p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, November 2025, 48(6):521-532
DOI: https://doi.org/10.5398/tasj.2025.48.6.521
Available online at https://journal.ipb.ac.id/index.php/tasj

Growth, Physiological, and Intestinal Responses of Low-Weight Day-Old Broiler Chicks to Dietary Infertile Egg Powder

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(Received 28-05-2025; Revised 13-08-2025; Accepted 20-08-2025)

ABSTRACT

The present study examined the consequences of supplementing the diets of low-weight day-old broiler chicks (DOC) with 4% infertile egg powder (IEP). The chicks were categorized into three groups namely; normal-weight DOC, low-weight DOC on a basal diet, and low-weight DOC on a diet that contained 4% IEP (T2). In hematological analysis, there was increase in hemoglobin levels, and mean corpuscular hemoglobin concentration (MCHC) compared to T2, but the MCV was less than T0 ($p<0.05$). There was improvement in the lipid metabolism, whereby triglyceride and HDL levels reduced and increased, respectively ($p<0.05$). T2 showed an improved antioxidant status; it had an increased activity of superoxide dismutase (SOD) and a decreased level of malondialdehyde (MDA) ($p<0.05$). Ileal lactic acid bacteria (LAB) and coliform counts were also substantially reduced in T2 vs. T0 ($p<0.05$), whereas both caecal coliform counts and LNE counts and the LAB-to-coliform ratio were significantly worse in T1 and T2 than T0 ($p<0.05$). Histological findings expressed the aspect of villus height-to-crypt depth (VH/CD) ratio that was greater in duodenum and ileum of T2 than T1, and deeper crypt in the jejunum in T0 than T1 ($p<0.05$). There was significant reduction in lesion score in duodenum and the jejunum as seen in T2 group ($p<0.05$). T2 also had much higher weight gain ($p<0.05$). per day, feed consumption and body weight at the end when compared to T1. To summarize the findings, the supplement of 4% IEP via the diet improved the physiological functioning and intestinal health status, causing the rise in body weights in low-weight broiler chicks.

Keywords: antioxidant; infertile egg; intestinal health; low-weight DOC; waste

INTRODUCTION

The initial weight of day-old chicks (DOC) plays a crucial role in determining the final harvest weight of broiler chickens. DOC with low initial weight have smaller yolk sac volumes than normal DOC (Cowieson & Parsons, 2024). Reduced nutrient reserves in the yolk sac may limit their ability to meet critical nutrient requirements during early growth (van Der Wagt *et al*., 2020). This implies that the proliferation and differentiation of satellite cells might be deferred, which can delay the development of vital organs such as the digestive, immune, and musculoskeletal system, and as a result, will inhibit growth. Low-weight DOC typically originate from younger breeder flocks as part of the natural egg production cycle. Instead of culling these chicks post-hatch, improving their growth performance through targeted nutritional strategies is more beneficial for hatcheries.

Alongside the rising of low-weight DOC, hatcheries struggle with fertility controls over the eggs which may have made up to 5-20% of fertilized eggs in incubators (Islam *et al*., 2017). Those that remain unfertilized are called infertile eggs and will usually be discarded and destroyed, because it is regulated that they cannot be sold as human food (Wang *et al*., 2024). Having high protein content, the eggs that are not fertilized are also environmental hazards when they are not disposed properly since high-nitrogen waste decays can result in nitrogen loss, groundwater contamination, and environmental pollution among eutrophication, air pollution and green house gases production (Glatz *et al*., 2011). Hence, recycling of infertile ova is highly promoted with the aim of saving environmental degradation. Some studies have provided their nutraceutical composition. Ratriyanto *et al.* (2020) found that infertile egg powder (IEP) contains 5,454.9 kcal/kg of metabolizable energy, 31.47% crude protein, 30.10% crude fat, 0.59% crude fibre, 1.99% ash, 0.55% calcium, and 0.18% phosphorus. Another study reported that IEP has a high protein digestibility coefficient of 86.81% (Dal Santo *et al*., 2020).

During the early period of life, protein synthesis and deposition are important processes due to its need to grow the chick at a high rate. Consequently, DOC need adequate consumption of high-quality and easy to digest protein. Since they are high in protein, infertile egg products could be used as an alternate source of protein to boost the growth of DOC with the low weight. Eggs are also a valuable dietary component

for broiler chickens due to their rich selenium content (Nimalaratne *et al*., 2015), making infertile eggs a potential source of antioxidants.

Previous studies have examined the use of infertile eggs in broiler diets. Esmailzadeh *et al.* (2016) reported that including IEP in broiler feed increased villus height and nutrient absorption in the small intestine. Ratriyanto *et al*. (2020) observed that it improved body weight gain and utilization of nutrients in the starter period. All these results imply that IEP in broiler rations is a potential alternative in boosting the growth of low-weight broilers at an early age and an eco-friendly measure to curb the problem of environmental contamination.

To date, no research has specifically investigated the use of infertile eggs for low-weight DOC. This study evaluated the responses of low-weight DOC to IEP as a protein-rich feed ingredient, focusing on hematological parameters, blood biochemistry, oxidative stress markers (SOD and MDA), bacterial populations, intestinal histology, and growth performance. These parameters were selected because they represent vital physiological systems often impaired in low-weight DOC. Such chicks commonly experience intestinal dysfunction, increased permeability, dysbiosis, and excessive inflammatory responses, making them more prone to oxidative stress and compromised gut integrity (Akram *et al*., 2024a). Assessing these characteristics was therefore essential to determine whether IEP could support physiological recovery and promote compensatory development in low-weight DOC. It was hypothesized that including IEP in the diets would improve hematological profiles, enhance antioxidant capacity, and maintain intestinal morphology, ultimately leading to growth performance of low-weight DOC comparable to that of normal-weight DOC.

