INFLUENCE OF BLACK SOLDIER FLY LARVAE MEAL-BASED DIETS ON GROWTH PERFORMANCE, HAEMATOLOGICAL AND SERUM BIOCHEMICAL PARAMETERS DURING THE STARTER AND FINISHER PHASES IN BROILER CHICKEN

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ABSTRACT

This study assessed the effect of feeding black soldier fly larvae meal-based diets on the haematological and serum biochemical parameters in broiler chickens during the starter and finisher phases. A total of 204 Cobb-500 broiler chickens were allocated to 4 dietary groups in the starter phase: a control group (T0) fed a standard diet containing soybean meal, and 3 treatment groups fed isonitrogenous diets with 10% (T1), 20% (T2), and 30% (T3) black soldier fly larvae meal (BSFLM) inclusion as replacement for soyabean meal. The finisher phase consisted of the same 4 dietary groups, except treatment group 3 (T3) which had 27.2% of BSFLM. Each treatment had three replicates of 17 birds per pen in a completely randomized design. The feeding trial lasted from 8-28 days for the starter phase and 29-49 days for the finisher phase. Growth performance, haematological and serum biochemical parameters were determined. Birds in group T3 had the lowest ($p<0.05$) daily weight gain and poorest feed conversion ratio in the starter and finisher phases. They also had the lowest ($p<0.05$) monocyte, mean corpuscular volume and mean corpuscular haemoglobin levels in the finisher phase. Furthermore, total proteins concentration were lowest ($p<0.05$) in the starter and finisher phases. The levels of the haematological and serum biochemical parameters obtained remained within the normal physiological range of values reported for poultry. The results showed that the inclusion of BSFLM up to 20%, as replacement for soybean meal, improved weight gain and feed conversion ratio, and did not adversely affect the health and physiological status of the chickens.

Keywords: *black soldier fly larvae meal, broiler chicken, growth performance, haematology, serum biochemical profile*

INTRODUCTION

The use of insects in poultry feeds is gaining much interest due to their high nutritional properties, including high protein content, good amino acid profile, rich micronutrients, and bioactive compounds with proven antibacterial and immunomodulatory effects, hypolipidemic efficiency, and growth-promoting effects, as indicated in a number of recent reviews (Hossain and Bhuiyan, 2023; Malematja *et al.,* 2003). This makes the use of insects in feed for monogastrics a good avenue to increase nutritional security in animals and humans.

The common protein sources used in poultry diet formulations, such as soybean meal (SBM) and fishmeal, face challenges related to availability, cost, and environmental impacts such as greenhouse gas emissions (Onsongo *et al.,* 2018; Benavides *et al.,* 2020), especially raising concerns for the viability of the poultry industry, particularly in developing nations (Chadd, 2007). The search for alternative protein sources has led to the use of insect-based ingredients such as the black soldier fly larvae meal (BSFLM) with its high crude protein content and good amino acid profile to provide a viable protein source in poultry diets (Schiavone *et al.,* 2017; Anankware *et al.,* 2018; Chobanova *et al.,* 2023; Fruci *et al.,* 2023; Heita *et al.,* 2023; Hossain and Bhuiyan, 2023; Nandhirabrata *et al.,* 2023). In addition to protein, BSFLM is rich in fats, minerals, and vitamins (de Souza Vilela *et al.,* 2021; Makokha *et al.,* 2023), thus providing a well-balanced nutritional profile.

The health, physiology, nutritional status, and productivity of poultry are frequently evaluated using a variety of haematological and serum biochemical indices (Jain, 1986; Harr, 2006; Mat *et al.,* 2022). In poultry medicine, pertinent haemato-biochemical indices encompass haemoglobin (Hb) concentrations, red blood cells (RBC) counts, white blood cells (WBC) counts, total proteins, albumin, globulins, lipoproteins, triglycerides, and uric acid (Odunitan-Wayas *et al.,* 2018). Variations in levels of the haemato-biochemical indices have an impact on poultry performance, and these indices are affected by a number of factors, including heredity, age, sex, diet, management practices, and environmental conditions (Obese *et al.,* 2018; Lin *et al.,* 2023).

