Effects of Yellow Mealworm *(Tenebrio Molitor)* Larvae Meal on Growth Performance, Hematological Parameters, Organ Characteristics, and Intestinal Morphometry of Sasso Broiler Chickens Reared in the Southwestern Area of Lomé, Togo

Nassi Guidi Hoeness Inaudi [1], Agboka Komi [2], Essen Paulinus Ogbonnaya [3*], Pitala Wéré [4]
[1, 3, 4] Laboratory of the Regional Center of Excellence in Avian Sciences (CERSA), University of Lomé, PMB 1515, Lomé, Togo
[2] Research Laboratory on Agro-resources and Environmental Health (LARASE), University of Lomé, PMB 1515, Lomé, Togo

Corresponding Author: **Essen Paulinus Ogbonnaya**

Article Info

E-ISSN: 3107-6599
Volume: 02
Issue: 02
March-April 2026
Received: 06-01-2026
Accepted: 04-02-2026
Published: 02-03-2026
Page No: 01-11

Abstract
The scarcity, high cost, and competition for conventional protein sources have made insects a valuable alternative for poultry feed, offering rich nutrition and a lower environmental footprint. This study evaluated the effects of two animal protein sources: fish meal (FM) and *Tenebrio molitor* larvae meal (TMLM) on the growth performance, organ development, and haematological indices of Sasso broiler chickens over an 85-day period. The study was carried out between August and October 2025 by distributing a total 360 dayold Sasso broiler chicks, randomly assigned to four treatment groups (TM4, TM6, TM8, FM8) in a completely randomized design (CRD), with five replicates of 18 chicks each. Data were collected based on parameters of interest which includes proximate analysis, growth performance, haematological indices, organ weights and morphometric indices. Statistical analyses were performed using GraphPad Prism software, version 9. Bromatological analysis showed no significant differences ($p > 0.05$) between FM and TMLM, though TMLM had higher crude protein, fat, and methionine + cysteine levels. Growth performance metrics, including feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR), varied significantly ($p < 0.05$) across treatments. Broilers fed TMLM diets (TM4%, TM6%, TM8%) exhibited improved FI, BWG, and lower FCR values compared to those fed FM8%. Relative organ weights and morphometric indices also differed significantly ($p < 0.05$), with TM8% yielding the highest heart, gizzard, and abdominal fat weights; TM6% showing superior liver, lung, and intestinal length; and TM4% leading in intestinal weight. Haematological indices revealed significantly higher WBC, RBC, Hb, and HCT values in TMLM-fed groups, particularly TM8%, TM6%, and TM4%, while FM8% consistently recorded the lowest values. Overall, *T. molitor* larvae meal enhanced growth performance, organ development, and blood profile in Sasso broiler chickens, demonstrating its potential as a viable alternative to fish meal in poultry nutrition.

DOI: https://doi.org/10.54660/.IJIADR.2026.2.2.01-11

Keywords: Growth Performance, Haematological Parameters, Intestinal Morphometry, Nutrition, Organ Characteristics, Sasso Broilers, *Tenebrio molitor*

Introduction

Poultry industry has experienced rapid growth in recent years (Khan *et al.*, 2018) [23]. However, one of the major constraints limiting its continued expansion is the availability of sufficient quantities of high-quality feed ingredients (Mahmoud *et al.*, 2025). Protein sources such as soybean meal (SBM) and fish meal (FM) are conventionally used in poultry feed formulations (Mottet and Tempio, 2017) [29]. Soybean meal is the most widely used plant-based protein due to its favorable amino acid profile and protein content (Allahoki, 2023) [4].), while fish meal remains the benchmark for animal-based protein in poultry diets (Van Huis, 2013) [48]. Despite their nutritional value, SBM and FM present several challenges. Increasing competition between human and animal consumption, coupled with fluctuating availability and rising costs, has made these ingredients less sustainable (Elahi *et al*., 2020) [15]. Moreover, their extensive use contributes to environmental degradation. In particular, the overharvesting of marine resources to produce fish meal poses a serious threat to aquatic ecosystems and fish populations (Kaul, 2021) [22].

To address these challenges, insects have emerged as promising alternative protein sources due to their high nutritional value and minimal environmental impact (Van Huis, 2013) [48]. Unlike conventional feed ingredients, insects do not compete with humans for food and can be reared on organic waste and agro-industrial by-products (Nabaterega *et al*., 2025) [30]. Research has demonstrated that insects are easy to produce at low cost and possess high protein content (Ramos-Elorduy *et al*., 2002; Choi *et al*., 2018) [39, 12].

Various studies have explored the use of insect larvae such as termites, cockroaches, crickets, grasshoppers, dipterans, and beetles in the diets of reptiles, small mammals, pets, livestock, pigs, and poultry (Devic *et al*., 2018) [13]. Specifically, silkworms (Bombyx mori), houseflies (Musca domestica), black soldier flies (Hermetia illucens), and yellow mealworms (Tenebrio molitor) have been studied as replacements for SBM and FM in poultry diets, including quail, Ross, and Anak broilers (Bovera *et al*., 2015; 2016) [11, 10].

Yellow mealworms (Tenebrio molitor) have received particular attention for their nutritional benefits. Studies have shown that incorporating Tenebrio molito larvae meal (TMLM) at levels of 1% to 10% in broiler diets can increase the weight of digestive organs such as the small intestine and gizzard (Bovera *et al*., 2016; Nascimento *et al*., 2020) [10, 31]. Additionally, Tenebrio molitor (yellow mealworms) contain approximately 3% chitin, lauric acid, and antimicrobial peptides, which have been shown to enhance growth performance, blood profile, immune function, intestinal morphology (including villi height and crypt depth), and carcass weight in poultry species such as quail, Ross and Anak broilers, and Barbary partridges (Loponte *et al*., 2017; Benzertiha *et al*., 2019; Zadeh *et al*., 2019) [25, 7, 50].

Although extensive literature exists on the use of TMLM in various poultry species, there is limited or no research specifically assessing its effects on Sasso broiler chickens. To address this gap, the present study aims to evaluate the impact of dietary inclusion of Yellow Mealworm (Tenebrio molitor) Larvae Meal on growth performance, hematological parameters, organ characteristics, and intestinal morphometry of Sasso broiler chickens reared in the southwestern region of Lomé, Togo.

