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Impacts of *Tenebrio molitor* Larval Meal on Zootechnical Performance, Biochemical Indices, and Intestinal Morphometry in Broiler Chickens

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ABSTRACT

Protein resources availability is a major challenge for poultry farming in Sub-Saharan Africa. Insects such as *Tenebrio molitor* (*T. molitor*), known as yellow mealworm, which are rich in high-quality protein, offer a promising solution. The present study aimed to evaluate the effects of *T. molitor* larvae meal as an alternative protein source in broiler chicken diets. A total of 375 day-old hybrid broiler chickens (Crossbreeding of Sasso and Faso) were randomly assigned to five dietary treatments. Each treatment included five replicates with 15 chickens. During the 12-week study period, from hatching to the finishing phase, five dietary treatments were administered, including *T. molitor* larvae meal at 4% (TM4%), 6% (TM6%), 8% (TM8), black soldier fly larvae meal at 8% (BSF8), and as the control diet, fish meal at 8% (FM8). Biochemical parameters, including total protein, albumin, triglycerides, and cholesterol, as well as the small intestinal weight and length, and zootechnic performance such as feed intake, body weight gain, and feed conversion ratio (FCR), were measured. The current results indicated that feed consumption did not differ significantly among the treatments. However, the live weight of broiler chickens in TM8 was significantly higher in the finisher phase compared to other treatments, and their weight gain was higher in the grower and finisher phases. The FCR was significantly lower in the TM8 during the finisher phase than in other treatments. Carcass yield and gut length were significantly higher in the TM8. Total protein and albumin did not differ significantly among all groups. Cholesterol and triglyceride levels in TM6 were significantly higher than other groups. The incorporation of *T. molitor* meal at 8% inclusion level is recommended for broiler chicken feed.

Keywords: Biochemical parameter, Black soldier fly, Broiler chicken, Carcass yield, *Tenebrio molitor*, Weight gain

INTRODUCTION

Proteins, such as amino acids, are the main components of poultry feed and the biologically active compounds in the body, which contribute to tissue synthesis, tissue renewal, and body growth (Shariat Zadeh et al., 2019; Kidd et al., 2021). Proteins exist in the form of enzymes and hormones, playing important roles in the physiological functioning of living organisms (Sleman et al., 2015). In poultry, proteins are mainly provided through feed. Thus, the protein sources used in poultry feed are from two origins, namely animal protein sources and plant-based protein (Pexas et al., 2023). Conventional protein sources, such as fish and soybean meal, not only compete for human consumption but also contribute to deforestation and threaten fishery resources through widespread production and the constant harvesting of seafood to feed poultry (Attivi et al., 2023). It is essential to find alternative protein sources that are not in competition with human consumption, economically viable, and can be used in poultry feed as replacements for soybeans and fishmeal (Heuel et al., 2021).

Several studies have identified insects as an economically viable source of animal-based protein that can be used in poultry feed with less negative impacts on the environment (Khusro et al., 2012; Veldkamp et al., 2012; Shumo et al., 2019). Insects such as termites, beetles, caterpillars, fleas, and ants have all been used as dietary supplements in poultry feed (Ravindran and Blair, 1993). According to Mushambanyi and Balezi (2002), the expensive animal protein source, which accounts for approximately 10% of poultry feed, can be effectively replaced with meal produced from cockroaches and termites. Other studies have indicated that silkworm pupae (by-product of the silk industry) or housefly larvae can totally or partially substitute fishmeal in the feed of laying hens and broiler chickens without negatively affecting their production performance. Moreover, housefly larvae are an excellent alternative source of protein in broiler

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chicken feeds (Pieterse et al., 2014; Khan et al., 2016) and laying hens (Akpodiete et al., 1998; Agunbiade et al., 2007). Several studies have been carried out on the use of soldier fly maggots in the feed of broiler chickens and laying hens, with convincing results, particularly improvements in growth performance and egg production (Attivi et al., 2023).

Tenebrio molitor (*T. molitor*), known as yellow mealworm, is one of the most widely consumed insects in the world and recognized for its valuable nutritional content, digestibility of its nutrients and essential amino acids, especially in monogastrics such as poultry and pigs (Bovera et al., 2016; Benzertiha et al., 2020; Elahi et al., 2020). Most studies have reported positive effects of *T. molitor* meal in broiler diets, particularly on body weight gain (Bovera et al., 2016; Biasato et al., 2018). However, most existing studies have not investigated the impact of *T. molitor* meal on blood biochemical parameters in broiler chickens. It should be noted that most studies on the use of *T. molitor* larvae in poultry feed have not recommended an appropriate inclusion rate of *T. molitor* larvae in the diet of broiler chickens, especially for breeds that are improved and adapted to tropical climates. Intermediate-growing broiler chickens, in particular the chickens resulting from the Sasso hens and local Faso cocks crossbreeding, are more adapted to the tropical climate (Bilalissi et al., 2022). The present study aimed to evaluate the effects of adding *T. molitor* to broiler chicken feed on digestive, biochemical, and zootechnical parameters, which are sensitive indicators of feed quality in animals.