The use of BSFLM has emerged as a promising alternative protein source due to its local production potential, lower cost of production, and reduced environmental footprint. Research assessing the effect of BSFLM on feed intake, growth performance, health, and metabolic status of broiler chickens has demonstrated its promise as a partial substitute for SBM or fishmeal in broiler diets in a number of studies (Opoku *et al.,* 2018; Attivi *et al.,* 2020; Kim *et al.,* 2021; Facey *et al.,* 2023; Fruci *et al.,* 2023). These studies, however, used more mature larvae and pre-pupa with higher chitin content in their dietary formulations, which can limit nutrient digestibility and affect overall performance at a higher inclusion rate. In this study, therefore, the effects of feeding BSFLM-based diets from larvae harvested at an earlier stage of development (4th instar larvae) on haematological and serum biochemical indices during the starter and finisher phases were evaluated in broiler chickens. It was hypothesized that the partial substitution of SBM with BSFLM in broiler chicken diets will not have a deleterious effect on the health and physiological status of broiler chickens.

MATERIALS AND METHODS

Experimental location

The study was conducted at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana. LIPREC lies within the Coastal Savannah zone on latitude 05^0 40' N and longitude 00^0 16' W (Ampong *et al.,* 2019). It experiences bimodal rainfall with an average of 881 mm (Adjorlolo *et al.,* 2014) and varying temperatures between 24.3 ^{o}C and 32.9 ^{o}C (Sarkwa *et al.,* 2020).

Source of Black soldier fly larvae and processing

The black soldier fly larvae were raised on fruit and vegetable wastes at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission. The 4th instar larvae were harvested and cleaned by soaking in bubbling water at 84^0C for 40 seconds. The cleaned larvae were oven-dried at 65 ^{O}C for 48 hours. A hammer mill with a 2.5 mm sieve was used to grind the weighed dried larvae.

Experimental Treatments

The BSFLM was utilized as a source of protein at varying inclusion levels in a broiler starter, and broiler finisher diet in a two-phase feeding regime.

A total of 204 unsexed Cobb-500 broiler chicks that were 7 days old were used in the experiment. The chickens were allocated to 4 dietary groups in the starter phase: a control group (T0) fed a standard diet containing soybean meal, and 3 treatment groups fed isonitrogenous diets with 10% (T1), 20% (T2), and 30% (T3) BSFLM inclusion as replacement for soybean meal. The finisher phase consisted of the same 4 dietary groups, except treatment group 3 (T3) which had 27.2% of BSFLM. Each treatment had three replicates of 17 birds per pen in a completely randomized design. The experimental starter diets (Table1) were given to the birds from days 8 to 28, and the finisher diets (Table 2) from days 29 to 49.

Table 1: Ingredient composition of experimental starter diets

Ingredient	Percentage (%) inclusion of ingredients			
	T0 (0% BSFLM)	**T1** (10% BSFLM)	**T2** (20% BSFLM)	**T3** (30% BSFLM)
Wheat bran	15.2	16.4	18.0	19.1
Maize	47.8	45.1	42.1	39.5
Soybean meal	34.0	25.5	16.9	8.40
Black soldier fly larvae meal	0.00	10.0	20.0	30.0
Common Salt	0.50	0.50	0.50	0.50
Oyster shell grit	1.50	1.50	1.50	1.50
Lysine	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.20	0.20	0.20	0.20
Toxin binder	0.20	0.20	0.20	0.20
Vitamin-trace mineral premix*	0.30	0.30	0.30	0.30
Total	**100.0**	**100.0**	**100.0**	**100.0**
	Calculated proximate composition (%) DM			
Dry matter	86.3	85.8	86.1	85.3
Ash	9.60	12.4	12.7	13.9
Ether extract	2.91	5.60	6.97	8.77
Crude protein	23.1	23.1	23.2	23.2
Crude fibre	8.08	8.45	9.18	9.67
Metabolizable energy (kcal/kg)	3001	3001	3000	3001

*Diets were supplemented with toxin binder (Biomin®) at the manufacturer's specified rate. T0 was the common starter diet fed to the birds from day 1 to day 7. *Composition of vitamin-mineral premix: gallate = 368 mg, butylated hydroxytoluene = 809 mg, citric acid = 594 mg, vitamin A = 4,000,000 IU, vitamin D3 = 800,000 IU, vitamin E = 4,000 IU, vitamin* K_3 *= 600 mg, vitamin* B_1 *= 600 mg, vitamin* B_2 *= 1,600 mg, calcium D-pantothenate = 3,200 mg, choline chloride = 48,000 mg, niacinamide = 800 mg, vitamin* B_6 *= 600 mg, folic acid = 200 mg, vitamin* B_{12} *= 6,000 mg, biotin = 60,000 mcg, ferrous sulphate, monohydrate, iron = 16,000 mg, calcium iodate, anhydrous, iodine = 800 mg, copper (II) sulphate pentahydrate, copper = 2,400 mg, manganese (II) oxide, manganese = 24,000 mg, zine oxide, zinc = 2,400 mg, and sodium selenium = 60 mg.*