It is hypothesized that TMLM can serve as a high-quality protein source with multiple nutritional benefits, thereby enhancing growth, health, and overall performance in Sasso broiler chickens. This research contributes to the ongoing exploration of insect-based feed alternatives and supports the development of sustainable protein sources for the poultry industry.

Materials and Methods

Experimental Ethical Approval

All experimental procedures involving animals were reviewed and approved by the Animal Ethics Committee of the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, Togo, operating under the National Ethics Committee for the Control and Supervision of Experiments on Animals. All efforts were made to minimize discomfort to the birds, and the study was conducted in compliance with the ARRIVE guidelines.

Study area and experimental duration

The research was conducted at the Experimental Unit of the Teaching and Research Farm, Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, located in the Maritime Region, Lomé, Togo. The site is situated at a latitude of 6.13° North and a longitude of 1.22° East, with an altitude of approximately 64 meters above sea level. It lies within the tropical savanna (Aw) agro-ecological zone of southeastern Togo. The region is characterized by an average annual rainfall of about 900 to 1,400 mm, distributed over 70 to 100 rainy days per year. Relative humidity during the rainy season typically ranges from 70% to 84%, while environmental temperatures vary between 23.4°C and 32.3°C throughout the year (UL, 2024; AccuWeather, 2024) [46, 1]. The experiment data collection period was considered from the 15th of July to the 15th of October, 2025.

Production, sourcing, and processing of test ingredients

Yellow mealworms (Tenebrio molitor L.) used in the experiment were reared on wheat bran and corn bran substrates, housed in 600 plastic trays. Fresh orange slices (50 g), peeled and cut into small pieces, were provided as a supplementary source of moisture and energy for the larvae. After three months of rearing, the larvae were harvested and dried at 65°C for 72 hours using a PRIORCLAVE CL012N autoclave (manufactured by Priorclave Ltd., United Kingdom). The dried larvae were then ground into meal using a GRINDOMIX GM 300 blender (Manufacturer: Retsch GmbH, Germany) to produce Tenebrio molitor Larvae Meal (TMLM).

The entire production process was conducted at the Research Laboratory on Agro-resources and Environmental Health (LARASE), University of Lomé, Togo. The resulting TMLM was packaged in airtight containers and stored at room temperature.

For comparison, the Danish fish meal used as the protein source in the control diet was purchased from a reputable feed ingredient supplier (HTS Farms Ltd) in Ibadan, Nigeria.

Egg collection and incubation

A total of 540 fertile eggs from the Sasso broiler strain were collected from the experimental unit of the Regional Centre of Excellence for Avian Sciences (CERSA) at the University of Lomé, Togo. All eggs originated from the same parent stock and were collected once. They were stored vertically with the broad end facing upward at ambient room temperature (16°C) and 85% relative humidity (RH) for four days prior to incubation.

Incubation was carried out simultaneously at the Poultry Hatchery Laboratory of CERSA using a Petersime incubator, maintained at a constant temperature of 37.7°C, 65% RH, and optimal ventilation. Automatic egg turning occurred every two hours, starting on day 3 and continuing until day 18 of incubation. On day 18, the eggs were candled to identify and remove infertile ones.

The viable eggs were then transferred to the hatcher for the final three days of incubation. Hatching was completed at 21.12 days (516 hours), yielding a total of 381 chicks. From this group, a sample of 262 chicks was individually weighed to determine their average initial body weight. The chicks were subsequently divided into three groups of 127 each, placed in plastic baskets, and transported to the rearing site located within the Open Housing System of CERSA.

Experimental design, management, and experimental diets

A total of 360day old Sasso broiler chicks were obtained from the Poultry Hatchery Laboratory of CERSA. The chicks were randomly assigned to four treatment groups (TM4, TM6, TM8, and FM8) using a completely randomized design (CRD). Each treatment group was replicated five times, with 18 chicks per replicate.

Prior to the arrival of the chicks, the housing facility was prepared by covering the floor with wood shavings at a depth of 4 cm. This litter depth was selected to ensure adequate insulation from cold surfaces, effective moisture absorption, and cushioning to prevent leg injuries. Each chick was tagged with a distinct color of poplin tape on its wing corresponding to its treatment group for easy identification.

All rearing equipment including feeders, drinkers, and heating devices were thoroughly washed, dried, and disinfected to minimize the risk of disease outbreaks. The housing system was equipped with feeding and watering troughs, charcoal pots for heat provision, and a digital thermo-hygrometer (HTC-2; China) to monitor and regulate environmental conditions such as temperature and relative humidity.

Upon arrival and after each handling, an anti-stress supplement (glucose and vitamins) was administered through their drinking water. Feed and water were provided ad libitum throughout the study. Adequate ventilation was maintained, and lighting was supplied for 12 hours during the day (natural daylight) and 12 hours at night using solar lamps. The chicks were vaccinated according to the standard broiler vaccination schedule outlined by Afrimash (2024) [2]. Additional management practices including sanitation, pest and rodent control, biosecurity measures, and litter management were strictly adhered to.

Experimental diets were formulated to meet the nutritional requirements of Sasso broiler chickens at different growth phases: starter, grower, and finisher. Each diet incorporated fish meal, Tenebrio molitor larvae meal, and other ingredients as presented in Table 1. The formulations were designed to align with the nutrient recommendations established by the National Research Council (NRC, 1994) [32] for poultry across various age groups.

Table 1: Experimental diets of Sasso broiler chickens fed yellow mealworm *(Tenebrio molitor)* larvae meals over 85 days, formulated according to their growth phases (starter, grower, and finisher)