MATERIAL AND METHOD

Ethical approval

All experimental protocols were reviewed and approved by the Ethics Committee of the Regional Center of Excellence in Poultry Sciences (CERSA) at the University of Lomé, Lomé, Togo, a branch of the National Ethics Committee for the control and supervision of experiments on animals. All the authors complied with the ARRIVE guidelines.

Biological materials

Tenebrio molitor larvae

The *T. molitor* used for larval production originated from strains imported from the United States in 2021. These strains were subsequently multiplied and reared at the Research Laboratory on Agro Resources and Environmental Health of the University of Lomé, Togo. For larval production, the method of Aguilar-Miranda et al. (2002) was carried out for four months. For mating, 20 males and 30 females of *T. molitor*, aged 5-11 days, were selected and introduced into 25 boxes (Length = 6.5 cm and Width = 4.5 cm) containing a mixture of 50 g wheat bran and maize bran (Aguilar-Miranda et al., 2002). Eggs of *T. molitor* were collected after one week and incubated in rearing trays (a blue, round plastic tray with a diameter of 80 cm and a height of 5 cm). The eggs effectively hatched after three weeks of incubation. The hatched larvae were fed with 4 kg of wheat-maize bran/box for three months. A total of 50 g of orange slices was placed in each rearing tray to provide larvae with water and energy (Aguilar-Miranda et al., 2002). The larvae were collected at the early pre-chrysalid stage based on specific characteristics, including reduced mobility, pale body, and slight cuticle hardening, indicating the onset of pupation. The larvae collected were starved for 24 hours to allow emptying of the gastrointestinal tract (GIT), minimizing internal contents that could affect subsequent analyses, then oven dried (CL012N, Belgium) at 65°C for 72 hours. After drying, the larvae were left in the open air at the ambient temperature (28 ± 1°C) for 15-20 minutes. Larvae were then ground in a GRINDOMIX GM 300 mixer (Retsch GmbH, Germany) to obtain 1.2 kg/box of *T. molitor* larvae powder, which has been stored at 4°C for further experiments.

Black soldier fly larvae

The reference method of Attivi et al. (2023) was used for the production of black soldier fly (BSF; *Hermetia illucens*) larvae. Incubation boxes were used to collect freshly laid eggs, and first-stage larvae were obtained after three days. A medium containing a mixture of 50% palm kernel cake and 50% beer dregs, supplied by KSY POPO Group (Lomé, Maritime Region, Togo), was used to grow the young larvae. A relative humidity of 60-70% and a temperature of 28 ± 1°C with a photoperiod of 12:12 (Light: dark) were maintained in the rearing room. A total of 100 kg of fresh larvae aged 14 days were produced, collected, and killed (by freezing) at -20°C. The harvested larvae were washed and dried at 70°C for 72 hours in an autoclave dryer (CL012N, Belgium) at the production unit of the Regional Centre of Excellence in Avian Sciences at the University of Lomé, Togo. After drying, a total of 30 kg of BSF larvae meal was used in the present study, with an inclusion rate of 8% in the diet, based on the findings of Attivi et al. (2023). Table 1 shows the chemical and nutritional composition of the meal of *H. illucens* larvae.

For feed manufacturing, all ingredients, including corn, wheat bran, soybean meal, black soldier larvae, brewers' grains, oyster shells, common salt, *T. molitor* larvae meal, and essential amino acids, were first ground in a grinder, and homogeneous mixing was performed using a vertical mixer with a grinder.

Fishmeal

The fishmeal (30 kg) used in the present study was imported from Senegal Halieutics SA, Senegal. Fishmeal is widely used by West African poultry farmers (Attivi et al., 2023). Table 1 shows the chemical and nutritional composition of fishmeal.

