± 51.74	667.20 ± 178.74
Percentage of Leg	32.80 ± 1.89	33.04 ± 2.17	34.40 ± 1.02	32.69 ± 1.99	33.50 ± 2.31

Note: T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%.

Table 4. Relative organ weight variables of Muscovy ducks fed diets supplemented with *Spirulina* and *Indigofera*

Relative organ weight	Treatments				
	T0	T1	T2	T3	T4
Proventriculus (g)	13.30 ± 1.25	21.50 ± 4.77	21.10 ± 2.38	21.55 ± 4.48	19.62 ± 0.47
Ventriculus (g)	87.64 ± 13.38	92.64 ± 10.56	90.51 ± 6.37	94.41 ± 5.40	89.34 ± 5.51
Spleen (g)	2.75 ± 0.27	3.23 ± 1.44	3.25 ± 0.56	3.22 ± 1.32	3.334 ± 0.72
Heart (g)	17.44 ± 2.21	20.58 ± 2.20	20.77 ± 3.96	19.97 ± 2.73	21.85 ± 7.54
Liver (g)	56.82 ± 1.08	66.24 ± 9.91	69.7 ± 2.97	67.66 ± 4.80	72.49 ± 7.41

Note: T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%.

Table 5. Intestinal environment variables of Muscovy ducks fed diets supplemented with *Spirulina* and *Indigofera*

Intestinal environment	Treatments				
	T0	T1	T2	T3	T4
Viscosity	3.20 ± 0.837^{b}	3.60 ± 1.14^{a}	2.20 ± 0.837^{b}	3.80 ± 1.30^{a}	5.20 ± 0.83^{a}
pH Duodenum	5.574 ± 0.18	5.58 ± 0.32	5.71 ± 0.25	5.82 ± 0.17	5.942 ± 0.10
pH Jejunum	6.222 ± 0.194	6.402 ± 0.19	5.986 ± 0.09	6.368 ± 0.12	6.334 ± 0.08
pH ileum	6.65 ± 0.31	6.996 ± 0.33	7.034 ± 0.27	6.87 ± 0.19	7.074 ± 0.06
Intestinal weight (g)	79.65 ± 6.44^{c}	95.584 ± 6.71^{b}	101.518 ± 3.76^{b}	119.278 ± 8.7^{a}	96.052 ± 12.24^{b}
Intestinal length (cm)	193.0 ± 6.28	199.6 ± 25.22	196.6 ± 10.87	211.4 ± 9.47	183.2 ± 15.80

Note: T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%.

Table 6. Intestinal microflora variables of Muscovy ducks fed diets supplemented with *Spirulina* and *Indigofera*

Intestinal microflora	Treatments				
	T0	T1	T2	T3	T4
Salmonella sp. (CFU/g)	5.683 ± 0.50	5.747 ± 0.39	5.324 ± 0.31	5.463 ± 0.30	5.642 ± 0.32
Escherichia coli (CFU/g)	6.435 ± 0.02^{a}	6.469 ± 0.01^{a}	6.477 ± 0.0^{a}	6.226 ± 0.05^{b}	6.292 ± 0.15^{b}
Lactic acid bacteria (CFU/g)	8.510 ± 0.50	8.707 ± 0.49	9.311 ± 0.36	9.135 ± 0.48	9.273 ± 0.13

Note: Means within a single measured variable with different superscripts differ significantly (p<0.05) or (p<0.01). T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%. CFU= colony forming units; g = grams.

Table 7. Blood biochemical variables of Muscovy ducks fed diets supplemented with *Spirulina* and *Indigofera*

Blood biochemistry	Treatments				
	T0	T1	T2	T3	T4
Cholesterol (mg/dL)	169.8 ± 18.56^{a}	148.0 ± 12.55^{a}	135.0 ± 10.95^{b}	140.2 ± 9.33^{b}	133.4 ± 6.91^{b}
LDL (mg/dL)	78.4 ± 8.08^{a}	71.0 ± 7.07^{a}	66.6 ± 3.36^{b}	65.8 ± 3.96^{b}	66.4 ± 5.36^{b}
HDL (mg/dL)	64.4 ± 1.81^{b}	67.6 ± 1.51^{b}	71.8 ± 3.11^{a}	68.0 ± 2.55^{b}	75.6 ± 2.30^{a}
Triglyceride (mg/dL)	104.8 ± 11.54^{a}	95.6 ± 7.98^{a}	91.8 ± 9.91^{a}	83.6 ± 12.89^{b}	88.0 ± 5.78^{a}