Ingredients	Start				Growth				Finishing			
	FM8	TM4	TM6	TM8	FM8	TM4	TM6	TM8	FM8	TM4	TM6	TM8
Maize	50.00	50.00	50.00	50.00	55.00	55.00	55.00	55.00	60.00	60.00	60.00	60.00
Wheat bran	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
BDG	10.00	14.00	14.00	10.00	10.00	14.00	14.00	10.00	10.00	14.00	14.00	10.00
SBM	27.00	27.00	25.00	27.00	21.15	21.15	19.15	21.15	16.00	16.00	14.00	16.00
TMLM	0.00	4.00	6.00	8.00	0.00	4.00	6.00	8.00	0.00	4.00	6.00	8.00
FDFM (72%)	8.00	0.00	0.00	0.00	8.00	0.00	0.00	0.00	8.00	0.00	0.00	0.00
Oyster shell	1.15	1.15	1.15	1.15	2.00	2.00	2.00	2.00	2.15	2.15	2.15	2.15
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.20	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutriments (%)												
Crude protein	20.91	20.89	21.23	21.57	19.02	19.43	19.85	20.27	18.02	18.43	18.85	19.27
Crude fat	4.84	5.42	5.91	6.40	4.45	5.04	5.53	6.02	4.25	4.84	5.33	5.82
Crude ash	6.83	6.45	6.36	6.27	6.79	6.41	6.32	6.23	6.79	6.41	6.32	6.23
Crude fiber	6.42	6.56	6.63	6.70	6.46	6.60	6.67	6.74	6.46	6.60	6.67	6.74
Lysine	1.26	1.26	1.29	1.32	1.17	1.20	1.23	1.26	1.07	1.10	1.13	1.16
Methionine	0.59	0.59	0.61	0.63	0.56	0.57	0.59	0.61	0.53	0.54	0.56	0.58
Meth. + Cystine	1.04	1.05	1.09	1.13	0.98	1.00	1.04	1.08	0.93	0.95	0.99	1.03
Tryptophan	0.29	0.30	0.31	0.32	0.27	0.28	0.29	0.30	0.25	0.26	0.27	0.28
Threonine	0.79	0.80	0.83	0.86	0.74	0.76	0.79	0.82	0.69	0.71	0.74	0.77
Calcium	1.43	1.23	1.23	1.23	1.41	1.21	1.21	1.21	1.52	1.32	1.32	1.32
Phosphorus	0.56	0.52	0.52	0.52	0.55	0.51	0.51	0.51	0.55	0.51	0.51	0.51
ME (kcal/kg)	2967.00	2992.00	3006.00	3020.00	2952.00	2981.00	2997.00	3013.00	2942.00	2971.00	2987.00	3003.00

Vitamin and trace mineral declaration 0.25kg of broiler premix contains: Vitamin A: 2400000 IU, vitamin D: 1000000 IU, vitamin E: 16000 IU, vitamin K: 800mg, vitamin B6: 1000 mg, vitamin B12: 6mg, Niacin: 8000mg, folic acid: 400mg, pantothenic acid: 3000mg, Biotin: 40mg, antioxidant: 3000mg, Cobalt: 80mg, copper: 2000mg, Iodine: 400mg, iron: 1200mg, Manganese: 1800mg, Selenium: 60mg, and Zinc: 14000mg. TMLM = *Tenebrio. molitor* larvae meal; FDFM = Foreign danish fish meal ; BDG = Brewer's dried grain ; SBM = Soybean meal ; ME = Metabolizable energy. Diet inclusion levels and treatment groups: FM8 = 8% fish meal; TM4 = 4% *Tenebrio molitor* larvae meal; TM6 = 6% *Tenebrio molitor* larvae meal; TM8 = 8% *Tenebrio molitor* larvae meal.

Data collection

A known quantity of fish meal (FM) and Tenebrio molitor larvae meal (TMLM) was sampled from the procured and milled ingredients, respectively, and taken to the Research Laboratory on Agro-resources and Environmental Health (LARASE), University of Lomé, Togo. Proximate analysis was conducted to determine parameters including dry matter, crude protein, crude fat (ether extract), crude fiber, metabolizable energy (ME), and essential mineral and amino acid components such as calcium, phosphorus, methionine + cysteine, and lysine. These analyses were performed prior to feed formulation, following the procedures outlined by AOAC (2023) [5].

Growth performance parameters

All measurements were performed in all treatment groups according to different growth phases. Data on the initial body weight of the experimental birds were taken at the beginning of the trial and weekly subsequently. The BW of birds in each pen was recorded using electronic sensitive scales (DIGITAL KITCHEN SCALE WP6291, manufactured by Winpeace Ltd., China). Feed rations were weighed before serving, and leftover feed was measured at the end of each feeding period. Growth performance indices were calculated as follows:

$$\text{Total body weight gain} = \text{Final body weight} - \text{Initial body weight}$$

$$\text{Total feed intake (g)} = \text{Quantity of feed given} - \text{Feed Leftover}$$

$$\text{Feed conversion (FCR)} = \frac{\text{Total feed consumed (g)}}{\text{Total body weight gain (g)}}$$

Organ weights and morphometric indices

Broiler chickens of the same age, previously selected for hematological analysis, were also used to evaluate organ weights and morphometric indices. Each bird was humanely euthanized by severing the jugular vein with a sterile knife. Following exsanguination (approximately 5 minutes), the carcasses were opened and the internal organs including the gizzard, intestine, heart, liver, lungs, and kidneys were carefully excised and weighed using a precision electronic scale (DIGITAL KITCHEN SCALE WP6291, manufactured by Winpeace Ltd., China). Additionally, the length of the intestines was measured.

All organ weights were expressed relative to the live body weight of each bird using the formula:

$$\text{Relative organ weight (ROW \%)} = \frac{\text{Absolute Organ weight (g)}}{\text{Live weight (g)}} \times \frac{100}{1}$$

Haematological indices

At the end of the 12-week experimental period, blood samples were collected from twenty (20) randomly selected broiler chickens in each treatment group. Approximately 1.5 ml of blood was drawn from the wing vein of each bird using a hypodermic sterile syringe fitted with a 21-gauge needle. The blood was transferred into labeled sterile tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Samples were placed in flasks containing ice and transported to the Research Laboratory on Agro-resources and Environmental Health (LARASE), University of Lomé, Togo, for haematological analysis.

Haematological parameters assessed included white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), and packed cell volume (PCV). These indices were measured using a DYMIND DH36 automatic hematology analyzer (Shenzhen Dymind Biotechnology Co., Ltd., China).

Statistical analysis

All collected data were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure in GraphPad Prism version 9 (GraphPad Software Inc., San Diego, California, USA). Mean comparisons were performed using Tukey's multiple range test at a significance level of $P < 0.05$. Charts were generated using GraphPad Prism version 9 (GraphPad Software Inc., 2022). The one-way statistical model is given as follows:

$$Yij = \mu + Ti + eij$$

Where;
Yij = Single observation;
μ = Over all mean;
Ti = Treatment effect; and
eij = Random error.