Experimental birds management

For a period of 7 days, all the chicks were raised together in the brooder house and were offered a commercial starter diet and water *ad libitum*. On the 8th day, the 204 one-week-old chicks were relocated to an open-sided poultry house, where they remained until the end of the 42-day feeding trial. To raise the birds, a total of 12 deep litter (wood shavings) pens, each measuring 2.5 m x 3.0 m, were used. Feed and water were supplied *ad libitum* throughout the trial. The birds were given routine medications and supplements (vitamins and minerals) through drinking water. On days 7 and 21, as well as on days 14 and 28, they received oral vaccinations against infectious bursal disease and Newcastle disease, respectively.

Chemical composition of SBM, BSFLM and formulated diets

Samples of BSFLM, SBM, and the formulated diets were ground to pass through a 1 mm screen for analysis. The prepared samples were analyzed for dry matter (DM), crude protein (CP),

Table 2: Ingredient composition of experimental finisher diets

Ingredient	**Percentage (%) inclusion of ingredients** **T0** (0% BSFLM)	**T1** (10% BSFLM)	**T2** (20% BSFLM)	**T3** (27.2% BSFLM)
Wheat bran	21.1	22.7	24.0	24.3
Maize	52.8	49.8	47.0	45.5
Soybean meal	23.1	14.5	6.00	0.00
Black soldier fly larvae meal	0.00	10.0	20.0	27.2
Common Salt	0.50	0.50	0.50	0.50
Oyster shell grit	1.50	1.50	1.50	1.50
Lysine	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.20	0.20	0.20	0.20
Toxin binder	0.20	0.20	0.20	0.20
Vitamin-trace mineral premix*	0.30	0.30	0.30	0.30
Total	**100**	**100**	**100**	**100**
	Calculated proximate composition (%) DM			
Dry matter	87.2	86.4	85.5	87.2
Ash	9.17	11.6	12.8	13.2
Ether extract	5.50	8.33	11.1	12.4
Crude Protein (%)	19.2	19.2	19.2	19.2
Crude fibre (%)	8.18	8.82	9.94	10.6
Metabolizable energy (kcal/kg)	3151	3150	3151	3151

Diets were supplemented with toxin binder (Biomin®) at the manufacturer's specified rate, composition of vitamin-mineral premix: gallate = 368 mg, butylated hydroxytoluene = 809 mg, citric acid = 594 mg, vitamin A = 4,000,000 IU, vitamin D3 = 800,000 IU, vitamin E = 4,000 IU, vitamin K_3 *= 600 mg, vitamin* B_1 *= 600 mg, vitamin* B_2 *= 1,600 mg, calcium D-pantothenate = 3,200 mg, choline chloride = 48,000 mg, niacinamide = 800 mg, vitamin* B_6 *= 600 mg, folic acid = 200 mg, vitamin* B_{12} *= 6,000 mg, biotin = 60,000 mcg, ferrous sulphate, monohydrate, iron = 16,000 mg, calcium iodate, anhydrous, iodine = 800 mg, copper (II) sulphate pentahydrate, copper = 2,400 mg, manganese (II) oxide, manganese = 24,000 mg, zine oxide, zinc = 2,400 mg, and sodium selenium = 60 mg.*

crude fibre (CF), ether extract (EE), and ash according to AOAC (2005). The metabolizable energy (ME) concentrations of the experimental diets were determined by the formula given by Wiseman (1987) as: ME (kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 ash.

Feed intake, weight gain and FCR

Body weights (g) were measured using an Electronic Scale (Wadfow India Private Limited) at the beginning of the experiment and then on weekly basis. Additionally, the amount of feed that was supplied each time was recorded, and the amount that was refused was weighed weekly on a pen basis. The data were used to determine average daily feed intake (ADFI) and average daily weight gain (ADG). Feed conversion ratio (FCR) was computed for each phase by dividing feed intake by body weight gain (feed/gain).