Table 1. Chemical and nutritional composition of different protein sources, including fish meal, black soldier fly larvae meal, and *Tenebrio molitor* larvae meal, for the hybrid broiler chickens' diet

Parameters \ Protein sources	Fish meal	Black soldier fly	*Tenebrio molitor*
Dry matter (%)	94.4	91.3	85.3
Crude protein (%)	40	47.1	52.09
Fat (%)	10	25	37.44
Calcium (%)	4	6.5	1.45
Phosphorus (%)	2.5	1.05	3.5
Methionine + Cysteine (%)	1.54	1.42	4.63
Lysine (%)	2	4.90	1.6
Energy (kcal/kg)	2.800	3.102	2.36

Nutritional analysis of fishmeal, *Tenebrio molitor, and Hermetia illucens*

Nutritional value of insect meal (*T. molitor and H. illucens*) and fishmeal was analyzed at the Togolese Institute of Agronomic Research, Lomé, Togo. Nutritional analyses focused on dry matter content, crude protein, lipid levels, and calcium, phosphorus, methionine, lysine, cysteine, and energy values. The protein content was determined by the Kjeldahl method according to NF V18-100 (1977). The dry matter content was expressed as a percentage of the weight of the fish meal, *T. molitor*, and the BSF larvae sample. The fat was determined using a Soxhlet apparatus (C. Gerhardt GmbH & Co. KG, Germany) according to the AOAC 960.39 method (AOAC, 1999). Calcium and phosphorus contents were determined using the AOAC 935.13 and 965.17 methods, respectively (AOAC International, 2005). Calcium was released by acid digestion and quantified by titration with EDTA using a suitable indicator, while phosphorus was measured after digestion and determined spectrophotometrically (PG Instruments, United Kingdom) as phosphate. The methionine and lysine content was achieved in accordance with TS EN ISO 17180. Cysteine content was determined by spectrophotometry using L-cysteine tablets (Now Foods, Bloomingdale, IL, USA). Metabolizable energy (ME) was calculated as described by the University of Georgia Poultry Science Extension (2025).

Experimental diets

A total of five rations were designed, including diets with 4% of *T. molitor* (TM4), 6% of *T. molitor* (TM6), 8% of *T. molitor* meal (TM8), 8% of black soldier flies' maggots (BSF8), and 8% of fish meal (FM8), representing the control group (Mlaga et al., 2022; Attivi et al., 2023). These diets have been formulated to contain similar levels of energy and protein across the developmental stages, including the starter (3 weeks), grower (6 weeks), and finisher phases (3 weeks; Table 2). Based on the nutritional content of the ingredients and the dietary needs of chickens raised in tropical conditions, specifically 17-22% of crude protein and 2700-3000 kcal/kg of metabolizable energy, as reported by Kpomasse et al. (2020), the inclusion rates of these ingredients were calculated using a locally developed Excel software. Water and feed were provided *ad libitum* according to Chokakkar et al. (2024) during the 12-week experiment.

Experimental design

Unsexed, day-old broiler chickens from the crossbreeding of 35-week-old Sasso hens, imported from France, and a 35-week-old local Faso rooster, imported from Burkina Faso, were used for the present study. A total of 375 day-old crossbred broiler chickens were randomly divided into five treatment groups, with five replicates per treatment, each containing 15 chickens. The treatment groups were fed with 4% of *T. molitor* larvae meal (TM4), 6% of *T. molitor* larvae meal (TM6), 8% of T. *molitor* larvae meal (TM8), 8% of BSF larvae meal (BSF8), and 8% of fish meal (FM8). The chickens were raised for 12 weeks (from day one to 12 weeks of age) in a semi-closed building, at an ambient temperature ranging from 27 to 35°C and a relative humidity from 50 to 60%. Lighting was provided for 24 hours per day during the first week and adjusted to 18 hours per day thereafter. Chickens were reared at a density of 10 Chickens/m². Chickens were fed a diet with a metabolizable energy value ranging from 2,734 to 2,934 kcal/kg and a crude protein content varying from 17.35% to 20.84%.

Table 2. Composition of experimental diets formulated for hybrid broiler chickens from day 1 to 12 weeks of age, across starter, grower, and finisher phases