Note: Means within a single measured variable with different superscripts differ significantly (p<0.05) or (p<0.01). T0 (Control): Basal diet without the addition of *Spirulina* or *Indigofera*; T1: Basal diet 89.5% + *Spirulina* 0.5% + *Indigofera* 10%; T2: Basal diet 89% + *Spirulina* 1% + *Indigofera* 10%; T3: Basal diet (84.5% + *Spirulina* 0.5% + *Indigofera* 15%); and T4: Basal diet 84% + *Spirulina* 1% + *Indigofera* 15%. HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 8. Intestinal histomorphology variables of Muscovy ducks fed diets supplemented with *Spirulina* and *Indigofera*

Intestinal histomorphology	Treatments				
	T0	T1	T2	T3	T4
Villus height (µm)	389.66 ± 118.57	458.70 ± 145.03	445.14 ± 149.05	436.82 ± 219.84	571.18 ± 92.78
Crypt depth (µm)	152.71 ± 25.86^{b}	251.18 ± 67.49^{a}	137.17 ± 19.97^{b}	109.60 ± 58.58^{b}	225.02 ± 42.31^{a}
Villus area (µm^{2})	89.46 ± 38.31	108.82 ± 23.67	100.24 ± 28.36	97.29 ± 36.54	84.22 ± 16.21
Basal villus width (µm)	98.24 ± 12.66	141.74 ± 61.46	109.54 ± 58.90	84.91 ± 37.19	120.27 ± 20.70
Apical villus width (µm)	66.02 ± 28.81	72.44 ± 42.46	89.61 ± 35.15	52.78 ± 19.27	79.68 ± 27.75

Note: Means within a single measured variable with different superscripts (a, b) differ significantly (p<0.05).

of HDL levels, T4 and T2 had the highest values. These treatments likely increase lipid metabolism by increasing the amount of beneficial cholesterol fractions. T3 had the lowest triglyceride concentration (83.6 mg/dL), which was significantly different (p<0.05) among all the other treatments. Moreover, T0, T1, T2, and T4 did not significantly differ, although T1 had the highest triglyceride content (104.8 mg/dL). The effects of dietary treatments on intestinal morphology in Muscovy ducks are presented in Table 8. Villus height and villus area were not significantly affected by the treatments, although the highest values were observed in T4 and T1–T2, respectively. Crypt depth was significantly greater in T1 and T4 than in T0, T2, and T3 (p<0.05). Basal and apical villus widths were not significantly influenced by the treatments, despite numerical variations among the groups.

DISCUSSION

The inclusion levels of *I. zollingeriana* (10% and 15%) were selected on the basis of previous studies demonstrating their suitability as alternative protein sources in poultry diets, whereas *S. platensis* was included at 0.5% and 1.0% based on reported beneficial effects on intestinal health, nutrient utilization, and metabolic responses without adversely affecting feed intake or growth performance (Rufino *et al.*, 2025; Tanjung *et al*., 2025). According to the findings of Rufino *et al.* (2025) and Feijó *et al.* (2024), the feed conversion ratio for Muscovy ducks is estimated to range from approximately 3.39 to 4.93. Furthermore, consistent with the observations of Rufino *et al.* (2025), the overall feed consumption may amount to as much as 10.76 kg across a duration of 90 days during the finishing phases. Previous research has indicated that Pekin ducks are larger than Muscovy ducks are, although this study revealed a less significant difference than Muscovy ducks did (Makram *et al*., 2021; Hassan *et al*., 2026; Hassan *et al*., 2018; West *et al*., 2022). Another finding from Oguntunji and Ayorinde (2014), Önk *et al*. (2018) mentioned that female ducks tend to achieve their full weight sooner, leading to a better distribution of meat cuts and quicker accumulation of fat on the carcass in Muscovy ducks. The existing use of protein, until 16% crude protein for Muscovy ducks between 2 and 7 weeks old, is evidently inadequate for contemporary breeds (El Abdel-Hamid & Abdelfattah, 2020). Based on the study by Dong and Ogle (2003), the intake of lysine and methionine was greater than that recorded in ducks that were given duckweed as a substitute for soybean meal in an earlier study involving a broken rice diet in the Mekong Delta. The present study revealed that *I. zollingeriana* and *S. platensis* are prospectively responsible for the lower FCR observed in the treatment groups, possibly because of their complementary nutritional and functional properties. Align with Linh *et al.* (2024), local farmers preferred to use alternative feeds, such as mosquito ferns (*Azolla* sp.), indigo plants (*Indigofera* sp.) (Widianingrum *et al*., 2020), and banana plant by-products to reduce feed costs.