Blood sampling and determination of blood parameters

Blood samples were taken from two birds per replicate (a total of 24 samples per phase) on days 26 and 47 of the study for analysis of haematological and serum biochemical parameters. Four milliliters of blood samples were obtained by puncture of the brachial left-wing vein using needles and syringes. For determination of haematological parameters, tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant were used to collect the blood samples. The tubes were gently shaken to enable mixing of the blood with the EDTA. Gel separator tubes were used to collect blood samples for analysis of the serum biochemical indices. These tubes contain a gel barrier that separates the serum from the cellular components of the blood, allowing for easier separation during analysis. The blood samples for both the haematological and serum biochemical indices were kept on ice after collection and immediately sent to the laboratories for subsequent analysis. Following a 30-minute clotting period, blood samples in the gel separator tubes were centrifuged for 5 minutes at room temperature at 1500 revolutions per minute (rpm). The sera were taken out and analyzed for the serum biochemical indices.

Haematological indices measured included total WBCs counts, heterophils, monocytes, lymphocytes, eosinophils, basophils, total RBCs counts, haemoglobin, PCV, MCV, MCH, MCHC, and platelets. These were determined using an automatic Prokan PE-7080 Veterinary Hematology Analyzer (Shenzhen Prokan Electronics Inc.).

The concentrations of serum biochemical constituents measured include total protein, albumin, uric acid, urea, total triglycerides, total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). The concentrations were determined by using the Mindray Semi-Auto Chemistry Analyzer BA-88A (Guangzhou Medsinglong Medical Equipment Co., Ltd.). Globulin concentration was estimated by the difference between total protein and albumin.

Data analysis

All data collected were subjected to the one-way analysis of variance (ANOVA) using the statistical software GenStat Release 12th Edition (https://vsni.co.uk). Significantly different means were separated using the Student-Neuman Keuls (SNK) Test at a significance level of 5%.

RESULTS AND DISCUSSION

Feed intake and growth performance in broiler chickens fed black soldier fly larvae meal-based diets

Table 3 shows growth performance of birds in the starter and finisher phases. The finial body weight of birds on T3 was lower ($p<0.05$) compared to those on T0, T1, and T2 in the finisher phase. The ADG of birds on dietary treatments T0, T1, and T2 were higher ($p<0.05$) than those on T3 in the starter phase, but similar in the finisher phase. The FCR was poorer ($p<0.05$) in birds on T3 than those on treatments T0, T1, and T2 in the starter and finisher phases. The lower ADG, and poor FCR in birds on the highest inclusion rate (30%) of BSFLM (T3) in the starter

Table 3: Effects of diets containing graded levels of BSFLM on average feed intake, average daily weight gain, and feed conversion ratio of broiler chickens

Parameter	Dietary treatments **T0** (0% BSFLM)	**T1** (10% BSFLM)	**T2** (20% BSFLM)	**T3** (30% BSFLM)	**SEM**	**p-value**
Starter phase, day 8-28						
Initial weight, g/bird	146	146	146	146	0.13	1.000
ADFI, g/bird	98.58	95.70	95.62	93.90	0.71	0.180
ADG, g/bird	66.70^a	64.61^a	64.81^a	57.22^b	1.19	0.001
FCR	1.48^b	1.48^b	1.48^b	1.64^a	0.03	0.019
	T0 (0% BSFLM)	**T1** (10% BSFLM)	**T2** (20% BSFLM)	**T3** (27.2% BSFLM)		
Finisher phase, day 29-49						
Final body weight, g/bird	3065^a	3209^a	3078^a	2821^b	49.5	0.012
ADFI, g/bird	167	176	169	177	1.91	0.110
ADG, g/bird	72.3	81.2	74.8	70.1	1.66	0.070
FCR	2.31^b	2.17^b	2.26^b	2.52^a	0.05	0.020

ADFI - average daily feed intake; ADG - average daily gain; Means in the same row with different superscripts (a, b) are significantly different ($p<0.05$). SEM - standard error of mean

and finisher phases compared to those on the other dietary treatments (T0, T1, and T2) may be attributed to lower protein digestibility, which may have reduced feed utilization. This may be a result of higher chitin levels in T3. Chitin, a bioactive compound and antinutritional factor present in the exoskeleton of black soldier fly larvae, has been reported to inhibit nutrient digestibility (protein digestion), feed utilisation, growth performance, and feed conversion efficiency in broiler chickens fed diets containing higher levels of BSFLM beyond 25% (Cutrignelli *et al.,* 2018; Chobanova *et al.,* 2023).