Ingredients	Starter phase					Grower phase					Finisher phase				
	FM8	BSF8	TM4	TM6	TM8	FM8	BSF8	TM4	TM6	TM8	FM8	BSF8	TM4	TM6	TM8
	Incorporation rate														
Togo-Benin maize (%)	43	58.5	55	54.65	53	49	57	53	52	53	50	56	54	53	53
Cubed bran/ wheat (%)	10.15	4	4.65	4	4.65	10.15	4	4.65	8.75	4.65	10.15	4.75	4.65	4	4
Roasted soybeans (%)	20	9.5	8	8	8	14	9.5	9	6	8	13	8.5	8	8	7
Soybean meal 45%	7	7.65	16	15	15	8	7.65	16	15	15	8	7.65	16	16.75	15.75
Beer spent grains (%)	8	5	5	5	4	7	6.5	6	5	4	7	6.5	6	5	5
Fishmeal 40%	8	0	0	0	0	8	0	0	0	0	8	0	0	0	0
Tenebrio molitor (%)	0	0	4	6	8	0	0	4	6	8	0	0	4	6	8
Black soldier fly maggots (%)	0	8	0	0	0	0	8	0	0	0	0	8	0	0	0
MMC 5%	100	5	5	5	5	1	5	5	5	5	1	1	5	5	5
Methionine (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lysine (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Oyster shell (%)	2	1.5	1.5	1.5	1.5	2	1.5	1.5	1.4	1.5	2	2	1.5	1.4	1.4
NaCl (g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Premix 2 (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	5	0.25	0.25	0.25
Total (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Nutritional values															
Crude protein (%)	20.81	20.67	20.83	20.29	20.4	17.91	17.91	17.45	17.93	17.4	18.56	18.93	19.00	18.91	18.98
Crude fat (%)	7.68	6.11	6.2	6.89	7.53	6.6	6.16	6.4	6.55	7.53	6.43	5.81	6.23	6.38	7.36
Crude ash (%)	2.23	2.08	1.31	1.34	1.4	1.88	2.14	1.41	1.51	1.4	1.83	2.14	1.36	1.46	1.35
Crude fiber (%)	5.87	4.95	5.3	5.16	5	5.71	5.11	5.42	5.39	5	5.7	4.96	5.4	5.38	4.98
Lysine (%)	1.4	1.21	1.14	1.11	1.1	1.31	1.22	1.17	1.09	1.1	1.29	1.09	1.15	1.07	1.08
Methionine (%)	0.55	0.53	0.53	0.52	0.51	0.52	0.53	0.54	0.52	0.51	0.52	0.41	0.53	0.51	0.51
Meth. + Cysteine (%)	0.83	0.7	0.79	0.77	0.76	0.79	0.71	0.8	0.77	0.76	0.79	0.56	0.79	0.76	0.75
Tryptophan (%)	0.13	0.06	0.06	0.05	0.05	0.1	0.06	0.06	0.06	0.05	0.09	0.06	0.06	0.05	0.05
Threonine (%)	0.41	0.35	0.18	0.18	0.17	0.31	0.37	0.21	0.18	0.17	0.3	0.36	0.19	0.16	0.16
Calcium (%)	1.17	0.83	0.84	0.83	0.82	1.17	0.83	0.84	0.8	0.82	1.17	0.84	0.84	0.8	0.82
Phosphorus (%)	0.7	0.54	0.6	0.58	0.57	0.69	0.55	0.6	0.62	0.57	0.69	0.41	0.6	0.61	0.57
Metabolizable energy (kcal/kg)	2868.11	2897.45	2892.98	2927.79	2934.2	2759.01	2790.77	2788.88	2752.17	2734.2	2858.66	2849.27	288853	2851.82	2833.85

FM8: 8% of Fish meal, BSF8: 8% of black soldier fly larvae meal, TM4: 4% of *T. molitor* larvae meal, TM6: 6% of *T. molitor* larvae meal, TM8: 8% of *T. molitor* larvae meal, MMC: Meat meal concentrate 5%.

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Vaccination program

The chickens underwent a prophylactic program adapted by CERSA, Togo, for Newcastle disease in accordance with the recommendations of Rauw et al. (2009). On days 5, 15, and 25 of age, they were vaccinated against Newcastle disease and infectious bronchitis with the AVINDHB1 vaccine (Laproved, Hungary) and the IB vaccine (Laproved, Hungary), respectively. On days 7, 14, and 21, they were vaccinated against Gumboro disease using the CEVAC IBD vaccine (Ceva Santé Animale, France). The antibiotic Alizeryl (Interchemie werken 'De Adelaar' B.V., Netherlands) was administered from days 16 to 20 and again from days 40 to 44. The anticoccidial Amprocox WS (Interchemie werken 'De Adelaar' B.V., Netherlands) was administered from days 30 to 34. Each treatment was accompanied by vitamin supplementation for two days.