Results

Proximate composition of test ingredients

Table 2 presents the results of bromatological analyses conducted on the protein sources: fish meal (FM) and *Tenebrio molitor* larvae meal (TMLM), used in the formulation of experimental diets for Sasso broiler chickens. No statistically significant differences ($p > 0.05$) were observed between the two protein sources across all measured parameters. However, proximate analysis of the test ingredients confirmed the presence of dry matter (91.30; 85.30%), crude protein (41.10; 52.09), crude fat (25.00; 37.44), ether extract (9.37; 3.29), crude fibre (7.00-5.60), metabolizable energy (2800.00; 2360.00), calcium (4.00; 1.45), phosphorus (2.50; 3.50), methionine plus cysteine (1.54; 4.63), and lysine (2.00; 1.60), respectively.

The FM source exhibited higher values for DM, Ash, CF, Ca, and ME. In contrast, TMLM showed elevated levels of CP, crude fat, and Met + Cys. Lysine content was approximately similar in both protein sources.

Table 2: Nutrient composition of fish meal and *Tenebrio molitor* used in formulation of experimental diets for Sasso broiler chickens reared over 85 days

Parameters (%)	Animal protein sources	
	Fish meal (FM)	*Tenebrio molitor* larvae meal (TMLM)
Dry matter	91.30	85.30
Crude protein	41.10	52.09
Crude fat	25.00	37.44
Crude ash	9.57	3.29
Crude fibre	7.00	5.60
Calcium	4.00	1.45
Phosphorus	2.50	3.50
Methionine + Cysteine	1.54	4.63
Lysine	2.00	1.60
ME (kcal/kg)	2800.00	2360.00

Growth performance parameters

Table 3 presents the growth performance metrics of Sasso broiler chickens fed two different animal protein sources: fish meal (FM) and *Tenebrio molitor* larvae meal (TM) at varying inclusion levels over an 85-day period. The evaluated parameters include initial body weight (IBW), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). The diets consisted of FM at 8% inclusion and TM at 4%, 6%, and 8% inclusion levels. All evaluated growth performance parameters showed statistically significant differences ($p < 0.05$), except for IBW, which remained consistent across treatments.

The initial body weights of the broiler chickens ranged between 41.90 and 42.02 grams. Total feed intake increased progressively across the production phases, ranging from 560.01–656.20 g in the starter phase, 1370.25–1525.44 g in the grower phase, and 2471.60–2994.63 g in the finisher phase. Correspondingly, body weight gain ranged from 320.82–340.09 g, 580.51–620.15 g, and 690.33–740.14 g across the starter, grower, and finisher phases, respectively. Feed conversion ratios also varied, with values between 1.65–1.93 (starter), 2.21–2.58 (grower), and 3.34–4.22 (finisher).

During the starter phase, broilers fed the TM8% diet recorded significantly higher FI ($p < 0.001$) compared to those fed TM4%, TM6%, and FM8%. In the grower and finisher phases, FI was significantly higher ($p < 0.001$) in birds fed TM4% and TM6% diets compared to those on FM8% and TM8%.

At the starter phase, broilers fed TM8% and FM8% diets showed similarly high BWG ($p < 0.001$) compared to other treatments. During the grower and finisher phases, BWG increased proportionally with TM inclusion level, following the trend: TM8% > TM6% > TM4% > FM8%.

Across all growth phases, broilers fed TM8% (Treatment 4) exhibited the lowest FCR values: 1.65 (starter, $p = 0.001$), 2.21 (grower, $p = 0.013$), and 3.34 (finisher, $p = 0.001$). In contrast, higher FCR values were observed in broilers fed FM8% (Treatment 1) during the starter phase (1.93), and in those fed TM4% (Treatment 2) during the grower and finisher phases (2.58 and 4.22, respectively).

Table 3: Growth performance of Sasso broiler chickens fed two different animal protein sources (Fish meal and *Tenebrio molitor* larvae meal) at varying inclusion levels over 85 days

Parameters	Treatments groups				P-value
	FM8%	TM4%	TM6%	TM8%	
IBW	42.00 ± 0.08	41.95 ± 0.30	41.90 ± 0.10	42.02 ± 0.08	0.086
	Total feed intake				
TFIS (g)	656.20 ± 0.25^{a}	633.60 ± 0.55^{b}	595.00 ± 0.47^{c}	560.01 ± 0.47^{d}	0.001
TFIG (g)	1386.50 ± 0.88^{c}	1525.44 ± 0.93^{a}	1500.02 ± 0.86^{b}	1370.25 ± 0.40^{d}	0.001
TFIF (g)	2556.01 ± 0.34^{c}	2994.63 ± 0.68^{a}	2854.20 ± 0.52^{b}	2471.60 ± 0.84^{d}	0.001
	Body weight gain				
BWGS (g)	340.00 ± 0.49^{ab}	330.29 ± 0.85^{b}	320.82 ± 0.95^{c}	340.09 ± 0.92^{a}	0.001
BWGG (g)	580.51 ± 0.88^{d}	590.25 ± 0.58^{c}	600.41 ± 0.58^{c}	620.15 ± 0.63^{a}	0.001
BWGF (g)	690.33 ± 0.22^{d}	710.28 ± 0.38^{c}	720.03 ± 0.94^{b}	740.14 ±0.57^{a}	0.001
	Feed conversion ratio				0.001
FCRS	1.93 ± 0.10^{a}	1.92 ± 0.10^{ab}	1.85± 0.10^{b}	1.65± 0.10^{c}	0.001
FCRG	2.39 ± 0.18^{c}	2.58 ± 0.18^{a}	2.50 ± 0.18^{b}	2.21 ± 0.18^{d}	0.013
FCRF	3.70 ± 0.45^{c}	4.22 ± 0.45^{a}	3.96 ± 0.45^{b}	3.34 ± 0.45^{d}	0.001

a,b,c,d values within the same row followed by different subscripts differ significantly ($p < 0.05$). IBW = Initial body weight ; FIS = Average feed intake at starter phase ; FIG = Average feed intake at grower phase ; FIF = Average feed intake at finisher phase ; BWGS = Body weight gain at starter phase ; BWGG = Body weight gain at grower phase ; BWG = Body weight gain at finisher phase ; FCRS = Feed conversion ratio at starter phase ; FCRG = Feed conversion ratio at grower phase ; FC = Feed conversion ratio at finisher phase

Table 4 presents the relative organ weights (heart, liver, lungs, kidney, and gizzard) and morphometric indices (abdominal fat, intestinal weight, and intestinal length) of Sasso broiler chickens fed two different animal protein sources: fish meal and *Tenebrio molitor* larvae meal at varying inclusion levels over an 85-day period. All measured parameters showed statistically significant differences ($p < 0.05$) across the treatment groups.