MATERIALS AND METHODS

Preparations for the Infertile Egg Powder

Following manual candling on day 7, infertile egg waste was collected from a local hatchery near the campus. After removing the shells, the egg whites and yolks were homogenized. The shelled eggs were placed in zip-lock plastic bags, frozen, and then freeze-dried. The process began with freezing for 6 hours at −18 °C to 0 °C, followed by heating from 0 °C to 55 °C over the next 6 hours, holding at 55 °C for 8 hours, and cooling to 40 °C for 4 hours. All steps were performed under vacuum conditions of −0.2 to 0 mbar, with a total drying time of 24 hours. This freeze-drying procedure followed the internal standard operating protocol of PT Industri Jamu Borobudur Indonesia and was conceptually consistent with the process described by Nowak & Jakubczyk (2020). The nutritional composition of the IEP was determined using proximate analysis in accordance with the AOAC Official Methods of Analysis (AOAC International, 2023). The powder was stored in aluminum foil packaging to preserve quality and prevent oxidation.

Table 1. Nutrient contents of infertile egg powder

Nutrients	Contents
Metabolizable energy (kcal/kg)[1]	4687
Crude protein (%)	34.3
Crude fat (%)	26.9
Crude fiber (%)	2.39
Ash (%)	2.10

Note: [1]Calculated according to Bolton (1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

Ethical Approval

The research procedures were approved by the Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (60-10/A-19/KEP-FPP).

Animals, Diets, and Management Practices

The study used 176 low-weight DOC (34.5 ± 0.25 g) and 88 normal-weight DOC (42.4 ± 0.86 g) from the Cobb JP 313 strain. Broilers were reared in a closed house with rice husk litter. Feed and water were provided ad libitum. From day 1, chicks received a formulated starter feed (Table 2), followed by a finisher feed from day 22 onward (Table 3). Both diets were prepared using locally sourced ingredients and formulated to meet the Indonesian National Standards for Broiler Feed (SNI, 2022a,b). At the hatchery, chicks were vaccinated against Newcastle disease and infectious bronchitis using the spray method.

Upon arrival, chicks were divided into groups based on initial body weight. DOC weighing less than 35.0 g were categorized as low-weight, while those weighing more than 40.0 g were categorized as normal-weight. This classification followed the Indonesian National Standard (SNI, 2024), which recommends a minimum body weight of 35 g per chick. This distinction allowed the evaluation of dietary responses in chicks with suboptimal initial conditions compared to those within the optimal weight range. The chicks were assigned to three treatment groups with eight replicates (11 chicks per replicate) and raised until day 32. The treatments were: normal-weight DOC receiving a control diet (T0), low-weight DOC receiving a control diet (T1), and low-weight DOC receiving a diet containing 4% IEP (T2).

A male broiler from each replicate, with body weight closest to the pen's average, was selected for blood sampling on day 31. The procedure for collecting and handling blood was based on the work of Sugiharto *et al.* (2016). Using a syringe, 5.0 mL of blood was drawn from the wing vein. One milliliter was transferred to EDTA-filled tubes for routine blood analysis, and 4 mL to non-EDTA tubes for serum production. Blood was clotted at room temperature for 2 hours and centrifuged at 2,000 rpm for 15 minutes. Serum was stored at −20 °C for later biochemical and oxidative stress analyses.

On day 32, a broiler from each replicate was randomly selected, weighed, and slaughtered following ethical procedures. After de-feathering, tissue samples

Table 2. Ingredients and nutritional composition of experimental broiler diets (day 1–21)

Ingredients (%)	T0, T1[1]	T2
Yellow corn	53.5	55.09
Palm oil	2.32	-
Soybean meal	40.13	36.86
DL-methionine	0.19	0.19
Bentonite	0.75	0.75
Limestone	1.00	1.00
Monocalcium phosphate	1.30	1.30
Premix[2]	0.34	0.34
Choline chloride	0.07	0.07
Salt	0.40	0.40
Infertile egg powder	-	4.00
Calculated composition (%, unless otherwise noted)		
ME[2] (kcal/kg)	2900	2900
Crude protein	22.0	22.0
Crude fat	2.40	3.40
Crude fiber	5.50	5.50
Ca	1.10	1.20
P (available)	0.60	0.60
Analyzed composition (%, unless otherwise noted)		
ME[3] (kcal/kg)	2947	2949
Crude protein	22.3	22.2
Crude fiber	3.88	2.79
Crude fat	2.69	2.20
Ash	0.60	0.60

Note : [1]Ingredients and nutritional compositions of feeds for T0 and T1 groups are similar.
[2]The following nutrients are provided per kilogram of feed: 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vitamin A, 250,000 IU vitamin D3, 1,350 g pantothenic acid, 1,875 g vitamin E, 250 g vitamin K3, 250 g vitamin B1, 750 g vitamin B2, 500 g vitamin B6, 2,500 mg vitamin B12, 5,000 g niacin, 125 g folic acid, and 2,500 mg biotin.
[3]ME (metabolizable energy) calculated according to: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen - free extract) + 2.5} T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

Table 3. Ingredients and nutritional composition of experimental broiler diets (day 22–32)

Ingredients (%)	T0, T1[1]	T2
Yellow corn	59.0	60.55
Palm oil	4.70	2.40
Soybean meal	32.25	29.00
DL-methionine	0.19	0.19
Bentonite	0.75	0.75
Limestone	1.00	1.00
Monocalcium phosphate	1.30	1.30
Premix[2]	0.34	0.34
Choline chloride	0.07	0.07
Salt	0.40	0.40
Infertile egg powder	-	4.00
Calculated composition (%, unless otherwise noted)		
ME (kcal/kg)	3100	3101
Crude protein	19.0	19.0
Crude fat	2.50	3.50
Crude fiber	5.40	5.50
Ca	1.10	1.10
P (available)	0.60	0.60
Analyzed composition (%, unless otherwise noted)		
ME[3] (kcal/kg)	3101	3091
Crude protein	20.9	20.6
Crude fiber	3.19	3.70
Crude fat	3.08	2.90
Ash	4.78	5.27

Note: [1]Ingredients and nutritional compositions of feeds for T0 and T1 groups are similar.
[2]The following nutrients are provided per kilogram of feed: 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vitamin A, 250,000 IU vitamin D3, 1,350 g pantothenic acid, 1,875 g vitamin E, 250 g vitamin K3, 250 g vitamin B1, 750 g vitamin B2, 500 g vitamin B6, 2,500 mg vitamin B12, 5,000 g niacin, 125 g folic acid, and 2,500 mg biotin.
[3]ME (metabolizable energy) calculated according to: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen - free extract) + 2.5} T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

from the duodenum, jejunum, and ileum (2 cm each) were preserved in 10% buffered formalin for histological analysis (de Souza *et al.*, 2021). Digesta samples from the ileum and caecum were collected in sterile containers for bacterial enumeration.