Zootechnical performance

Throughout the duration of the experiment from August 15 to November 15, 2024, the Feed offered and leftovers per replicate were recorded weekly. Feed consumption was determined to be the difference between the food offered and the leftovers. Chickens were weighed at the end of each week, and weight gain was calculated as the difference between final weight and initial weight during the rearing period. Body weights and feed consumption were used to calculate the feed conversion ratio (FCR) according to the following formula (Attivi et al., 2023).

$$\text{Feed conversion ratio} = \frac{\text{Feed intake}}{\text{Weight gain}} \quad \text{(Formula 1)}$$

Biochemical parameters

At 12 weeks of age, blood samples (6 mL) were collected from 10 randomly selected chickens per treatment group into plain tubes without anticoagulant via the wing vein to evaluate the effect of different feed rations on the biochemical parameters of the chickens. The blood collected was centrifuged at 3000 rpm for 15 minutes by a Vacutainer brand centrifuge (Kouame et al., 2019). The serum obtained helped to analyze the biochemical parameters, including total proteins, albumin, cholesterol, and triglycerides, using the Synergy H1 microplate reader device (Agilent Technologies, United States). Different methods were used to perform the biochemical analyses of protein (Gomall et al., 1949), total cholesterol (Allain et al., 1974), and triglyceride concentrations (Fossati and Prencipe, 1982) in broiler chicken serum, respectively. The albumin concentration was determined according to the recommendations of Doumas et al. (1971) and the hydrolysis method of p-nitrophenyl phosphate at pH 10.4 (Krawczun et al., 2020).

Slaughtering

The methodology of Mlaga et al. (2020) was used for chicken culling. At 12 weeks of age, for slaughter, 10 chickens from each treatment were randomly selected and individually weighed using a digital weighing scale (ADVENTURER, Atlanticlabo, France) to ensure uniformity in live weights. Before slaughtering, chickens were fasted for 14 hours, but water was provided *ad libitum*. The selected chickens were first subjected to electrical stunning (Electric stunner VE memory, SOPRODA, France) and then were slaughtered by bleeding after an incision of the jugular vein. Once bled out, the slaughtered chickens were hung on hooks to allow the blood to flow completely. Afterwards, the carcasses were scalded in a hot water bath maintained at a temperature of 55 to 65°C. Defeathering was performed by mechanical plucking using an automatic plucking machine (Flexevi model, manufactured by BAYLE SA, France).

Carcass cutting

The carcasses were cut using the method described by Tougan et al. (2013). After the slaughter, plucking, evisceration, and cleaning, the following steps were employed. Initially, the legs were severed at the level of the Tibiotarsus-metatarsal joint, then the head was detached at the junction between the atlas and the occipital. After that, organs from the abdominal and thoracic cavities were extracted, along with the removal of abdominal fat. The weight of hot carcasses was measured, as well as the weight of viscera, including gizzard, liver, and intestines. The intestinal length was also measured using a flexible measuring tape after the intestine was laid flat without stretching. Finally, each carcass was cut to measure the weight of the different parts, namely the thigh, drumstick, wing, and keel, as well as the rest of the carcass.

Statistical analysis

All the data collected were subjected to a one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of GraphPad Prism Statistical Software, version 8.02. All comparisons between means were tested using the Tukey method at a p-value less than 5% ($p < 0.05$) significance level of acceptance.

RESULTS

Zootechnical performance

Table 3 presents the effects of *T. molitor* meal on the zootechnical performance in chickens. The results indicated that the feed consumption did not differ significantly ($p > 0.05$) among the different treatments (TM4, TM6, TM8, BSF8,

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FM8) from the starter to the finisher phases. Similarly, live weight exhibited no significant difference ($p > 0.05$) among the different treatments (TM4, TM6, TM8, BSF8, FM8) in the starter and grower phases (0-9 weeks). However, in the finisher phase, live weight was significantly higher ($p < 0.05$) in TM8 (1385 g) and BSF8 (1325 g), compared to FM8 (1272 g), TM6 (1268 g), and TM4 (1213 g). Moreover, weight gain and FCR did not differ significantly ($p > 0.05$) among the different groups (TM4, TM6, TM8, BSF8, FM8) during the starter phase. However, the highest weight gain and the lowest FCR were observed in TM8 during the grower (15.79; 2.82) and finisher phases (20.66; 5.70).

Carcass characteristics

Table 4 illustrates carcass, drumstick, thigh, wing, and keel yields according to the different treatments. Among all diets, broiler chickens in TM8 and BSF8 indicated a significant difference in wing yield. However, the highest yields were observed in carcass (67.62%), thighs (9.91%), drumsticks (9.98%), and Keel yield (16.66%) in TM8. In contrast, the lowest yields in carcass (60.88%), thigh (7.94%), drumstick (7.70%), and Keel yield (12.12%) were observed in the FM8.

Length and relative weight of the small intestine

The relative weight of the intestine, gizzard, and liver indicated no significant differences ($p > 0.05$) among different groups (FM8, BSF8, TM4, TM6, and TM8). However, the length of the intestine was significantly higher ($p < 0.05$) in TM8 (167.8cm) compared to TM4 (159.5cm), TM6 (158.6cm), BSF8 (153.3cm), and FM8 (150.8cm; Table 5).