Haematological parameters of broiler chickens fed black soldier fly larvae meal-based diets

The effects of BSFLM dietary treatments on the haematological parameters in the starter and finisher phases are presented in Table 4. During the starter phase, monocyte levels were higher ($p<0.05$) in birds on the control diets T0 than those fed the diets containing BSFLM (T1, T2, and T3). Birds on T1 had higher ($p<0.05$) eosinophils than those on T2 and T3. Basophil levels were lower ($p<0.05$) in birds on T1 than those on diets T2, T3, and T0. The packed cell volume levels in birds on diets T0 and T2 were higher ($p<0.05$) than those on T1 and T3. The MCH levels were higher ($p<0.05$) in birds in T1 than those on diets T0, T2, and T3. The platelet levels were elevated ($p<0.05$) in birds on T2 than those on the control (T0). All other parameters measured were not affected ($p>0.05$) by the dietary treatments.

Heterophil levels were higher ($p<0.05$) in birds on the control diet (T0) than those fed the BSFLM-based diets T1, T2, and T3 in the finisher phase. The levels observed for all the treatments T0, T1, T2, and T3 were higher than the normal physiological range of 12-30% reported for poultry (Bounous and Stedman, 2000). Heterophils are the first line of defense against infection and inflammation in chickens (Guriec *et* *al.,* 2018); thus, higher levels than normal obtained in the present study may signify infection in the broilers at the finisher stage. However, no physical symptoms or signs of infection were observed in the birds at this stage. Lymphocytes play an important role in the immune status of birds. Although the level in the finisher phase was significantly lower ($p<0.05$) in birds on the control diet (T0) than those fed the BSFLM-based diets, all fell within the normal physiological range of 26.9-70.6% reported for poultry (Bounous and Stedman, 2000) suggesting that the birds on the control and those on the BSFLM -based diets had normal immune status. The heterophil/lymphocyte ratio was higher ($p<0.05$) in birds on the control diet (T0) than those on the BSFLM-based diets T1, T2, and T3. Chickens with a low heterophil/lymphocyte ratio tend to exhibit superiority over those with a higher heterophil/lymphocyte ratio in terms of survival, immunological response, and resistance to *Salmonella* (Thiam *et al.,* 2022). Therefore, the lower heterophil/lymphocyte ratio observed for birds on the BSFLM-based diets than the control suggests better resistance to disease and stress, and better immune response than the control. Birds on diet T3 had lower ($p<0.05$) levels of monocytes than those on diets T1, T2, and T0. The values for monocytes recorded for birds on all the dietary treatments in the starter and finisher phases fell within the normal range of 0.0-6.4% reported for poultry (Bounous and Stedman, 2000), indicating no adverse effect of feeding BSFLM on monocyte levels and hence no infection in birds fed BSFLM-based diets. Eosinophil levels were higher ($p<0.05$) in birds on T1 and T2 than those on the control diet (T0). Eosinophils play a major role in detoxifying toxins produced by parasites and also counteract allergies. The higher levels than the normal physiological range of 0.0-11.5% (Bounous and Stedman, 2000) in birds on T1 and T2 may suggest the presence of parasitic infection in birds on these treatments in the starter phase. The levels, however, fell to the normal range (0-11.5%) by the end of the finisher phase, signifying no adverse effect of parasitic infection due to the feeding of BSFLM. The levels of basophils in both the starter and finisher phases were within the normal physiological range (0 – 6.4%) reported for poultry (Clinical Diagnostic Division, 1990; Bounous and Stedman).

The MCV levels were higher ($p<0.05$) in birds on T2 than those on T3. Also, the MCH levels were higher ($p<0.05$) in birds on T2 than those on T3. However, the birds on T2 had the lowest MCHC levels compared to those on T0, the control. The red blood cell indices, MCV, MCH, and MCHC, represent the size, haemoglobin content, and haemoglobin concentration of RBC (Perkins, 2009; Vajpayee *et al.,* 2011; Mondal and Lotfollahzadeh, 2023), and can be used to diagnose a variety of blood disorders, including anaemia. The higher MCH value in birds on diet T1 compared to the other treatments (T0, T2, and T3) during the starter phase could be attributed to its higher MCV level during the starter phase. Also, the higher MCV values of birds on T2 contributed to their higher MCH than those on T3 during the finisher phase. The levels of MCV and MCH for all treatments in the finisher phase were within the normal physiological range of 90.0 to 140.0 fL and MCH (33.0-47.0 pg) respectively for poultry (Bounous and Stedman, 2000), suggesting the birds did not suffer from anaemia during this phase. Although the higher than normal level of NCHC in the birds on all the dietary treatments during the finisher phase may suggest hemolysis, however, the birds on the various treatments looked normal.

In the present study, the levels of RBC, and Hb in birds fed the control diets were similar to those on the BSFLM-based diets in both the starter and finisher phases. The haemoglobin values fell within the normal range of 7.0-13.0 g/dL reported for poultry (Bounous and Stedman 2000), suggesting the feeding of BSFLM did not adversely affect the respiratory activities of the birds. The values obtained in the present study were comparable to those reported by Opoku *et al.,* (2018) when BSFLM-based diets were fed to broilers.