The values obtained in this study ranged as follows: RHW (Relative Heart Weight): 0.29–0.36%, RLW (Relative Liver

Weight): 1.32–2.09%, RLW2 (Relative Lung Weight): 0.42–0.68%, RKW (Relative Kidney Weight): 0.17–0.29%, RGW (Relative Gizzard Weight): 2.11–2.42%, RIW (Relative Intestinal Weight): 4.04–5.12%, RIL (Relative Intestinal Length): 7.06–8.17 cm, and AF (Abdominal Fat): 0.05–0.35%.

Broilers fed diets containing *T. molitor* larvae meal (TM4%, TM6%, and TM8%) generally exhibited higher relative organ weights and morphometric indices compared to those fed the fish meal diet (FM8%). Specifically, TM8% (Treatment 4) and abdominal fat ($p = 0.0017$, $p = 0.0235$); TM6% (Treatment 3) showed the highest values for liver, lungs, abdominal fat, and intestinal length ($p < 0.0001$, $p = 0.0052$); TM4% (Treatment 2) had the highest intestinal weight ($p < 0.0001$) and FM8% (Treatment 1) recorded the highest kidney weight, although values from TM6% and TM8% were comparable. TM4% had the lowest kidney weight.

In contrast, the lowest relative heart weight was observed in TM6%, while lungs and kidney weights were lowest in TM4%. FM8% recorded the lowest values for liver, gizzard, abdominal fat, intestinal weight, and intestinal length.

Table 4 : Organ weights and morphometric indices of Sasso broiler chickens fed two different animal protein sources (Fish meal and *Tenebrio molitor* larvae meal) at varying inclusion levels over 85 days

Parameters	Treatments groups				P-value
	FM8%	TM4%	TM6%	TM8%	
RHW (%)	0.31 ± 0.00^{bc}	0.32 ± 0.00^{b}	0.29 ± 0.00^{c}	0.36 ± 0.01a	0.0017
RLW (%)	1.32 ± 0.00^{c}	1.50 ± 0.05^{b}	2.09 ± 0.03^{a}	1.50 ± 0.03^{b}	<0.0001
RLW_2 (%)	0.48 ± 0.02^{bc}	0.42 ± 0.02^{c}	0.68 ± 0.00^{a}	0.54 ± 0.01^{b}	<0.0001
RKW (%)	0.29 ± 0.00^{a}	0.17 ± 0.00^{c}	0.25 ± 0.00^{b}	0.26 ± 0.00^{ab}	<0.0001
RGW (%)	2.11 ± 0.04^{bc}	2.33 ± 0.09^{b}	2.34 ± 0.06^{ab}	2.42 ± 0.08^{a}	0.0235
RIW (%)	4.04 ± 0.05^{c}	5.12 ± 0.08^{a}	4.76 ± 0.10^{b}	4.86 ± 0.04^{ab}	<0.0001
RIL (cm)	7.06 ± 0.70^{bc}	7.10 ± 0.22^{b}	8.17 ± 0.38^{a}	7.64 ± 0.16^{ab}	0.0052
AF (%)	0.05 ± 0.00^{d}	0.27 ± 0.01^{b}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	<0.0001

a,b,c,d values within the same row followed by different subscripts differ significantly ($p < 0.05$). RHW = Relative heart weight ; RLW = Relative liver weight ; RLW_2 =Relative lungs weight ; RKW = Relative kidney weight ; RGW = Relative gizzard weight ; RIW = Relative intestinal weight ; RIL = Relative intestinal length AF = abdominal fat.

Haematological Indices

Table 5 presents the haematological indices of Sasso broiler chickens fed two different animal protein sources: fish meal and *Tenebrio molitor* larvae meal at varying inclusion levels over an 85-day period. All measured parameters showed statistically significant differences ($p < 0.05$) across the treatment groups.

A similar trend observed in organ weight evaluation was also evident in the blood profile. Broilers fed diets containing *T. molitor* larvae meal (TM4%, TM6%, and TM8%) generally exhibited higher haematological values compared to those fed the fish meal-based diet (FM8%).

The values obtained in this study ranged as follows: WBC: 72.51–94.16 $\times 10^3$/ µL, RBC: 2.35–2.73 $\times 10^9$/L, Hb: 13.52–15.33 g/dL and HCT: 28.85–33.93 %.

Treatment 2 (TM4%) recorded the highest and significantly different ($p < 0.0001$) WBC count, compared to the other groups. Treatment 4 (TM8%) showed the highest HCT value ($p < 0.0001$), while Treatment 3 (TM6%) had the highest Hb concentration ($p < 0.0001$). In contrast, Treatment 1 (FM8%) consistently recorded the lowest values across most haematological parameters, including WBC, Hb, and HCT.

Table 5 : Haematological indices of Sasso broiler chickens fed two different animal protein sources (Fish meal and *Tenebrio molitor* larvae meal) at varying inclusion levels over 85 days
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Parameters	Treatment groups				P-value
	FM8%	TM4%	TM6%	TM8%	
WBC ($\times 10^3$/µL)	72.51 ± 0.73^{d}	94.16 ± 1.46^{a}	79.65 ± 3.75^{b}	78.81 ± 2.87^{c}	<0.0001
RBC ($\times 10^9$/L)	2.53 ± 0.20^{bc}	2.35 ± 0.06^{c}	2.64 ± 0.30^{ab}	2.73 ± 0.03^{a}	0.0042
Hb (g/dL)	14.75 ± 0.73^{ab}	14.25 ± 0.50^{ab}	15.33 ± 0.2^{a}	13.52 ± 0.76^{b}	<0.0001
HCT (%)	30.72 ± 0.38^{c}	28.85 ± 0.68^{d}	31.45 ± 1.58^{b}	33.93 ± 0.50^{a}	<0.0001

a,b,c,d values within the same row followed by different subscripts differ significantly ($p < 0.05$). WBC = White blood cell; RBC = Red blood cell; Hb = Hemoglobin; HCT = Hematocrit; NR = Normal range

Discussion

The proximate composition of two different animal protein sources: fish meal and Tenebrio molitor larvae meal, revealed high nutrient content on a dry matter basis, underscoring their potential as viable feed ingredients. The elevated dry matter content suggests strong nutritive value and extended shelf-life, enhancing storage stability (Akinsola *et al*., 2021) [3].