I. zollingeriana has emerged as a protein-rich species that provides essential amino acids, vitamins, and macro- and microminerals to support growth and nutrient utilization (Tanjung *et al*., 2025). Furthermore, this plant contains β-carotene, a pigment known to influence footpad and egg yolk color. Additionally, *S. platensis* contains bioactive compounds that enhance intestinal health and improve the gut microbial environment. As reported by Salahuddin *et al.* (2025), *S. platensis* successfully upregulated nucleotides and amino acids, which enhanced nutrient utilization and absorption. Moreover, several taxa, such as *Lactobacillus* and *Limosilactobacillus*, contribute to the stability of the gut environment (Salahuddin *et al*., 2025). These microorganisms synergize to produce metabolites that can reduce the pH and create a less favourable environment for pathogenic bacteria such as *E. coli*. As a consequence of this condition, the birds fed supplemented diets required less feed per unit of body weight gain, and their FCR was significantly lower than that of those fed the control diet.

In another study by Ali *et al.* (2014), duckweed was fed to Muscovy ducks. Duckweed supplementation greatly improved Muscovy duck carcass quality by changing the fat composition of the Muscovy duck meat. Apart from lowering total fat, duckweed changed the fatty acid profile of the meat. Ducks whose diet contained duckweed tended to have decreased saturated fatty acids, particularly palmitic acid, while their linoleic acid levels increased (Ali *et al*., 2014). This change toward a higher ratio of unsaturated fatty acids suggests that duckweed may enhance the nutritional composition of duck meat by encouraging a better fat composition. These modifications are important since unsaturated fatty acids are related to increased nutritional benefits and improved meat performance (Ali *et al*., 2014). On the other hand, in a study by Rahman *et al.* (2024), which focused particularly on improving meat composition while maintaining acceptable carcass performance, green forage resources such as *Lemna minor* and *Asystasia gangetica* have shown promise as alternative feed components for Muscovy ducks. The dietary inclusion of duckweed has been linked to a decrease in intramuscular fat deposition and an improvement in the fatty acid profile, as indicated by lower saturated fatty acid concentrations and increased linoleic acid concentrations, suggesting an increase in the nutritional value of duck meat (Rahman *et al.*, 2024). These findings suggest that the high-fiber composition of duckweed probably affects lipid metabolism and fat deposition in muscle tissue, hence promoting leaner carcass features. Conversely, the addition of *A. gangetica* leaves at 15% of the diet did not significantly change the carcass yield or main proximate components of the meat, although the dry matter of the breast meat increased, indicating a somewhat decreased moisture content and denser muscle structure (Rahman *et al.*, 2024). Despite these variations in compositional response, both plant materials generally tended to preserve production characteristics since neither treatment significantly impacted final body weight, dressing percentage, or the organ weight (Rahman *et al*., 2024). Although birds in the T3 group (receiving 0.5% *Spirulina* and 15% *Indigofera*) presented numerically higher carcass weights and percentages, these differences were not statistically significant across the treatments. While the experimental diets improved feed efficiency and provided sufficient nutrients for overall growth, these specific inclusion levels may be inadequate to significantly enhance carcass traits. Instead, they appear to favor a balance between intestinal health and metabolic regulation. Furthermore, as noted by Tanjung *et al*. (2025), specific dietary compounds such as β-carotene can directly influence carcass quality and the organ weight.

Rich in crude fiber, the meal obtained from *Indigofera* sp. improved the complexity of the diet and increased digestive effort and intestinal physiological adaptation. A relatively high fiber content is known to reduce digesta retention time and may stimulate digestive system mechanical activity, therefore promoting tissue growth and expanding the absorbing surface area (Jha & Berrocoso, 2015; Singh & Kim, 2021). Although bulky fibrous feeds such as *I. zollingeriana* tend to shorten this transit time, which can potentially limit nutrient absorption, the concurrent addition of *S. platensis*, a polysaccharide-rich biomass, may counteract this effect by increasing the water-holding capacity and overall viscosity of the digesta (Tanjung *et al*., 2025). Furthermore, as mentioned earlier, this specific combination alters the gut microbiota environment, which subsequently influences overall digestive characteristics.