Data Measurement and Sample Analysis

Hematological and blood biochemical analysis. Hematological and serum biochemical analyses followed the procedures of Sugiharto *et al.* (2016) with minor modifications. A complete blood count (CBC) was performed using a Prima Fully-Auto Hematology Analyzer (PT Prima Alkesindo Nusantara, Jakarta, Indonesia) in accordance with the manufacturer's protocols. Blood cholesterol levels were determined using the enzymatic CHOD-PAP method, and triglyceride concentrations (mg/dL) were measured via the CPO-PAP method. HDL and LDL levels were assessed using the CHOD-PAP enzymatic technique. The total protein content was analyzed using the biuret method, and albumin levels were measured using the bromocresol green method. Globulin concentrations were calculated by subtracting albumin from total protein. Uric acid levels were determined using the urease method, and creatinine was measured by the Jaffe method. Serum biochemical analyses were standardized using commercial assays from DiaSys Diagnostic Systems GmbH (Holzheim, Germany).

Oxidative stress marker analysis. The levels of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) were measured by following Agusetyaningsih *et al*. (2022). The determination of MDA levels was done with the help of thiobarbituric acid reactive substances (TBARS) assay. Blood samples were added to the solution made of 8.1% sodium dodecyl sulfate (SDS) incubated at room temperature and left for 10 minutes, then combined with 0.6% thiobarbituric acid (TBA) and 20% acetic acid. Water bath incubation was done on the mixture at 90-95 °C at hour and cooled down before extracting the

supernatant. The extract was added to butanol:pyridine solution (15:1, v/v) to which the mixture was mixed and centrifugalized. The supernatant thus obtained which had the MDA-TBA complex was spectrophotometrically analyzed at 532 nm. The SOD activity was determined on the basis of inhibition of the pyrogallol auto-oxidation. These samples were combined with a 50 mM Tris-HCl (pH 8.2) and 1 mM diethylenetriamine pentaacetic acid (DTPA), which was a buffer. It supplemented 0.2 mM pyrogallol as a substrate, and it also involved the use of the rate of oxidation in determining the SOD activity.

Bacterial enumeration. The presence of lactose-negative enterobacteria and total coliform was counted by means of MacConkey agar (Merck KGaA, Darmstadt, Germany) as per the approach of Sugiharto *et al*. (2019). Red colonies were characterized as coliforms after a 24-hour aerobic incubation at 38 °C, whereas, colorless colonies were lactose negative enterobacteria. Lactic acid bacteria (LAB) were quantified by inoculation into de Man, Rogosa and Sharpe (MRS) (Merck KGaA) and following anaerobic incubation at 38 °C for 48 hours.

Histological analysis. Histological evaluation of intestinal tissue at each segment was done by making microtome sections (5 μm) and staining in hematoxylin and eosin (H&E). Stained slides were examined under ordinary optical microscope equipped with digital camera. The intestine was measured via measurement of villus height and crypt depth in 3 segments of each of the intestinal sections in terms of mucosal structure (Erya *et al*., 2020).

Histopathological scoring. Lesions in the small intestine were evaluated using a histopathological scoring system from 0 to 4: 0 (no lesions), 1 (presence of lesions), 2 (more than 10 lesions per section), 3 (overlapping lesions), and 4 (extensive lesions with epithelial cell sloughing), following Johnson & Reid (1970). The assessment included focal or multifocal damage, cilia loss, epithelial cell hypertrophy or hyperplasia, inflammatory infiltration, and necrosis.

Growth performance assessment. Body weight and cumulative feed intake were monitored throughout the study. Feed conversion ratio (FCR) was calculated as the total feed consumed (grams) divided by the total body weight gained (grams) (Agusetyaningsih *et al*., 2022).

Statistical Analysis

Data are presented as the standard error of the mean (SEM). Planned orthogonal contrasts were used to test specific hypotheses about treatment effects, allowing independent comparisons between treatment means. Contrast coefficients were derived from the experimental design and met orthogonality criteria, whereby the sum of the cross-products of the coefficients equaled zero. Statistical significance was set at ($p \leq 0.05$) using SPSS 16.0. Histopathological lesion scores were analyzed non-parametrically using the Kruskal–Wallis test.

RESULTS

Complete Blood Counts

Table 4 presents the complete blood counts of broilers. Erythrocyte levels did not differ among groups, whereas hemoglobin levels were significantly higher in T2 compared to T1 ($p<0.05$), with no significant differences between T0 and the other groups. Mean corpuscular hemoglobin concentration (MCHC) was significantly higher in T2 than in T1 ($p<0.05$), with no differences between T2 and T0 or between T0 and T1. Mean corpuscular volume (MCV) was significantly lower in T2 than in T0 ($p<0.05$), with no differences between T2 and T1 or between T0 and T1. Other parameters, including haematocrit, leukocyte, lymphocyte, and heterophil counts, as well as the heterophil-to-lymphocyte (H/L) ratio, showed no significant differences across groups.