Table 3. Feed intake, average weight gain, and feed conversion ratio in hybrid broiler chickens during the starter, grower, and finisher phases

Parameters \ Treatments	FM8	BSF8	TM4	TM6	TM8	P value
Starter phase (0-3weeks)						
Live weight (g)	281.0 ± 3.48	297.7 ± 4.86	272.7 ± 11.02	277 ± 8.88	287.7 ± 3.06	0.1329
Feed intake (g/day)	16.96 ± 2.96	20.26 ± 1.6	17.85 ± 1.51	18.85 ± 1.51	19.78 ± 1.52	0.2292
Weight gain (g)	11.71 ± 0.17	12.51 ± 0.24	11.30 ± 0.53	11.52 ± 0.43	12.03 ± 0.15	0.1313
FCR	1.42 ± 0.29	1.60 ± 0.12	1.57 ± 0.12	1.62 ± 0.10	1.64 ± 0.07	0.3453
Grower phase (4-9 weeks)						
Live weight(g)	865.3 ± 15.72	911.3 ± 0.421	854.0 ± 32.58	873.3 ± 37.25	951.0 ± 20.09	0.1441
Feed intake (g/day)	44.33 ± 2.01	42.54 ± 2.02	41.11 ± 2.09	40.15 ± 1.86	44.45 ± 2.29	0.4888
Weight gain (g)	13.92 ± 0.45^{c}	14.61 ± 0.128^{b}	13.85 ± 0.51^{c}	14.21 ± 0.68^{bc}	15.79 ± 0.41^{a}	0.0448
FCR	3.20 ± 0.10^{a}	2.91 ± 0.06^{b}	2.97 ± 0.01^{b}	2.84 ± 0.07^{c}	2.82 ± 0.06^{c}	0.0061
Finisher phase (10-12 weeks)						
Live weight (g)	1272 ± 17.30^{b}	1325 ± 26.23^{a}	1213 ± 42.81^{b}	1268 ± 22.79^{b}	1385 ± 39.80^{a}	0.04097
Feed intake (g/day)	120.1 ± 6.03	118.8 ± 6.48	111.4 ± 6.82	113.7 ± 7.11	116.9 ± 6.31	0.8729
Weight gain (g)	19.39 ± 1.11^{b}	19.70 ± 1.27^{a}	17.08 ± 0.51^{b}	18.77 ± 0.69^{b}	20.66 ± 0.94^{a}	0.0026
FCR	6.28 ± 0.31^{ab}	6.03 ± 0.07^{b}	6.53 ± 0.08^{a}	6.08 ± 0.18^{b}	5.70 ± 0.24^{c}	0.0018

FM8: 8% of Fish meal, BSF8: 8% of black soldier fly larvae meal, TM4: 4% of *T. molitor* larvae meal, TM6: 6% of *T. molitor* larvae meal, TM8: 8% of *T. molitor* larvae meal, FCR: Feed conversion ratio. [a,b, and c] Means within the same row followed by different subscript letters differ significantly ($p < 0.05$).

Table 4. Carcass characteristics, thighs, drumstick, wing, and keel at 12 weeks of age in hybrid broiler chickens

Parameters \ Treatments	FM8	BSF8	TM4	TM6	TM8	P value
Carcass yield (%)	60.88 ± 1.28^{c}	63.89 ± 0.56^{b}	61.69 ± 0.58^{c}	63.38 ± 0.31^{b}	67.62 ± 0.77^{a}	< 0.0001
Thigh yield (%)	7.94 ± 0.95^{c}	9.58 ± 0.25^{a}	9.03 ± 0.14^{b}	9.56 ± 0.28^{a}	9.91 ± 0.26^{a}	0.0440
Drumstick yield (%)	7.70 ± 0.95^{c}	9.36 ± 0.18^{ab}	9.13 ± 0.35^{b}	9.78 ± 0.40^{ab}	9.98 ± 0.17^{a}	0.0185
Wing yield (%)	7.36 ± 0.74^{c}	8.81 ± 0.15^{ab}	8.53 ± 0.18^{b}	8.67 ± 0.11^{b}	9.17 ± 0.25^{a}	0.0222
Keel yield (%)	12.12 ± 1.26^{b}	15.57 ± 0.53^{a}	12.61 ± 1.60^{b}	15.82 ± 0.54^{a}	16.66 ± 0.70^{a}	0.0067

FM8: 8% of Fish meal, BSF8: 8% of black soldier fly larvae meal, TM4: 4% of *T. molitor* larvae meal, TM6: 6% of *T. molitor* larvae meal, TM8: 8% of *T. molitor* larvae meal. [a,b, and c] Means within the same row followed by different subscript letters differ significantly ($p < 0.05$).