Table 4: Effects of diets containing graded levels of BSFLM on haematological parameters of broiler chickens

Parameter	Dietary treatment				SEM	p-value
	T0 (0% BSFLM)	T1 (10% BSFLM)	T2 (20% BSFLM)	T3 (30% BSFLM)		
Starter phase						
White blood cells (10^9/L)	10.57	8.97	9.83	10.63	0.26	0.075
Heterophils (%)	63.03	58.1	63.4	61.45	1.51	0.620
Lymphocytes (%)	22.75	16.82	25.24	27.67	1.68	0.115
Heterophil/lymphocyte	3.12	4.92	2.99	2.22	0.47	0.232
Monocytes (%)	1.18^{a}	0.73^{b}	0.90^{b}	0.72^{b}	0.05	<0.001
Eosinophils (%)	12.75^{ab}	13.88^{a}	9.93^{b}	9.5^{b}	0.06	0.011
Basophils (%)	0.62^{a}	0.30^{b}	0.55^{a}	0.70^{a}	0.04	<0.001
Red blood cells(10^{12}/L)	2.76	2.53	2.61	2.56	0.04	0.080
Haemoglobin (g/dL)	12.87	12.22	12.38	11.82	0.17	0.170
Packed cell volume (%)	33.93^{a}	30.93^{b}	33.77^{a}	31.82^{b}	0.46	0.039
MCV (fL)	112.4	116.2	114.3	113.7	0.54	0.087
MCH (pg)	46.90^{b}	48.40^{a}	47.48^{b}	47.17^{b}	0.17	0.006
MCHC (g/dL)	41.77	41.68	41.67	41.48	0.12	0.877
Platelets (10^9/L)	1.83^{b}	3.67^{ab}	5.00^{a}	3.5^{ab}	0.42	0.050
	T0 (0% BSFM)	T1 (10% BSFLM)	T2 (20% BSFLM)	T3 (27.2% BSFLM)		
Finisher phase						
White blood cells (10^9/L)	11.88	4.17	7.1	4.59	1.31	0.134
Heterophils (%)	56.42^{a}	36.98^{c}	46.62^{b}	35.77^{c}	2.1	<0.001
Lymphocytes (%)	41.07^{c}	59.70^{a}	49.37^{b}	60.98^{a}	2.1	<0.001
Heterophil/lymphocyte	1.38^{a}	0.62^{c}	0.99^{b}	0.62^{c}	0.08	<0.001
Monocytes (%)	0.25^{a}	0.28^{a}	0.25^{a}	0.05^{b}	0.03	0.002
Eosinophils (%)	1.83^{b}	2.87^{a}	3.25^{a}	2.55^{ab}	0.16	0.008
Basophils (%)	0.45	0.20	0.55	0.67	0.06	0.051
Red blood cells(10^{12}/L)	2.61	2.51	2.58	2.45	0.05	0.644
Haemoglobin (g/dL)	11.00	10.42	11.12	9.83	0.24	0.207
Packed cell volume (%)	26.98	26.67	27.52	25.28	0.53	0.524
MCV (fL)	104.0^{ab}	105.6^{ab}	106.5^{a}	103.1^{b}	0.48	0.047
MCH (pg)	42.35^{ab}	41.25^{bc}	43.10^{a}	40.10^{c}	0.33	0.003
MCHC (g/dL)	40.70^{a}	39.07^{ab}	36.62^{b}	38.97^{abc}	0.47	0.012
Platelets (10^9/L)	2.83	1.33	1.78	3.00	0.27	0.077

MCV- mean corpuscular volume;
MCH - mean corpuscular haemoglobin;
MCHC - mean corpuscular haemoglobin concentration. Means in the same row with different superscripts (a, b, and c) are significantly different ($p<0.05$);
SEM - standard error of mean.