Blood Biochemical Parameters

Triglyceride levels were significantly lower in T2 than in T0 ($p<0.05$), with no differences between T2 and

Table 4. Complete blood counts of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
Erythrocytes, 1012/L	1.85	1.80	1.94	0.06	0.457	0.205	0.051
Hemoglobin, g/dL	7.42^{ab}	6.97^{b}	7.82^{a}	0.13	0.123	0.168	0.006
Hematocrit, %	32.45	31.31	33.22	0.49	0.347	0.520	0.121
MCV, fl	174.56^{a}	173.84^{ab}	171.92^{b}	0.66	0.392	0.013	0.082
MCH, pg	39.99	38.59	40.16	0.41	0.173	0.862	0.128
MCHC, g/dL	22.26^{ab}	21.65^{b}	22.83a	0.23	0.268	0.298	0.039
Leukocytes, 109/L	63.27	58.45	55.16	1.92	0.365	0.091	0.481
Lymphocytes, 109/L	62.35	57.34	54.39	1.85	0.268	0.085	0.510
Heterophils, 109/L	0.92	1.11	0.77	0.04	0.502	0.696	0.293
H/L ratio	0.014	0.018	0.013	0.001	0.308	0.896	0.252

Note: Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis. MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; MCH = mean corpuscular hemoglobin; H/L Ratio = heterophils-to-lymphocyte ratio. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

T1 or between T0 and T1. HDL levels were significantly higher in T2 than in T1 ($p<0.05$) and in T0 compared to T1 ($p<0.05$), with no difference between T2 and T0. LDL levels were significantly higher in both T1 and T2 than in T0 ($p<0.05$), with no difference between T1 and T2. Uric acid levels were significantly lower in T2 than in T0 ($p<0.05$), with no differences between T1 and T2 or between T0 and T1. Other parameters, including total cholesterol, protein, albumin, globulin, and creatinine, showed no significant differences across groups (Table 5).

Oxidative Stress Marker

Superoxide dismutase (SOD) activity was significantly higher in T2 compared to both T0 and T1 ($p<0.05$). T0 also showed higher SOD activity than T1 ($p<0.05$). Malondialdehyde (MDA) concentration was significantly lower in T2 than in T1 ($p<0.05$), with no difference between T2 and T0. T1 had significantly higher MDA levels than T0 ($p<0.05$). Serum MDA and SOD values are presented in Table 6.

Ileum and Caecum Microbiota

Table 7 presents the selected bacterial populations in the gastrointestinal tract. In the ileum, lactic acid bacteria (LAB) counts were significantly lower in T2 than in T0 ($p<0.05$), and coliform counts did not differ among treatment groups. In the caecum, coliform counts were significantly higher in both T1 and T2 compared to T0 ($p<0.05$). Lactose-negative Enterobacteriaceae (LNE) counts were also higher in T1 and T2 than in T0 ($p<0.05$). The LAB-to-coliform ratio was significantly lower in T1 and T2 compared to T0 ($p<0.05$). No significant differences were found between T2 and T1 for any bacterial counts.

Table 5. Blood biochemical parameters of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
Total cholesterol, mg/dL	126.51	134.15	132.07	2.33	0.197	0.344	0.720
Triglycerides, mg/dL	91.29^{a}	81.46^{ab}	56.49^{b}	0.08	0.459	0.012	0.058
HDL, mg/dL	87.12^{a}	81.75^{b}	89.50^{a}	1.23	0.054	0.378	0.008
LDL, mg/dL	21.13^{b}	36.10^{a}	31.27^{a}	0.07	0.003	0.014	0.497
Total protein, g/dL	2.81	2.78	2.88	0.05	0.847	0.574	0.447
Albumin, g/dL	1.25	1.28	1.31	0.02	0.519	0.177	0.467
Globulin, g/dL	1.50	1.56	1.57	0.03	0.563	0.914	0.494
Uric acid, mg/dL	6.08^{a}	4.9^{ab}	4.33^{b}	0.06	0.092	0.034	0.615
Creatinine, mg/dL	0.23	0.23	0.25	0.004	0.894	0.056	0.069

Note: Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis.HDL = high density lipoprotein; LDL = low density lipoprotein. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

Table 6. Oxidative stress markers of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
SOD, u/L	14.58^{b}	7.01^{c}	19.55^{a}	0.10	<0.001	0.051	<0.001
MDA, nmol/mL	14.49^{b}	25.50^{a}	13.95^{b}	1.29	<0.001	0.758	<0.001

Note: Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis.SOD = superoxide dismutase; MDA = malondialdehyde. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

Table 7. Selected bacterial counts of ileal and cecal digesta of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
Ileum, log cfu/g							
LAB	6.13^{a}	4.90^{ab}	4.09^{b}	0.30	0.069	0.004	0.214
Coliform	5.01	4.91	3.84	0.24	0.869	0.051	0.071
LNE	3.05	3.64	2.6	0.23	0.293	0.406	0.068
LAB/coliform	1.30	1.05	1.23	0.11	0.375	0.808	0.517
Cecum, log cfu/g							
LAB	9.54	9.43	9.58	0.08	0.617	0.836	0.481
Coliform	5.15^{b}	5.83^{a}	6.20^{a}	0.15	0.038	0.003	0.243
LNE	3.50^{b}	4.49^{a}	5.03^{a}	0.18	0.006	0.001	0.116
LAB/coliform	1.90^{a}	1.62^{b}	1.56^{b}	0.05	0.020	0.005	0.549

Note: Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis.LAB = lactic acid bacteria; LNE = lactose negative Enterobacteriaceae; LAB/coliform = lactic acid bacteria-to-coliform ratio. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

Small Intestine Histomorphometry

The VH/CD ratio in the duodenum was significantly higher in T2 than in T1 ($p<0.05$), with no differences between T0 and the other groups. In the jejunum, crypt depth was significantly lower in T2 than in T0 ($p<0.05$), with no differences between T2 and T1. The VH/CD ratio in the ileum was significantly higher in T2 than in T1 ($p<0.05$), with no differences between T2 and T0 or between T0 and T1. Other variables including villus height and crypt depth in the duodenum, jejunum, and ileum, showed no significant differences among groups (Table 8).

Histopathologic Scoring of Small Intestines

As illustrated in Table 9, lesion scores in the duodenum were significantly lower in T2 than in both T0 and T1 ($p<0.05$), with no differences between T0 and T1. Similarly, jejunal lesion scores were significantly lower in T2 than in both T0 and T1 ($p<0.05$). Ileal lesion scores did not differ significantly among groups. The microscopic photographs of the small intestines of 32-day-old broiler chickens can be seen in Figures 1-3.