Table 5. Length and relative weight of the small intestine, gizzard, and liver at 12 weeks of age in hybrid broiler chickens

Parameters \ Treatments	FM8	BSF8	TM4	TM6	TM8	P value
Relative intestinal weight (g/cm)	0.55 ± 0.04	0.62 ± 0.12	0.56 ± 0.04	0.57 ± 0.06	0.60 ± 0.04	0.9463
Intestinal length (cm)	150.8 ± 4.28^{b}	153.3 ± 3.64^{b}	159.5 ± 4.73^{b}	158.6 ± 3.97^{b}	167.8 ± 0.88^{a}	0.0215
Relative weight of gizzard (%)	1.812 ± 0.07	1.985 ± 0.08	2.069 ± 0.125	1.865 ± 0.09	1.742 ± 0.11	0.1481
Relative weight of the liver	1.867 ± 0.08	1.785 ± 1.00	1.977 ± 0.08	1.988 ± 0.12	1.684 ± 0.09	0.1353

FM8: 8% of Fish meal, BSF8: 8% of black soldier fly larvae meal, TM4: 4% of *T. molitor* larvae meal, TM6: 6% of *T. molitor* larvae meal, TM8: 8% of *T. molitor* larvae meal. $^{a \text{ and } b}$ Means within the same row followed by different subscript letters differ significantly ($p < 0.05$).

Biochemical parameters

Blood parameters, including total protein and albumin in the chickens, did not demonstrate significant differences ($p > 0.05$) among all groups. Triglyceride concentrations in the BSF8 (59.5 g/dL) and TM6 (59.75 g/dL) treatments were significantly higher compared to the FM8 treatment (38.75 g/dL), which recorded the lowest values. Total cholesterol concentration was higher in TM6 (140.5 g/dL) compared to the TM4 (130g/dL), FM8 (108.3 g/dL), and BSF8 (103.5 g/dL).

Table 6. Biochemical analysis at 12 weeks in hybrid broiler chickens fed different diets from day 1 to 12 weeks of age

Parameters \ Treatments	FM8	BSF8	TM4	TM6	TM8	P value	Normal range
Total protein (g/L)	47.43 ± 2.19	40.80 ± 1.30	44.50 ± 3.75	45.73 ± 2.29	45.88 ± 6.26	0.3630	30-60
Albumin (g/L)	16.60 ± 0.38	14.68 ± 0.56	16.70 ± 1.00	16.78 ± 0.76	15.00 ± 1.00	0.1993	10-30
Triglyceride (g/dL)	38.75 ± 1.28^{c}	59.5 ± 5.59^{a}	46.25 ± 4.26^{b}	59.75 ± 8.07^{a}	54. 75 ± 8.99^{ab}	0.0005	30-100
Cholesterol (g/dL)	108.3 ± 5.05^{c}	103.5 ± 5.45^{c}	130 ± 7.82^{ab}	140.5 ± 8.69^{a}	125.8 ± 5.54^{b}	0.0011	100-180

^{a,b,c} Means within the same row followed by different subscript letters differ significantly ($p < 0.05$). FM8: 8% of Fish meal, BSF8: 8% of black soldier fly larvae meal, TM4: 4% of *T. molitor* larvae meal, TM6: 6% of *T. molitor* larvae meal, TM8: 8% of *T. molitor* larvae meal

DISCUSSION

The current results indicated that *T. molitor* did not have a substantial effect on feed consumption in chickens across different treatment groups. This result could be due to the fact that *T. molitor* larvae have a palatability similar to that of BSF larvae and fishmeal, and they lack any adverse effects that could affect feed intake in poultry diets. The current findings are similar to those of Bovera et al. (2016), who included 29.65% of *T. molitor* larvae in poultry feed and observed no significant differences in feed consumption among different treatments. The present results demonstrated that during the starter phase (0-3 weeks), there were no notable differences in body weight, weight gain, and FCR. The current results suggested that short-term use of *T. molitor* at rates less than or equal to 8% did not affect the growth performance in broiler chickens. However, after six weeks, the inclusion of *T. molitor* at 8% in the diet resulted in higher weight gain and a lower FCR than other treatments. These improvements in weight gain and the reduction in FCR might be attributed to the nutritional quality of the larvae, which provided an optimal balance of energy, amino acids, vitamins, and mineral salts essential for superior growth in poultry. These findings are consistent with those of Benzertiha et al. (2020), who reported enhanced growth and efficiency FCR in broilers fed diets containing even very low levels of *T. molitor* (0.2% and 0.3%) as a functional additive. Similarly, Shariat Zadeh et al. (2019) observed an increase in body weight in quails when fishmeal was replaced with *T. molitor* meal at inclusion levels of 22.5 g and 30 g. Loponte et al. (2017) reported improved growth performance in partridges following the inclusion of *T. molitor* flour at 250-500 g in their feed ration.

