GSAR Journal of Agriculture and Veterinary Sciences
ISSN: 3048-9075 (Online)
Abbreviated key title: Glob.J. Agri.Vet.Sci.
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journal-gjavs-home/

EFEECTS OF FEEDING FULL FAT BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL ON ABDOMINAL FAT AND SERUM BIOCHEMISTRY PROFILE OF BROILER CHICKENS AT FINISHING PHASE

By

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Article History

Received: 02/03/2026
Accepted: 07/03/2026
Published: 09/03/2026

Vol – 3 Issue –3

PP: -05-14

Abstract

Feed quality and quantity are major factors that affect the health of chicken and ultimately their production performance. Therefore, health is one of the parameters used to evaluate the suitability of ingredients used for feed formulation in broiler chicken. This study evaluated the effects of inclusion of full- fat black soldier fly (BSF) (Hermetia illucens) larvae meal on the health of broiler chicken by assessing their biochemical indices at finisher phase. Five isonitrogenous and iso-calorific dietary treatments with three replications were randomly assigned to fifteen experimental cages containing 6 Cobb 500 broiler chicken of mixed sex at 21days of age in a completely randomized design experimental setup. The meal was included at 0, 7.5%, 15%, 22.5% and 30% levels in the diets. At 49 days of age (28 days of experiment), 3 birds from each dietary treatment, that is, 1 bird from each replicate were randomly selected and starved overnight. Blood samples were collected via wing vein for analysis of serum lipid, liver enzymes and protein profiles. Thereafter, they birds were slaughtered to obtain weight of the abdominal fat. Data was analyzed using Statistical Analysis System software (SAS, 2009) and significant means were separated using Tukey Honest Significant Difference (HSD). The BSF larvae meal significantly (P<0.05) increased the abdominal fat, total cholesterol, triglyceride and low protein lipoprotein. However, these values were within the acceptable references and therefore BSF larvae may be utilized for broiler feeding at inclusion level of 30%. Methods of reducing the level of abdominal fat need to be assessed since fat is a waste product with low economic value and represents dietary energy loss. In addition, it reduces the carcass yield and lowers consumer acceptance of meat

Key Words: *Broilers, Black soldier Fly Larvae, abdominal fat, serum, lipids, enzymes, proteins.*

INTRODUCTION

The poultry industry is considered as one of the most vital sources of animal protein for human nutrition security. Much attention has been focused on the chicken in the poultry industry segment for its compatibility with dietary legislation with religious and ethnic groups (Shah *et al.,* 2021). Among the chicken, (*Gallus gallus domesticus*) broiler is bred and raised specifically for meat production (Joshua *et al.,* 2022). However, it is faced with challenge in finding a sustainable protein feed source for feed formulation. (Dörper *et al.,* 2021).

Feed accounts for over 70% in broiler production and the protein fraction bears a bigger proportion of that cost compared to the other nutrients. Research studies are being conducted to assess non-conventional protein sources for chicken without human competition. The larvae of black soldier fly (BSF) *Hermentia illucens* has been identified as sustainable alternative protein sources for broilers. It contains high content of crude protein, good amino acids and fatty acids profiles. The larvae can be reared using waste streams and can therefore be used as a bioconversion agent hence a promising solution to many global environmental concerns (Lalander *et al,* 2019). It does not compete with human for food resources and has little negative effect on the environment in terms of space, water consumption and emission of green-house gases (GHGs) (Kinasih *et al,* 2018). Although the crude protein content is varied, it can be optimized by modulating the proximate composition of the rearing substrate among other factors (Liland, 2017).

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The absence of adverse effects of dietary inclusion of BSF larvae in broiler feed makes it a promising alternative energy and protein source (Purnamasari *et al.*, 2022). The meal of BSF registers high fat content and it is well established that the amount and type of fat accumulated by chicken is related to the exogenous source and this is used to evaluate the suitability of their meat by the final consumer (Mutisya *et al.*, 2022). Fat content in meat has been considered to have a negative impact on human health and due to this, the consumer society has developed fat/cholesterol phobia (Zeraatkar *et al., 2019*). Fat also presents dietary energy waste, reduces carcass yield and has little economic value.

However, majority of the available studies on BSF larvae meal are based on production performance with paucity of information on biochemical indices and ultimately their health. Serum biochemical indices are used to assess the clinical and physiological responsiveness of chickens and equally to evaluate the suitability of ingredients for feed application (Bello *et al.,* 2022). Animals with good blood composition are likely to show good production performance (Gadde *et al.,* 2017). The most commonly used serological indices indicate lipid profiles, kidney and liver functions.

Marono *et al*. (2017) reported an increased level of globulin and calcium in laying chicken fed diets containing 17% of defatted BSF larvae meal. The same authors reported reduced albumin to globulin ratio, cholesterol and triglycerides and recommended further investigations for complete replacement of soybean meal with BSF larvae meal. On the other hand, Mat *et al.* (2022) reported reduced total protein in broilers fed diets containing 4%, 8% and 12% of defatted BSF larvae meal. Lastly, Dabbou *et al.* (2018) reported non-significant effects on the hematological and biochemical parameters on broilers fed partially defatted BSF larvae meal at inclusion level of 5, 10 and 15%.