Growth Performance of Broiler Chickens

Table 10 presents the data on growth performance results. Final body weight (BW) was significantly higher in T2 compared to both T0 and T1 ($p<0.05$), with no difference between T0 and T1. Body weight gain (BWG) was also significantly greater in T2 than in T0 and T1 ($p<0.05$), while BWG did not differ between T0 and T1. Cumulative feed intake (FI) was significantly higher in T2 than in T0 and T1 ($p<0.05$), with no variation between T0 and T1. Feed conversion ratio (FCR) did not vary among groups during the study period.

Table 8. Small intestine histomorphometry of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
Duodenum							
Villi height, μm	1438.09	1327.70	1455.35	60.03	0.473	0.910	0.407
Crypt depth, μm	254.86	277.09	219.70	14.54	0.533	0.328	0.117
VH/CD	5.73^{ab}	5.09^{b}	6.67^{a}	0.25	0.243	0.093	0.007
Jejunum							
Villi height, μm	1120.51	1006.94	782.15	87.98	0.599	0.127	0.303
Crypt depth, μm	218.59^{a}	186.00^{ab}	144.03^{b}	12.52	0.252	0.014	0.144
VH/CD	5.17	5.32	5.33	0.29	0.315	0.841	0.994
Ileum							
Villi height, μm	694.46	594.56	642.21	28.26	0.163	0.458	0.498
Crypt depth, μm	123.07	121.91	112.49	4.99	0.927	0.408	0.461
VH/CD	5.63^{ab}	4.94^{b}	5.84^{a}	0.18	0.106	0.610	0.039

Note: Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis.VH/CD = villi height-to-crypt depth ratio. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

Table 9. Histopathologic scoring of the small intestines of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
Duodenum	14.13^{b}	17.94^{b}	5.44^{a}	0.19	0.260	0.010	<0.001
Jejunum	14.00^{b}	16.63^{b}	6.88^{a}	0.21	0.440	0.036	0.004
Ileum	9.50	13.69	14.31	0.17	0.130	0.206	0.734

Note: Data are presented as mean ranks. Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP.

Table 10. Growth performance of low-weight DOC receiving experimental diets

Variables	Treatments			SEM	P value		
	T0	T1	T2		T0 vs. T1	T0 vs. T2	T1 vs. T2
Final BW, g	1742^{b}	1695^{b}	1914^{a}	25.2	0.263	<0.001	<0.001
BWG, g	1597^{b}	1536^{c}	1677^{a}	15.4	0.025	0.004	<0.001
Cumulative FI, g	2350^{b}	2281^{b}	2447^{a}	20.6	0.083	0.020	<0.001
FCR	1.47	1.48	1.46	0.01	0.554	0.594	0.266

Note: Values within a row with different superscript letters (a, b) differ significantly ($p<0.05$) based on orthogonal contrast analysis.BWG = body weight gain, calculated as final BW minus initial BW; Cumulative FI = cumulative feed intake, calculated as the total feed consumed (day 0–32); FCR = feed conversion ratio, calculated as feed intake per unit gain (day 0–32); Final BW = final body weight at day 32. T0 = normal-weight DOC receiving control diet; T1 = low-weight DOC receiving control diet; T2 = low-weight DOC receiving diet containing 4% IEP. SEM = standard error of the mean.

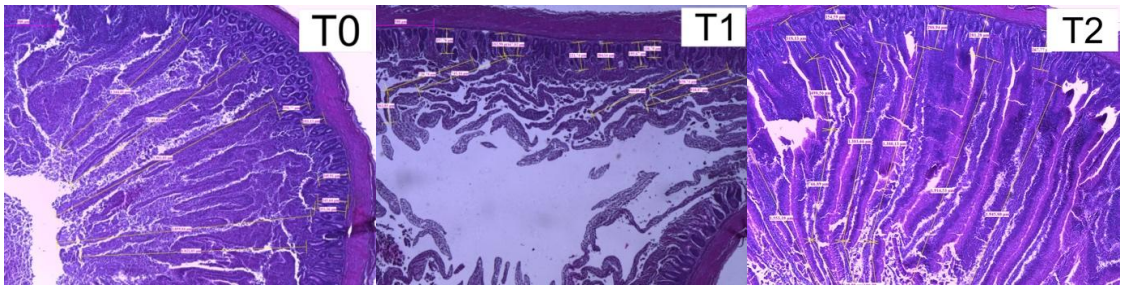

Figure 1. Duodenal microscopic photographs of 32-day-old broiler chickens. T0 = normal-weight DOC receiving control diet (note: mild focal lesions with partial cilia loss and minimal inflammation), T1 = low-weight DOC receiving control diet (note: moderate multifocal lesions with significant cilia loss, hyperplasia, and inflammation), T2 = low-weight DOC receiving diet containing 4% IEP (note: minimal lesions with preserved epithelium and minor inflammation).

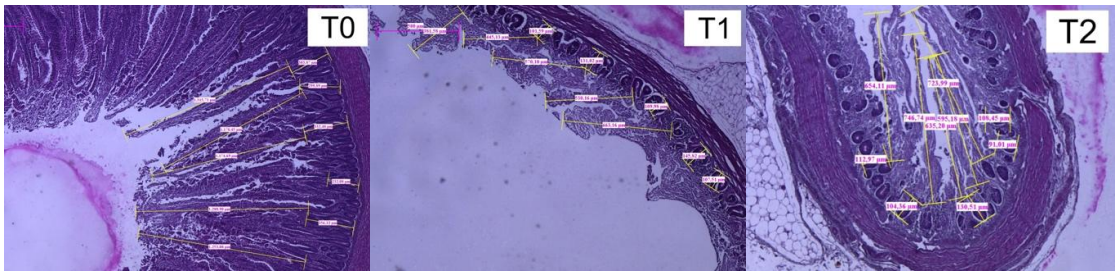

Figure 2. Jejunal microscopic photographs of 32-day-old broiler chickens. T0 = normal-weight DOC receiving control diet (note: mild lesions with partial cilia loss and minimal epithelial disruption), T1 = low-weight DOC receiving control diet (note: moderate lesions with marked cilia loss, epithelial hyperplasia, and noticeable inflammation), T2 = low-weight DOC receiving diet containing 4% IEP (note: minimal lesions with well-preserved epithelium and very slight inflammation).