The current findings indicated that an increase in carcass yield was associated with enhanced growth performance in chickens fed *T. molitor* at 8%, which might be due to higher nutrient availability in *T. molitor*, providing a balance between energy and protein utilization and thereby boosting growth. The current results are similar to those of Khatun et al. (2003), Hwangbo et al. (2009), and Ballitoc and Sun (2013), who reported improvements in carcass weight, compared to the weight of fillet muscle, and thigh, respectively, by adding silkworm pupae, house fly maggot, and *T. molitor* larvae in broiler feed. In poultry, intestinal development begins during embryonic life and reaches its peak between 6 and 10-days post-hatch (Noy and Sklan, 1998). Based on the present results, the total intestinal length increased in chickens fed insects, which led to longer feed retention in the digestive tract, improved digestion, and greater absorption. The extended contact between digesta and absorptive surfaces improves digestion and nutrient absorption; these factors are

essential for greater growth performance (Alyileili et al., 2020), as observed in the current experiments with broiler chickens fed *T. molitor* and BSF larvae diets.

The results of the blood parameters offered insights into the biochemical markers important for assessing the quality of *T. molitor* larvae. The present study found that total protein and albumin did not differ notably among treatments, suggesting that the proteins from *T. molitor* larvae were effectively metabolized and did not impair liver function in broiler chickens. These results are consistent with those of Jiang et al. (2024), who found no remarkable differences in biochemical parameters, including total protein and albumin, when *T. molitor* larvae diets at 3%, 5%, 7%, and 9% concentrations were incorporated into broiler feed. Sedgh-Gooya et al. (2021) found no notable effects on total protein and albumin when evaluating the impact of including *T. molitor* larval meal at 2.5% and 5% on growth performance, carcass characteristics, gut microbiota, and blood parameters in broiler chickens.

Kohout et al. (2013) stated that triglycerides originate from hepatic synthesis and dietary fats. Current findings on triglyceride changes suggested that appropriate inclusion levels of *T. molitor* larval meal in the diet may have promoted broiler growth by improving lipid metabolism. This finding is consistent with Jiang et al. (2024), who found elevated triglyceride concentrations in broiler chickens fed diets containing more than 3% of *T. molitor* larvae. However, the present results on increased triglyceride levels contradicted those of Kim et al. (2014), who found no significant differences in triglyceride concentrations in broiler chickens fed diets containing *T. molitor* larvae meal.

CONCLUSION

The inclusion of *T. molitor* larvae meal in broiler chickens' diets demonstrated promising effects on zootechnical performance, carcass traits, and selected biochemical parameters. Including *T. molitor* larvae meal at 8% improved weight gain and decreased FCR, but feed consumption remained unchanged in broiler chickens during the grower and finishing phases. Likewise, *T. molitor* at 8% enhanced carcass yield and intestinal length in broiler chickens. Although total protein and albumin levels remained stable across treatments, elevated triglyceride levels were observed in chickens fed black soldier fly larvae at 8% and *T. molitor* at 6%. Therefore, *T. molitor* larvae meal can be recommended at an inclusion rate of 8% in broiler diets. Future studies should examine the feed digestibility of *T. molitor* in chickens to determine its influence on production performance and to assess its effect on the nutritional quality of chicken meat.

DECLARATIONS

Availability of data and materials

All data generated or analyzed during the present study are available from the corresponding author upon reasonable request.

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Authors' contributions

Nassi Guidi Hoeness Inaudi was responsible for experimental implementation, including project administration and methodology. Agboka Komi participates in the correction of the protocol, funding acquisition, and reading of the manuscript. Attivi Komi contributed to the revision of the study, participated in Funding acquisition, and reviewed the manuscript. Mlaga Kodjo Gnatépé participated in the experimental procedures and contributed to the manuscript review. Tona Kokou provided critical reading and revision of the manuscript. All authors have read and approved the final edition of the manuscript submitted for publication.

Ethical considerations

All ethical issues, including plagiarism, consent to publication, research misconduct, data fabrication or falsification, and duplicate publication or submission, were carefully considered and verified by all authors prior to submission. There was no use of artificial intelligence in preparing the study and writing this article.

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Competing interests

The authors declared no competing financial interests or personal relationships.

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