TMLM demonstrated a notably higher crude protein level, positioning it as a promising alternative to conventional protein sources. Its protein density may better support growth and muscle development in broiler chickens. Given that protein is the most expensive component in animal feed (Zirari *et al*., 2024) [51], the use of TMLM could help reduce production costs, mitigate reliance on inconsistent protein sources, and ease competition between human and animal consumption.

Additionally, the higher crude fat content in TMLM indicates its value as a rich energy source, potentially improving broiler performance and offering greater flexibility in feed formulation. The substantial fat content may reduce the need for supplemental fat sources such as vegetable oils, thereby simplifying feed design and lowering costs. However, its high fat levels necessitate careful handling to prevent spoilage and maintain dietary balance. The ether extract values further affirm the ingredient's energy contribution to metabolic and daily activities (Romes *et al*., 2019; Zirari *et* *al*., 2024) [41, 51].

The appreciable ash content in TMLM reflects the presence of essential minerals that support bone development, metabolic functions, and overall production performance (Umeoka, 2024) [47]. Notably, minerals such as calcium, phosphorus, and amino acids like lysine, methionine, and cysteine enhance its suitability for broiler feed formulations. These nutrients are vital for maintaining normal physiological functions (Rempel *et al*., 2021) [40].

Moderate crude fibre levels in TMLM contribute to gut health, motility, and improved digestive efficiency, making it a suitable poultry feed ingredient. While primarily rich in protein and amino acids, TMLM also provides metabolizable energy essential for rapid growth, thermoregulation, and metabolic activity in broilers (Essen *et al*., 2025) [17].

Langston *et al*. (2024) examined the proximate composition of T. molitor larvae reared on four different substrates over two generations. Their findings: dry matter (82.25%–87.90%), crude fibre (4.87%–8.67%), crude ash (2.07%–3.74%), and calcium (2.07%–4.14%) were comparable to those observed in the present study. However, their reported crude protein (28.57%–36.24%) and crude fat (22.84%–36.07%) levels were lower than those obtained here.

In contrast, the current study's results for dry matter, crude ash, crude fat, and crude protein closely align with those of Matielle *et al*. (2025) [27], who analyzed T. molitor subjected to various processing techniques and drying durations. Their reported values ranged from 88.42%–96.21% for dry matter, 2.36%–3.53% for crude ash, 27.16%–34.25% for crude fat, and 46.82%–50.41% for crude protein.

In alignment with the present experimental trial, Attivi *et al*. (2022) [6] assessed the chemical and nutritional composition of commercial fish meal and Hermetia illucens (black soldier fly) larvae meal. Their findings revealed higher dry matter contents of 94.40% and 91.30%, and crude ash values of 18.20% and 9.57% for fish meal and H. illucens larvae meal, respectively, compared to the values obtained in this study.

Conversely, both protein sources exhibited lower crude protein levels: 40.00% for fish meal and 41.10% for H. illucens larvae meal, relative to the higher crude protein content recorded in Tenebrio molitor larvae meal (TMLM) in the current study. Similarly, the crude fat content of fish meal (10.00%) and H. illucens larvae meal (25.00%) was lower than that of TMLM. Crude fibre values were comparable across the three protein sources, with fish meal and H. illucens larvae meal recording 5.5% and 7.00%, respectively, aligning closely with the crude fibre content observed in this study. Regarding mineral composition, calcium content in fish meal (4.00%) was similar to that of TMLM, while H. illucens larvae meal showed a higher calcium level of 6.5%. A similar trend was observed for phosphorus, where fish meal recorded a comparable value of 2.50%, whereas H. illucens larvae meal had a lower phosphorus content of 1.05% compared to the present findings. For methionine + cystine, fish meal reported a similar value of 2.00%, while H. illucens larvae meal had a lower value of 1.45% relative to the higher concentration found in TMLM. In terms of lysine, fish meal showed a comparable value of 1.91%, whereas H. illucens larvae meal exhibited a higher lysine content of 4.90% compared to TMLM. Lastly, metabolizable energy (ME) content was lower in fish meal (2,600 kcal/kg) but higher in H. illucens larvae meal (3,102 kcal/kg) relative to the ME value recorded in this study.

These comparisons underscore the nutritional variability between insect-based and conventional protein sources, reinforcing the potential of insect meals such as T.molitor as viable alternatives in animal feed formulations. In particular, insect-derived proteins offer promising nutritional profiles that can rival or exceed those of traditional sources like fish meal.

The consistency in nutritional composition across multiple studies further highlights the reliability and cost-effectiveness of Tenebrio molitor larvae rearing. Its production supports sustainable food and feed systems, aligns with the United Nations Sustainable Development Goals (SDGs), and presents a more environmentally friendly option compared to conventional animal protein sources (Errico *et al*., 2022) [16].

This sustainability advantage contributed to T. molitor becoming the first insect approved by the European Food Safety Authority (EFSA) as a novel food under EU Regulation 2015/2283 (EFSA NDA, 2021) [14].

Based on these findings, T. molitor larvae meal demonstrates strong potential as a highly suitable and sustainable feed ingredient for poultry, particularly broiler chickens.

Growth performance metrics, including feed intake, body weight gain, and feed conversion ratio, varied significantly ($p < 0.05$) across treatments. Broiler chickens fed TMLM diets (TM4%, TM6%, TM8%) exhibited improved feed intake, body weight gain, and best feed conversion ratio, values compared to those fed FM8%.

The initial body weight of the chicks did not differ significantly across treatment groups, indicating that no selection bias was introduced and confirming that the birds were randomly assigned. This observation aligns with the report of Olubisi *et al*. (2025) [33], who reported no significant variation in initial body weight among four broiler breeds (Cobb, Ross, Marshall and Arbor-Acres) supporting our claim in the validity of randomization in treatment allocation.