Serum biochemical parameters of broiler chickens fed black soldier fly larvae meal-based diets

Table 5 presents the effect of diets with varying levels of BSFLM on some serum biochemical parameters in broiler chickens in the starter and finisher phases. During the starter phase, birds on T2 had a higher ($p<0.05$) concentration of total proteins than those on T3. Total protein concentration was higher ($p<0.05$) in birds on T2 than those on T3 and T0 in the finisher phase. Total protein concentration reflects digestible protein intake. The lower total protein concentration in birds on T3 in the starter phase may be due to the lower apparent ileal digestibility of crude protein of birds on that treatment, as reported in an earlier study (Adam *et al.,* 2024), and this may have contributed to their lower total protein and albumin concentrations in the finisher phase. Albumin concentration was higher ($p<0.05$) in birds on T2 and T3 than those on the control (T0) in the starter phase. Albumin concentration in birds on T2 was higher ($p<0.05$) than those on T0 which was also higher ($p<0.05$) than those on T1, and T3 in the finisher phase. The total protein and albumin concentration obtained in the starter and finisher phases fell within the normal physiological ranges of 21.0-44.0 g/L and 2.0-24.0 g/L respectively, reported for poultry (Harr, 2008), indicating that all the birds on the control, and BSFLM-based diets were in good protein status.

Globulin concentrations were higher ($p<0.05$) in birds on T0 and T2 than those on T3 in the starter phase, and were lower in birds on the control (T0) and T3 in the finisher phase. The globulin concentration was higher ($p<0.05$) in birds on T1 and T2 than those on T0. Globulins play a major role in the immune system's vigilance and antibody production (Abdel-Fattah *et al*., 2008). The globulin concentration in the various dietary treatments in the starter and finisher phases all fell within the normal physiological range of 12.0 to 36.0 g/L reported for poultry, suggesting the birds on all the dietary treatments had a good ability to resist diseases and infections. The albumin/globulin ratio is an important indicator of the nutritional and health status of broiler chickens, can be affected by diet and nutrition in broiler chickens (Tothova *et al.,* 2019). The higher ($p<0.05$) albumin/globulin ratio in birds fed diet T3 than those on T0, T1, and T2 in the starter phase may be due to their relatively high albumin coupled with very low globulin concentrations. However, birds on T3 rather had a lower ($p<0.05$) albumin/globulin ratio in the finisher phase. The albumin/globulin ratio for birds on the dietary treatments during the starter and finisher phases all fell within the normal range of 0.42-3.0, indicating birds on the control, and BSFLM-based diets had good nutritional and health status. Other studies have also reported no adverse effects on concentrations of albumin, globulin, and their ratio on inclusion of BSFLM in the diets of chickens (Seyedalmoosavi *et al.,* 2022; Facey *et al.,* 2023), and guinea fowls (Wallace *et al.,* 2018).

Uric acid, a by-product of purine and protein metabolism, is affected by diet (Harr, 2002). Interestingly, dietary treatments in this study did not influence the concentration of uric acid, which remained within the normal physiological range of 2.0 to 11.0 µmol/L (Bueno *et al.,* 2017) during the starter phase. However, uric acid concentration was lower in T3 during the finisher phase compared to T0, T1 and T2 in the finisher phase. The lower protein utilization in birds on T3 might explain the observed decrease in uric acid concentration. In contrast, serum urea concentration was higher in birds on T3 in the starter phase which could be attributed to the lower amino acid utilization by birds on T3, leading to increased urea production as reported in an earlier study (Adam *et al.,* 2024).

The VLDL concentration was higher ($p<0.05$) in birds on T2, and T3 than those on T0 and T1.

The higher ($p<0.05$) concentration of total cholesterol in birds on diet T3 than T2 in the starter phase (Table 5) may be due to the higher EE content of diets T2 and T3 (Table 3). Also, triglyceride concentrations were generally higher in birds on diets containing BSFLM (T1, T2 and T3) than those on the control during this phase

Table 5: Effects of diets containing graded levels of BSFLM on serum biochemical parameters of broiler chickens