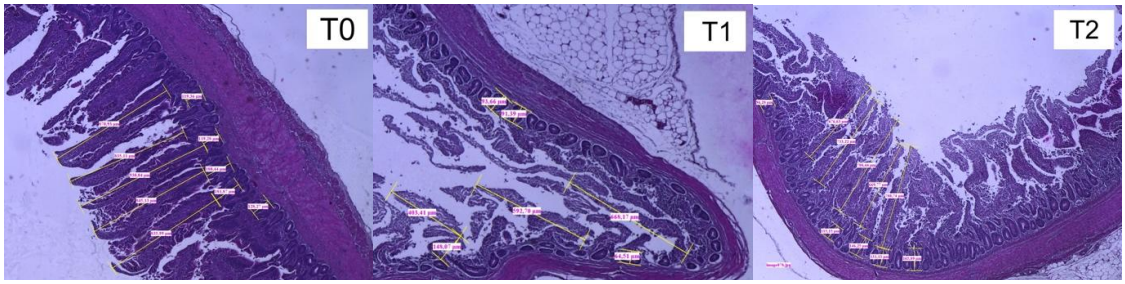

Figure 2. Ileal microscopic photographs of 32-day-old broiler chickens. T0 = normal-weight DOC receiving control diet (note: mild focal damage with partial cilia loss and minimal inflammation), T1 = low-weight DOC receiving control diet (note: multifocal lesions with severe cilia loss, epithelial thickening, and prominent inflammation), T2 = low-weight DOC receiving diet containing 4% IEP (note: minimal lesions with preserved epithelium and minor inflammation).

DISCUSSION

The hematological findings of this study indicate that dietary inclusion of IEP significantly improved erythropoietic function in low-weight DOC. Concentrations of erythrocytes and hemoglobin in each treatment group were lower than the physiological range of broilers (2.5–3.9 × 10^{12}/L and 10.2–15.1 g/dL; Samour, 2015) and were probably caused by oxidative stress-induced hemolysis, as identified by Ogbuagu *et al*. (2018). Importantly, low-weight DOC with the 4% IEP (T2) exhibited substantial increases of erythrocytes and hemoglobin as compared to T1, which received the basal diet, thus indicating a greater erythropoiesis. Such positive changes can be probably attributed to high bioavailability of essential amino acids (e.g., histidine and lysine) and hematopoietic micronutrients like iron, copper, folate, and vitamins B2 and B12 that IEP contains and are essential in the synthesis of globin structures, heme production, and the growth of erythroid progenitor cells (Oyay *et al*., 2021).

The enhanced quality of red blood cells are further observed when measuring the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) which varies significantly. Whereas the average values of MCHC in all treatments were in the rates lower than in normal physiological levels (30.2–36.2 g/dL; Samour, 2015), the birds of T2 group revealed a statistically higher MCHC when compared to T1, indicating better hemoglobin loading per 1 erythrocyte. Moreover, the T2 group had a significantly lower MCV than T0 although the values

were above the normal range (104–135 fL; Samour, 2015), which means better maturation or erythrocytes and a trend toward the normocytic profile. All these findings indicate that hematological effects of dietary addition of 4% IEP on low-weight DOC were most beneficial in terms of red cell production and levels of hemoglobin, compared to those obtained on the basal diet (T1) and in returning targeted parameters to the range of the normal-weight control (T0).

Totals of leukocyte and lymphocyte were beyond the normal physiological limits of broiler chickens (3.7–11.9 × 10^9/L and 1.2–4.2 × 10^9/L, respectively; Benerjee, 2008), pointing to the fact of possible immune system activation, probably by some stress or subclinical infection. Conversely, heterophil numbers and heterophil to lymphocyte (H/L) ratio remained within the normal physiologic range (0.5–7.6 × 10^9/L and ≤0.8, respectively; Gross & Siegel, 1983) and there was no sign of systemic stress responses. The bioactive extracts of IEP that consist of phospholipids and functional proteins are less likely to induce primary leukocyte profile alteration and have a long-term effect on the possible immunomodulation. Effect of IEP as an immunomodulator Although it has the potential, the effects of IEP may be insignificant in the event of pre-activated immune reactions. These findings concur with Agunbiade *et al*. (2011) who concluded that the addition of hatchery waste meal did not significantly influence the leukocyte profile of broiler chickens.

Although all the maintained blood biochemical variables fell within the readings in the normal physiology regions as published by Arzour-Lakehal *et al*. (2021), they note that there were some differences following the distribution assigned to the treatment groups. In the IEP group that comprised low-weight DOC, there was a lower level of triglycerides compared to normal-weight DOC. The evidence is also compatible with the result obtained by Esmailzadeh *et al*. (2012) that the addition of egg powder to the diet of broilers decreased triglyceride levels. The proteins and essential fats present in IEP have quality protein and other important fats like phospholipids that are significant in regulating metabolism of fats. Phospholipids activate lipoprotein lipase (LPL) which catalyzes breakdown of triglycerides into glycerol and free fatty acids and thus increases the use of triglyceride as a source of energy. Moreover, low-weight DOC that received IEP had high levels of high-density lipoprotein (HDL) then when compared to basal diet. Phospholipids of egg source have a high level of bioaccessibility (absorbed in amounts greater than 90%) and are preferentially transporting into HDL lipoproteins and not in the apo B-lipoproteins and other lipid carrier compartments (Kullenberg *et al*., 2012). Increased presence of HDL may also be connected to the micronutrient composition of IEP, especially, that of choline and vitamin B12. Choline is used as the molecular compound in the production of phosphatidylcholine that is subsequently used in producing HDL, stimulating the activity of lecithin-cholesterol acyltransferase (LCAT) (Liu *et al*., 2020b). This enzyme is responsible in the process of esterification of free cholesterol into cholesterol esters, which are combined or included inside HDL particles. Sufficient choline has proven to boost the functionality of HDL (Ramalho de Lima *et al*., 2024). Similarly, vitamin B12 plays a crucial role in the metabolism of lipids, and deficiency in the given vitamin has also been attributed with the alterations in the lipid profile, including lower levels of HDL (Saraswathy *et al*., 2018).