According to Essen *et al*. (2025) [17], feed intake is the single most important factor in influencing the growth rate of animals. During the starter phase, diets containing TMLM did not influence feed intake compared to the control group (FM8%), probably due to differences in palatability, digestibility, and nutrient composition. This result is consistent with the findings of Nascimento *et al*. (2021) [31] but contrasts with Elahi *et al*. (2020) [15] who observed significantly increased feed intake in Ross 308 broiler chicks fed yellow mealworm meal at a 4% inclusion level.

During the grower and finisher phases, feed intake progressively decreased as the inclusion level of TMLM increased from 6% to 8%. Notably, the control group (FM8%), also recorded reduced feed intake, comparable to the 8% TMLM group. Group 2, which received lower inclusion level 4% TMLM, exhibited the highest feed intake during these phases, suggesting that higher inclusion levels of both TMLM and fish meal may suppress voluntary feed consumption. Parlar and Ustundag (2024) [36] similarly reported that increasing TMLM inclusion in broiler diets led to reduced feed intake and growth performance, particularly at levels above 6%. The authors attributed these factors to reduced palatability, increased dietary fat content, and the presence of anti-nutritional compounds such as chitin. Biasato *et al*. (2018) [9] concluded that lower inclusion levels of TMLM may be more suitable for optimizing body weight, feed intake, feed efficiency, and intestinal morphology.

Contrary to expectations, broiler chickens fed TMLM-based diets exhibited higher body weight gain than those in the control group across all growth phases. This improvement may be attributed to the higher nutrient availability and the presence of high-quality amino acids in insect protein sources, which enhance their suitability for broiler feed formulations. Notably, Group 4 (TM8%), recorded the highest weight gain and the lowest (best) feed conversion efficiency (FCR). This trend, observed consistently across different growth stages, supports the findings of Shadi *et al*. (2020) [43] and Elahi *et al*. (2020) [15], who reported improved FCR and enhanced body weight gain in broilers fed TMLM-based diets compared to conventional control diets.

Collectively, the findings affirm the potential of TMLM as a high-quality and sustainable protein source in poultry nutrition. Its inclusion in broiler diets not only enhances growth performance but also supports environmentally responsible feed strategies. Notably, increasing the dietary inclusion level of TMLM particularly up to 8% was associated with improved feed efficiency and body weight gain in Sasso broiler chickens, highlighting its viability as an alternative to conventional protein sources.

The weights of internal organs such as the liver and kidneys are commonly used in feeding trials to assess potential toxicity. An abnormal increase in organ weight may indicate a heightened metabolic response aimed at detoxifying harmful substances present in the diet (Ewa *et al*., 2023) [19]. In the current study, the organ weight values remained within a consistent range across treatment groups, suggesting the absence of toxic effects from the test ingredients.

Regarding organ evaluation, the results obtained in this study did not align with the findings of Ewa *et al*. (2023) [19], who administered graded levels of Zingiber officinale to Ross 308 broiler chickens over a 49-day period. Their study reported no significant differences in organ characteristics across treatments, although the values recorded: heart (0.43–0.48%), liver (1.98–2.44%), kidney (0.59–0.71%), gizzard (1.72–2.04%), and intestinal weight (3.23–4.20%), were higher than those observed in the present study. Similarly, Attivi *et al*. (2022) [6] investigated the effect of replacing fish meal with black soldier fly larvae meal on the relative organ weights of Isa Brown layers fed varying inclusion levels of larvae meal. While no significant differences were found across treatments, the authors reported notably higher organ weights during the grower phase (20 weeks of age): liver (16.86–19.81%), heart (3.61–3.88%), and gizzard (19.11–22.71%). These elevated values were attributed to differences in age, breed, and the high-quality feed provided.

The observed improvement in relative intestinal length among Sasso broiler chickens fed diets containing Tenebrio molitor larvae meal suggests enhanced intestinal development, likely due to longer villi and an increased luminal surface area. This morphological enhancement may support improved digestive enzyme activity and nutrient absorption, as previously reported by Laudadio *et al*. (2012) [24] and Sedgh-Gooya *et al*. (2021) [42] who found that moderate dietary protein levels and T. molitor inclusion positively influenced intestinal morphology and nutrient utilization in broilers.

Our findings contrast with those of Biasato *et al*. (2016) [8], who reported no significant changes in intestinal morphology following the inclusion of 75 g/kg yellow mealworm in free-range chickens. The discrepancy may be due to differences in bird genotype, rearing conditions (free-range vs. controlled), or inclusion levels. Notably, Biasato's study focused on histological features under free-range conditions, where environmental factors and parasitic exposure may have masked dietary effects.

The significant increase in abdominal fat observed in Sasso broiler chickens with higher inclusion levels of TMLM may be attributed to the high lipid content, elevated energy density, and altered nutrient partitioning associated with the test ingredient, all of which can promote fat deposition. This finding aligns with the report by Benzertiha *et al*. (2019) [7], who demonstrated that full-fat T. molitor inclusion increased dietary energy and contributed to greater carcass fat deposition in female Ross 308 broiler chickens. Conversely, Popova *et al*. (2025) [37] investigated the effects of incorporating low-fat T. molitor meal as a partial replacement for soybean meal in Ross 308 broiler diets. They concluded that inclusion levels up to 5% had no adverse effects on meat quality and, in fact, reduced abdominal fat deposition compared to soybean-based diets. In contrast to our findings, Vasilopoulos *et al*. (2024) [49] reported no significant changes in abdominal fat or overall carcass traits when whole dried T. molitor larvae meal was included at 5–10% in the diets of Ross 308 broilers over a 35-day period. These discrepancies may be due to differences in insect meal composition (full-fat vs. low-fat), inclusion levels, broiler genotype, or duration of feeding.

Blood is a vital body fluid responsible for transporting essential substances such as nutrients and oxygen to cells, while also removing metabolic waste products. Blood analysis provides valuable insights into the physiological, nutritional, and pathological status of animals. It plays a critical role in disease diagnosis and treatment. Moreover, dietary intake has measurable effects on blood composition, making hematological evaluation a reliable indicator of long-term nutritional status (Ewa *et al*., 2023) [19].

In the present study, hematological results did not follow a consistent trend and differed from the findings of Kokore *et al*. (2021), who reported no significant differences ($p > 0.05$) in hematological indices after evaluating broilers and local chickens over a 90-day period in Korhogo. In contrast, the results of Ewa *et al*. (2023) [19] support the current findings, as they observed significant changes in all hematological parameters in Ross broilers fed diets containing graded levels of Zingiber officinale rhizome meal over 49 days.