These trials evaluated defatted form of BSF larvae meal. However, how high inclusion level impacts the health of broilers is yet to be documented and more so when the larvae meal is utilized in full fat form. This study investigated the impact of feeding full fat BSF larvae meal to broilers on serum biochemical profiles with the aim to establish the optimal inclusion level.

MATERIALS AND METHODS

Study site

The experiment was conducted at the University of Nairobi, Animal Production Department Poultry Unit. It is located at latitude 1.25287867437 and Longitude of 36.7298431783.

Experimental diets

The BSF larvae used in this study were reared in a private farm (Zihanga) specializing in commercial production of BSF located in Lower Kabete in Kiambu County- Kenya. Five iso-caloric and iso-nitrogenous diets were formulated using varying levels of BSF larvae meal at inclusion of 0, 7.5, 15, 22.5 and 30% to replace soybean meal to meet the National Research Council requirements for broiler chickens during the finishing phase; 3100 Kcal/kg and 20% CP (NRC, 1994).

Chemical analysis

The ingredients used in the current study were analyzed for DM, CP, fat, fibre and ash before being incorporated into the experimental diets. Dry matter (DM) was determined by drying in a hot air oven at 105°C for 24 h following standard method 925.09 (AOAC, 2006), Ash by burning the samples in a muffle furnace at 550°C for eight hours following standard method 923.03 (AOAC, 2006), ether extract by the Soxhlet method (using ether) following standard methods 920.39 (AOAC, 2006). Total nitrogen for crude protein (N x 6.25) determination was obtained using the micro-Kjeldahl method following standard methods 920.87 (AOAC, 2006). The nutritive composition of the BSF larvae meal and the experimental diets used in the present study are presented in Tables 1and 2 respectively.

Table 1. Analyzed proximate composition (%) of the black soldier fly larvae meal utilized in the experimental diets

Nutrient	BSF larvae meal
Dry matter	34.97
Crude protein	48.5
Crude fibre	16.2
Crude fat	23.5
Crude ash	12.31
ME Kcal/kg	3456

Management of the chicken

One hundred and fifty, day old broiler chicks of mixed sex (Cobb-500) were obtained from Ken-Chick® Ltd and reared under standard management. At twenty- one day of age, ninety chicken of mixed sex were randomly distributed to 15 experimental cages (1 by 1 m^2) in a deep litter system. The five broiler diets were then randomly assigned to the experimental units in a complete random design arrangement with three replications per treatment having six chicken per cage. The birds were fed on the experimental diets for 28 days and were offered *ad libitum* access to feed and clean drinking water.

Table 2. Proximate composition of the experimental diets for the broiler chicken

Ingredient (%)	T1	T2	T3	T4	T5
Maize grain	65.0	65.0	65.0	65.0	65.0
BSFL meal[1]	0	7.5	15.0	22.5	30.0
Soya bean meal[2]	30.0	22.5	15.0	7.5	0
Lysine	0.4	0.4	0.4	0.4	0.4
Methionine	0.3	0.3	0.3	0.3	0.3
DCP (granular 24%)	0.5	0.5	0.5	0.5	0.5

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Limestone	2.0	2.0	2.0	2.0	2.0
Salt	0.2	0.2	0.2	0.2	0.2
Premix [3]	0.3	0.3	0.3	0.3	0.3
Calculated nutrients					
Crude Protein	19.6	19.6	19.7	19.7	19.8
ME (Kcal/kg)	3176	3159	3152	3146	3138
Ether Extract	4.9	6.1	7.1	8.2	9.2
Crude Fibre	6.0	6.7	7.4	8.1	8.9
Ash	2.4	2.7	2.8	3.2	3.4

BSFL; Black soldier fly larvae, Soy bean meal; 20% full fat, 80% de-oiled, Premix; Supplied the following per 2Kg of diet: Each 2Kg contains: Vitamin A, 8,500,000 IU; Vitamin D3, 1,600,000 IU; Vitamin E, 4,000 IU; Vitamin K3, 2,000 mg; Vitamin B2, 5,000 mg; Vitamin B3, 20,000 mg; Vitamin B5, 8,800 mg; Vitamin B6, 1,200 mg; Vitamin B9, 00 mg; Vitamin B12, 8 mg; Chlorine chloride, 200,000 mg; Antioxidant, 125,000 mg; Fe, 5,000 mg; Mn, 80,000 mg; Zn, 50,000 mg; Cu, 2,000 mg; I, 1,200 mg; Co, 200 mg; Se, 100 mg.