	Dietary treatment					
Parameters	**T0** (0% BSFLM)	**T1** (10% BSFLM)	**T2** (20% BSFLM)	**T3** (30% BSFLM)	**SEM**	**p-value**
Starter phase						
Total protein (g/L)	28.29^{ab}	27.14^{ab}	32.48^{a}	23.27^{b}	1.16	0.032
Albumin (g/L)	11.60^{b}	13.25^{ab}	14.82^{a}	14.49^{a}	0.40	0.009
Globulin (g/L)	16.69^{a}	13.89^{ab}	17.65^{a}	8.78^{b}	1.12	0.013
ALB: GLB	0.78^{b}	0.98^{b}	0.88^{b}	2.04^{a}	0.18	0.029
UA (µmol/L)	450.5	545.6	484.0	488.4	26.1	0.661
Urea (mmol/L)	1.41^{ab}	1.20^{abc}	0.86^{ac}	1.44^{a}	0.08	0.047
TC (mmol/L)	3.62^{ab}	3.06^{b}	3.81^{ab}	4.01^{a}	0.13	0.043
Triglycerides (mmol/L)	0.50^{b}	0.55^{b}	0.79^{a}	0.71^{ab}	0.04	0.037
HDL (mmol/L)	1.65^{c}	1.66^{c}	2.76^{b}	3.38^{a}	0.18	<0.001
LDL (mmol/L)	1.75^{a}	1.15^{b}	0.69^{c}	0.30^{c}	0.13	<0.001
VLDL (mmol/L)	0.22^{b}	0.25^{b}	0.36^{a}	0.32^{ab}	0.02	0.040
	T0 (0% BSFLM)	**T1** (10% BSFLM)	**T2** (20% BSFLM)	**T3** (27.2% BSFLM)		
Finisher Phase						
Total protein (g/L)	28.64^{b}	30.91^{ab}	33.57^{a}	27.73^{b}	0.64	0.001
Albumin (g/L)	16.57^{b}	14.76^{c}	18.47^{a}	14.10^{c}	0.41	<0.001
Globulin (g/L)	12.06^{c}	16.41^{a}	15.10^{ab}	13.64^{bc}	0.49	0.004
ALB:GLB	1.39^{a}	0.91^{b}	1.25^{a}	1.03^{b}	0.05	<0.001
UA (µmol/L)	464.6^{a}	556.0^{a}	469.9^{a}	293.4^{b}	29.10	0.005
Urea (mmol/L)	1.43^{a}	0.30^{b}	0.53^{b}	1.18^{a}	0.13	0.001
TC (mmol/L)	2.60	2.72	2.68	2.82	0.08	0.800
Triglycerides (mmol/L)	0.87	0.53	0.80	0.95	0.07	0.186
HDL (mmol/L)	1.51	1.75	1.58	1.60	0.06	0.631
LDL (mmol/L)	1.06	0.73	0.74	0.79	0.08	0.418
VLDL (mmol/L)	0.28^{b}	0.24^{b}	0.36^{a}	0.43^{a}	0.02	<0.001

ALB:GLB - albumin/globulin; UA - uric acid;
TC- total cholesterol; HDL - high-density lipoprotein,
LDL - low-density lipoprotein;
VLDL - very low-density lipoprotein. Means in the same row with different superscripts (a, b, and c) are significantly different ($p<0.05$);
SEM standard error of mean.

due to the higher EE contents of the BSFLM-based diets (Table 3). However, cholesterol and triglyceride concentrations of birds on the control and BSFLM-based diets were within the normal physiological ranges of 2.60-6.50 mmol/L and 0.55-0.92 mmol/L respectively, reported for poultry (Bueno *et al.,* 2017). Feeding BSFLM-based diets to chickens did not significantly (p>0.05) affect the total cholesterol and triglyceride concentrations of birds compared to those on the control diet in the finisher phase, as was also observed by Schiavone *et al.* (2017; 2018). The total cholesterol and triglyceride concentrations obtained were within the normal physiological range reported for poultry (Bueno *et al.,* 2017). Generally, the dietary inclusion of BSFLM in the diet of the birds increased HDL and reduced LDL in serum compared to those on the control in the starter and finisher phases. A decrease in LDL levels and an increase in HDL levels have been linked to a decreased risk of cardiovascular diseases (Wade *et al*., 2013), thus suggesting an advantage in consuming meat from broilers raised on BSFLM. The concentrations of the HDL during the finisher phase fell within the normal range of 1.44 to 2.26 mmol/L reported for poultry (Bueno *et al.,* 2017). In another study, Wallace *et al.* (2018) included BSFLM in the diet of guinea fowls and observed no adverse effects on HDL concentrations. The VLDL concentrations in birds on T2 and T3 were higher than those on T0 and T1 during both starter and finisher phases (Table 5). However, the concentrations were within the normal range of values 0.33 to 0.45 mmol/L reported for poultry (Bueno *et al*., 2017), thus suggesting the birds on all the dietary treatments effectively utilized their energy resources, as VLDL is known to play a major role in providing energy and maintenance in poultry by transporting triglycerides to tissues (Scanes, 2022).

CONCLUSION

Results from the present study indicate that inclusion of BSFLM in broiler chicken diets up to 20% had no adverse effect on broiler chicken growth, physiology and health status. The haematological and serum biochemical parameters measured were within the normal physiological ranges for poultry and similar to the results obtained in other studies. Further research may be needed to refine BSFLM inclusion for optimal broiler meat tenderness, juiciness, and acceptability.

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