The increase in hematological parameters observed in Sasso broilers fed diets supplemented with TMLM may be attributed to bioactive compounds such as chitin and antimicrobial peptides. All measured blood parameters remained within standard reference ranges, except for total WBC counts, which were elevated. This elevation likely reflects immune stimulation due to dietary antigens or the immunomodulatory effects of TMLM. Importantly, in the absence of clinical symptoms such as lethargy, poor growth, or diarrhea, elevated WBC levels are indicative of physiological adaptation rather than pathology. These findings suggest that TMLM inclusion may enhance immune responsiveness without compromising bird health.

White blood cell values varied significantly among treatment groups. As WBCs are essential for fighting infections, phagocytosing foreign organisms, and transporting antibodies, animals with low WBC counts are more susceptible to infections due to weakened immunity.

Conversely, animals with higher WBC counts particularly those with active phagocytic cells like neutrophils and macrophages demonstrate stronger innate immune responses. These cells play a key role in pathogen elimination and contribute to improved disease resistance and adaptability to environmental stressors (Mario *et al*., 2025) [26].

These findings are consistent with those of Kamely *et al*. (2020) [21], Qiu *et al*. (2023) [38], and Korver and Stewart-Brown (2023), who reported that protein deficiency in poultry diets impairs hematopoiesis, leading to bone marrow hypoplasia and structural changes that compromise both innate and adaptive immunity. Etim (2014) [18] also emphasized that hematological parameters are influenced by genetic and non-genetic factors, including nutrition, and serve as indicators of poultry health and physiological status.

Red blood cell (RBC) counts in this study ranged within the normal limits of 2.5–3.5 × 10^9/L for healthy broilers (Mitruka and Rawnsley, 1977) [28]. RBC values were significantly higher in TMLM-supplemented groups compared to the control, with a positive correlation between inclusion levels and RBC counts. However, these values were lower than those reported by Ewa *et al*. (2023) [19] for Ross broilers (2.86–3.96 × 10^9/L), but similar to those reported by Owen *et al*. (2022) [34] (2.60–2.78 × 10^9/L).

Hemoglobin, the oxygen-carrying pigment in blood, reflects the oxygen transport capacity and overall immune competence of the animal. High hemoglobin concentrations suggest efficient oxygen delivery and carbon dioxide removal (Soetan *et al*., 2014) [45]. The hemoglobin values obtained in this study fell within the standard ranges of 14.30–17.70 g/dL (Kokore *et al*., 2021) and 4.50–15.80 g/dL (Gambo *et al*., 2021) [20], indicating that the birds had sufficient hemoglobin for healthy physiological function. These results imply that TMLM positively influenced the health status of Sasso broilers.

Hematocrit (HCT) values observed in this study were within the normal range of 22%–35% for healthy broilers (Mitruka and Rawnsley, 1977) [28]. These values were significantly higher than those reported by Owen *et al*. (2022) [34] (22.00%–28.00%) in Hubbard broilers fed kaolin-supplemented diets over 56 days. HCT is a recognized index of animal well-being, and reductions below the normal range may indicate poor nutrition or ill health (Oyawoye and Ogunkule, 1998; Siddique *et al*., 2024) [35, 44]. Therefore, the elevated HCT values in this study suggest that the birds were well-nourished and in good health.

In this study, TMLM inclusion did not negatively affect hematological parameters, suggesting that the diets provided adequate nutrition to support normal blood cell development and immune function.

Conclusion

The inclusion of *Tenebrio molitor* larvae meal (TMLM) in broiler diets demonstrated significant improvements in growth performance, particularly at the 8% inclusion level. Enhanced feed intake, body weight gain, and feed conversion efficiency highlight its potential as a sustainable alternative to conventional protein sources.

Tenebrio molitor larvae meal supplementation did not induce organ toxicity, as internal organ weights remained within normal ranges. Improvements in intestinal development and abdominal fat deposition suggest enhanced nutrient absorption and energy density, influenced by inclusion level, feed composition, and bird genotype.

Additionally, TMLM positively affected hematological parameters, with elevated WBC, RBC, hemoglobin, and hematocrit values remaining within physiological norms. These findings indicate enhanced immune responsiveness and nutritional adequacy, affirming TMLM’s safety and functionality as a protein source in poultry nutrition.

Declarations

Ethical approval

All experimental procedures involving animals were reviewed and approved by the Animal Ethics Committee of the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, Togo, operating under the National Ethics Committee for the Control and Supervision of Experiments on Animals. All efforts were made to minimize discomfort to the birds, and the study was conducted in compliance with the ARRIVE guidelines.

Consent to publish

All authors confirm that they have contributed to the work, reviewed and approved the final version of the manuscript, and consent to its publication in the *Journal of World’s Poultry Research*.

Competing interests

This research was supported and funded by World Bank interventions (IDA 5424) through the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, Lomé, Togo. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The first author acknowledges receipt of funding from this organization. All other authors declare that they have no competing interests.

Authors' contributions

NGHI and EPO conceived and designed the study, carried out the methodology, data curation, formal analysis, investigation, validation, and project administration. They also performed data interpretation, visualization, and prepared the original draft of the manuscript, as well as contributed to writing, reviewing, and editing.

AK and PW supervised the study, contributed to conceptualization, validation, and formal analysis, and participated in writing, reviewing, and editing of the manuscript.

Acknowledgements

The authors sincerely acknowledge the World Bank for its financial support provided through the Regional Centre of

Excellence in Avian Sciences (CERSA), University of Lomé, Togo. We also extend our gratitude to the Research Laboratory on Agro-resources and Environmental Health (LARASE), University of Lomé, for generously providing the facilities, technical assistance, and workspace that were essential for the successful execution of this research.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to Cite This Article
Inaudi NGH, Komi A, Ogbonnaya EP, Wéré P. Effects of yellow mealworm (Tenebrio molitor) larvae meal on growth performance, hematological parameters, organ characteristics, and intestinal morphometry of Sasso broiler chickens reared in the southwestern area of Lomé, Togo. Int J Insect Anim Divers Res. 2026;2(2):1–11. doi:10.54660/IJIADR.2026.2.2.01-11.