The gut microbiota produces short-chain fatty acids, notably acetate, propionate, and butyrate, by fermenting with non-starch polysaccharides; these compounds act as critical energy sources for enterocytes and support the development and differentiation of epithelial cells (Wang *et al.*, 2024; Shehata *et al.*, 2022; Jha *et al.*, 2019). This may have helped to promote quick turnover of mucosal cells and, therefore, assist in tissue healing and regeneration, contributing to this adaptive response. Enterocytes mostly rely on amino acids, particularly glutamine, which is essential for maintaining the health of the intestines. Therefore, the combined action of fiber fermentation and improved nutritional availability might help to produce a thickened intestinal wall and increase organ size. Furthermore, because increased nutrient flow and enzyme synthesis in the intestines increase nutrient absorption, an increase in feed intake can favor the growth of the digestive system. Together, these physiological changes support the growth of digestive organs and could increase the digestive effectiveness of Muscovy ducks. In addition, the mechanical stimulation of the digestive tract by *I. zollingeriana* and *S. platensis*, which contain relatively high amounts of fibre, promotes intestinal tissue development. Together, these structural adaptations increase relative organ weight, potentially improving the overall digestive and absorptive capacity of Muscovy ducks.

The present study demonstrated that dietary treatments influenced selected aspects of intestinal morphology in Muscovy ducks, particularly crypt depth, while other variables remained largely unaffected. The absence of significant differences in the villus height and area suggests that the treatments did not markedly alter the absorptive surface of the small intestine. Nevertheless, the numerically greater villus height observed in T4 may indicate a tendency toward improved nutrient absorption capacity, as taller villi are generally associated with increased surface area for nutrient uptake. In contrast, the significant increase in crypt depth in T1 and T4 indicates a higher rate of epithelial cell turnover. Deeper crypts are commonly associated with enhanced proliferative activity within the intestinal epithelium, reflecting an adaptive response to dietary modulation (Rufino *et al.*, 2025). This response may be beneficial for maintaining intestinal integrity and promoting rapid renewal of epithelial cells. However, increased crypt depth has also been linked to greater energy expenditure for tissue maintenance, which may reduce the net efficiency of nutrient utilization (Rufino *et al.*, 2025). The lack of significant effects on the basal and apical villus widths further suggests that the structural dimensions of the villi were relatively stable across treatments. Although numerical variations were observed, particularly in T1 and T2, these variations were not sufficient to indicate consistent morphological adaptation. Most of the morphological variables were not significantly altered, and the observed increase in crypt depth in response to specific treatments may reflect enhanced intestinal activity and epithelial turnover. Such changes could contribute to improved gut health and resilience under dietary interventions. Nevertheless, further investigations incorporating functional indicators, such as nutrient digestibility, enzyme activity, or gut microbiota composition, are needed to confirm the biological relevance of these morphological responses.

The significantly reduced levels of LDL cholesterol across T2, T3, and T4 may be linked to better utilization of lipids and more favorable metabolic processes. As reported by Adli *et al.* (2026), the upregulation of specific genes, such as *PPARα*, enhances lipid metabolism by promoting the conversion of fatty acids into energy rather than their storage in circulating metabolism pathways. Additionally, these genes may be involved in lipoprotein lipase (LPL) activity, which facilitates lipid mobilization and utilization. Conversely, a reduction in stearoyl-CoA desaturase (SCD) is associated with decreased lipid storage and lipid oxidation (Adli *et al.*, 2026). Therefore, the combination of *I. zollingeriana* and *S. platensis* may have promoted a metabolic shift toward lipid utilization rather than lipid accumulation, contributing to the lower serum cholesterol and LDL concentrations observed in the current research. Moreover, HDL may be associated with FAT (CD36) genes, which reverse cholesterol transport and promote HDL formation, thereby increasing circulating HDL concentrations while simultaneously reducing LDL and total cholesterol levels (Adli *et al.*, 2026).

CONCLUSION

The combined inclusion of *Indigofera zollingeriana* and *Spirulina platensis* enhanced feed efficiency and improved intestinal-related variables, including intestinal morphology, digesta characteristics, and selected microbial populations, without compromising growth performance or carcass traits. Among the treatments, the combination containing 15% *Indigofera* showed relatively favorable responses in several measured variables. These findings demonstrate the potential of an *Indigofera–Spirulina* strategy as a sustainable functional protein alternative to support efficient and resilient local meat-type Muscovy duck production. Further studies involving digestibility, gut microbiota sequencing, and molecular gut health indicators are recommended to confirm the underlying mechanisms.

CONFLICT OF INTEREST

We certify that there are no conflicts of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used AI tool to enhance readability and language clarity in this manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

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