Low-weight DOC fed IEP raised the uric acid score in basal diets that were fed to normal-weight DOC. This suggests that IEP may either inhibit the proliferation of uric acid or enhance its elimination. This is similar to what Akinola *et al*. (2020) found, which was that giving chickens diets that included infertile egg meal helped lower the amount of uric acid in their bodies. The high protein content of IEP likely improved nitrogen utilization, thereby reducing purine metabolism, a primary precursor of uric acid. The observation of the improved nutritional status of the IEP group may have mitigated some oxidative stress, which has been identified as a contributing factor to uric acid accumulation (Lu *et al*., 2018).

Remarkably, IEP inclusion did not have a significant influence on the levels of total protein, albumin, and globulin of broilers. This observation might indicate that the protein quality and bioavailability of IEP were similar to those of traditional protein-source ingredients with the capacity of sustaining a balanced homeostasis of proteins (Saleh *et al*., 2021). The similar protein measures indicate that IEP well met the requirements of the birds on systemic protein without interfering with it. As opposed to our current research, Akinola *et al*. (2020) noted an increase in the total protein, albumin, and globulin in the case of including infertile egg meal in the feed of broilers. Those differences could be on account of the varying proportions of components of the basal diet, protein, or its digestibility which may influence the serum protein metabolism. In sum, the information of biochemical parameters shows that low-weight DOC fed IEP-based diets have a more normalized metabolic status with better lipid and nitrogen metabolism albeit it did not affect the degree of LDL. These improvements show that the IEP-fed group is in better physical shape, which probably helps low-weight DOC grow and stay healthy.

Activity of oxidative stress markers was measured to determine the antioxidant effect of each of the treatment groups. DOC with low weight given the basal diet (T1) showed the lowest activity of SOD and the highest concentrations of MDA, which makes the levels of oxidative stress the highest. This indicates the pronounced susceptibility of low-weight DOC to oxidative stress, which in all probability is an outcome of lower physiological fitness and environmental/metabolic susceptibility to stressors at early stages of development. On the other hand, incorporation of IEP into diet among low-weight DOC (T2) resulted in a substantial upgrade of antioxidant profile with the highest level of SOD activity and MDA as high as it is in normal-weight controls (T0). These results are in consonance with Esmailzadeh *et al*. (2012), who found a reduction in the concentrations of the MDA in chickens exposed to increased amounts of egg powder. The constituents of eggs usually have

valuable antioxidant effects. Proteins like ovalbumin, ovotransferrin, and phosvitin contain antioxidant activity (Benedé & Molina, 2020), whereas the bioactive peptides of such proteins can lessen the oxidative state of poultry by activating the inherent antioxidant enzymes such as SOD. Should this happen, SOD could act upon any superoxide radicals by transforming them into hydrogen peroxide, which would then be degraded into water with the help of catalase (CAT) or glutathione peroxidase (GPx) (Ibrahim & Miyata, 2023). The process decreases the levels of MDA which refers to the level of lipid peroxidation hence describing less oxidative damage to the cells (Surai *et al*., 2019).

In addition to antioxidant proteins, eggs contain carotenoid, especially lutein and zeaxanthin, which are high in yolk (Zaheer, 2017). The carotenoids are useful in neutralizing the free radicals, stabilizing the cell membranes, and preventing peroxidation of lipids as effective antioxidants that enhance the antioxidant protection system during peak body growth and increased metabolic work. These include antibodies and selenium, which have high concentration in the yolks of the eggs (Liu *et al*., 2020a). The synthesis of the selenoproteins and antioxidant enzymes, such as GPx, thioredoxin reductase (TRxR), and iodothyronine deiodinase (DIO), requires selenium, which safeguards the cells against lipid oxidation of the cell membranes and oxidative stress (Barchielli *et al*., 2022). On the whole, low-weight DOC exposed to IEP (T2) manifested the best mitigation in oxidative stress revealed by increasing the activity of antioxidant enzymes and the normalization of lipid peroxidation products. These findings reflect the idea that IEP incorporation has a great effect on the Ox-status and physiological resilience of low-arrive at chicks.

Low-weight DOC receiving IEP also showed reduced LAB population in the ileum compared to normal-weight DOC, which is probably mediated by the presence of egg-derived lysozyme, which has antimicrobial activity with specificities to Gram-positive bacteria, such as LAB. This finding is consistent with Anton *et al.* (2006), which indicated that lysozyme in egg albumen degrades bacterial peptidoglycan by cleaving the β-(1,4) bond between N-acetylmuramic acid and N-acetylglucosamine, critical constituents of Gram-positive bacterial cell walls. Lysozyme is especially effective against Gram-positive bacteria because they have more peptidoglycan (40%–90%) than other types of bacteria, which makes them more likely to break down in water.

Coliform counts were also lower in low-weight DOC receiving IEP than in normal-weight DOC, which is interesting because it suggests that something other than lysozyme is having an effect on bacteria. This decrease is probably because of other bioactive compounds in IEP that attack Gram-negative bacteria by messing with their ability to get nutrients and the strength of their membranes. Ovotransferrin, for example, inhibits coliform growth by chelating iron (Fe^{3+}), an essential nutrient for bacterial metabolism, thereby starving iron-dependent Gram-negative bacteria such as *E. coli* (Rathnapala *et al*., 2021; Giansanti *et al*., 2015). Phosvitin also increases this effect by binding more metal ions (Fe, Zn, Mg) required in the process of enzymatic action and the multiplication of bacteria, thus inhibiting the growth of coliform (Yilmaz & Ağagündüz, 2020). Moreover, the direct action of the antimicrobial peptides (AMPs) on Gram-negative bacteria is their bactericidal effect based on elevation of the outer membrane permeability and consequent lysis of the cells (Zhang *et al*., 2021). The cecal microbiota on the contrary exhibited opposite trends in which there were greater populations of coliform and LNE with a lower ratio of LAB to population of coliform with both low-weight groups relative to the normal-weight group. These findings corroborate previous research demonstrating that low-weight DOC are more vulnerable to microbial imbalances resulting from early-life stress and protracted intestinal development (Akram *et al*., 2024b; Lundberg *et al*., 2021). Such imbalances can hinder gut maturation, promoting the proliferation of opportunistic bacteria (Meinen-Jochum *et al*., 2024). In general, the gut microbial profile shows that the normal-weight DOC group (T0) had the most balanced composition, which means their intestines were healthier. But the composition of microbes alone does not fully show how healthy the gut is.

The histomorphological measurements provide a more concise evaluation of intimate functionality and intestinal absorbance. The IEP-treated group (T2) had a villus height-to-crypt depth (VH/CD) ratio that was greater than that in the low-weight basal diet group (T1) in duodenum and ileum. This is in line with the insights of Esmailzadeh *et al*. (2016) who showed higher levels of VH/CD when these broilers were fed with egg powder diets. An increased VH/CD ratio indicates improved nutrient uptake and preserved mucosal integrity (Obianwuna *et al*., 2024). Lower crypt depth in low-weight DOC receiving IEP relative to normal-weight group could reflect lower rates of epithelial cell turnover, which in turn reflects a lesser degree of inflammation and better intestinal health. The depth of crypts is less, the energy spent on cell renewal is less, and cells spend more resources absorbing nutrients and growing (Salari *et al*., 2024). Taken together, these improvements suggest that while T2 may not outperform T0 in all aspects, it exhibits an intestinal histomorphological profile comparable to, or in certain parameters better than the normal-weight group, and significantly better than the untreated low-weight group. This supports the potential of IEP inclusion to enhance nutrient absorption, particularly in low-weight broiler chicks.

In the present study, broilers in the low-weight group fed with IEP showed markedly lower lesion scores in both the duodenum and jejunum compared with their normal-weight counterparts on a basal diet and the untreated low-weight group. In most circumstances, lesion score could be a direct measurement of intestinal damage, where elevated values indicate inflammation, epithelial disruption, and weakened barrier integrity (Johnson & Reid, 1970). The reduced scores in the IEP-treated group highlight its potential protective role in safeguarding mucosal

structure, particularly within key absorptive sites of the intestine. From a physiological perspective, higher lesion scores reflect damage to the intestinal lining, which can lead to shortened or blunted villi, loss of brush-border enzyme and impaired enterocyte activity (Ramchandani *et al*., 2024; De Meyer *et al*., 2019). Such alterations diminish the absorptive surface area and interfere with the active transport processes critical for nutrient uptake. The enhanced histological profile in broilers receiving IEP is likely attributable to the high bioavailability of essential amino acids and bioactive compounds such as phospholipids. Essential amino acids play a vital role in enterocyte proliferation, tissue repair, and the preservation of intestinal integrity (Yvon *et al*., 2024). Meanwhile, phospholipids as integral components of cell membranes, help to maintain membrane fluidity and structural stability in enterocytes, thereby supporting the gut barrier (Boontiam *et al*., 2017).

With enhanced absorptive surface area and preserved epithelial integrity, these physiological benefits are expected to translate into better nutrient assimilation, ultimately, superior growth outcomes. In the present study, dietary inclusion of 4% IEP significantly increased body weight gain and final body weight in low-weight DOC, with values exceeding those of the normal-weight control group. These results indicate that IEP can help overcome the initial disadvantage of low hatch weight while also promotes growth beyond that of normally developed chicks. This aligns with the findings of Aldhanki and Atiyah (2022), who observed improved growth performance in broilers receiving IEP.

The excellent growth response of the IEP group may be associated with the high nutrient bioavailability of IEP, supplying high quality protein, phospholipids and bioactive substances with efficiency nutritional absorption and stimulating secondary muscle proteins (Anton *et al*., 2006). The observed increase in feed intake in the IEP group (relative to the control and basal diet groups) in the present work could be due to the enhanced quality of dietary protein, a condition because the poultry possess physiological means that place greater emphasis on the essential protein ingestion to promote tissue maturation and growth (Gilbert *et al*., 2008). In regard to feed conversion ratio (FCR) in particular, the parameter observed was statistically consistent across the groups, which would show that the feed utilization efficiency was not affected by the IEP treatment. The finding was consistent to what Akinola *et al*. (2020) stated that there was no significant difference in FCR with IEP supplementation in broiler chicken. One possible reason is that eating more protein may raise metabolic energy expenditure, keeping FCR stable even though growth is better. Furthermore, increased lean tissue accretion, driven by improved amino acid availability, may elucidate the augmented weight gain without necessarily influencing feed efficiency.

The physiological responses observed in this study exhibited a cohesive adaptation that promotes recovery and weight gain in low-weight broiler chicks administered diets containing IEP. The increase of hematological values, including the rise of erythrocyte and hemoglobin levels, was the evidence of promoted erythropoiesis and the growth of oxygen transport capacity to maintain the functioning of cells and promote tissue expansion (Astuti *et al*., 2022). These enhancements are congruent with the enhanced antioxidant defences because there is an increase in SOD activity and low levels of MDA. This redox integrity probably maintains the integrity of the erythrocyte and is synergistic with oxygen delivery (Ogbuagu *et al*., 2018). It was evident in the current study that the lipids metabolism of low-weight DOC was positive, being improved after IEP administration. This advancement could be described by the reduced levels of triglyceride alongside the increased levels of HDL, an expression of more effective use of lipids to satisfy the energy requirements during initial development and physiological adjustment. The relationship between better gut structure, oxygen transport, oxidative balance, and lipid metabolism is a coordinated physiological response that helps low-weight chicks fed diets with IEP recover and grow.

CONCLUSION

Including 4% IEP in the diet promoted erythropoiesis, optimized lipid metabolism, and strengthened antioxidant defenses in low-weight broiler chicks. These physiological improvements coincided with improved small intestinal structure and lower lesion scores, leading to enhanced nutrient uptake and better intestinal health. Overall, dietary administration of IEP improved physiological conditions, intestinal function and growth performance of low-weight DOC.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors acknowledge Universitas Diponegoro for funding support and thank the undergraduate students for their assistance during data collection.

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

We declare that generative AI and AI-assisted technologies were used for language refinement and reference organization. All content has been critically reviewed and edited to ensure it reflects the authors' understanding, analysis, and academic integrity.

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