Data collection and statistical analysis

At seven weeks of age (28 days of experimental period), 3 birds from each dietary treatment, that is, 1 bird from each replicate were randomly selected, totaling 15 birds from the five dietary treatments. The birds were starved overnight and blood samples were collected via the wing vein into 5ml tubes without anticoagulant for analysis of biochemical parameters. The samples were centrifuged for 15 minutes at 3000 rpm to collect the serum. The serum was assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl-transferase (GGT), total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein and albumin. Globulin was calculated as the difference between the assayed total protein and albumin. The albumin to globulin ratio was also calculated. Thereafter, the chickens were slaughtered and the abdominal fat was extracted and weighed.

The data collected was analyzed using analysis of variance (ANOVA) in a CRD set up and treatment means were separated using Tukey's Honestly significant difference (HSD) test at significant level of $p<0.05$ where applicable.
The analysis was based on the following equation**;**

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Where: Y_{ij} = Observation (abdominal fat, serum biochemical profile values)

μ = Overall mean

T_i = Inclusion level of BSF larvae meal (0, 7.5, 15, 22.5, 30 %)

ε_{ij} =Random error component

RESULTS

Effect of feeding black soldier fly larvae meal as a substitute for soy bean meal on abdominal fat and lipid profile of broiler chicken

The results of abdominal fat and lipid profile of broiler chickens are shown in Table 3. The abdominal fat ranged between 20.3-61.5 g while the serum lipid profile ranged between 56.52-102, 18.18-34.38, 20.34-30.6, 36.36-56.7, 0.416-0.623 mg/dl for total cholesterol, triglycerides, HDL, LDL and HDL: LDL respectively. Dietary treatment significantly increased the abdominal fat, total cholesterol, triglycerides and LDL p<0.05. However, the inclusion of the larvae meal did not have significant effect (P<0.05) on HDL and the ratio of HDL:LDL P>0.0

Table 3. Abdominal fat (g) and serum lipid profile (mg/dl) of broiler chickens fed graded levels of black soldier fly larvae meal at finisher phase.

Lipid parameters	Treatments (BSFL meal inclusion level%)					SEM±	P-Value
	T1 0%	T2 7.5%	T3 15%	T4 22.5%	T5 30%		
Abdominal fat (gms)	$20.33^{a}\pm1.11$	$38.77^{b}\pm3.16$	$35.53^{b}\pm3.99$	$39.47^{b}\pm2.63$	$61.50^{c}\pm4.82$	3.74	0.002 S
T. cholesterol(mg/dL)	$62.28^{ab}\pm2.45$	$56.58^{a}\pm2.32$	$75.44^{bc}\pm8.86$	$83.78^{bc}\pm4.05$	$98.50^{c}\pm7.24$	4.55	0.002 S
Triglycerides (mg/dL)	$19.28^{a}\pm2.09$	$18.51^{a}\pm1.69$	$20.52^{a}\pm3.38$	$31.95^{b}\pm1.62$	$34.41^{b}\pm0.91$	2.00	0.001 S
HDL (mg/dL)	$21.80^{a}\pm4.5$	$18.54^{a}\pm2.55$	$27.51^{a}\pm4.12$	$23.60^{a}\pm1.94$	$30.75^{a}\pm3.53$	1.74	0.184 NS
LDL (mg/dL)	$36.58^{a}\pm2.86$	$36.46^{a}\pm2.87$	$50.09^{b}\pm3.33$	$53.75^{b}\pm1.85$	$56.76^{b}\pm1.63$	2.49	0.000 S
HDL: LDL	$0.62^{a}\pm0.18$	$0.53^{a}\pm0.12$	$0.54^{a}\pm0.06$	$0.44^{a}\pm0.07$	$0.54^{a}\pm0.04$	0.04	0.805 NS

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BSFL= black soldier fly larvae; SEM=standard error mean; T=Total; HDL=high density lipoprotein; LDL=low density lipoprotein; S=significant; NS= not significant

Means within same row with different superscript are statistically different at P < 0.05 according to Tukey's Honesty significant difference test.

Effect of feeding black soldier fly larvae meal as a substitute for soy bean meal on serological liver enzymes and protein profiles of broiler chicken at finishing phase

The results of the serum liver enzymes and proteins are indicated in Table 4. The AST, ALT, GGT and ALP ranged between 128.47-182.67, 158.48-208.36, 399.46-594.17 and 116.43-174.64 mmols /L while the values for total protein, albumin, globulin and total bilirubin were 6.37-7.87, 3.53-4.83, 2.6-3.43 and 0.633-0.800 g/dl. The AST:ALT ratio was less than 1 in inclusion level of 7.5, 15, 22.5 and 30%% but slightly higher than 1 in the control diet. On the other hand, the ratio of albumin: globulin was1.08-1.51. There was no significant (P>0.05) dietary effect on the serological enzymes and protein profile under evaluation in the current study. There was a numerical increase in total protein and albumin as the inclusion level of BSF larvae meal increased in the trial diets.

Effect of substituting soy bean meal with black soldier fly larvae meal on serum metabolites and electrolytes profiles on broiler chicken at finishing phase

The kidney function was assayed by analyzing the serum metabolites and electrolytes. The serum enzymes and electrolytes parameters of the broiler chickens are summarized in Table 5. There was no significant effect (P > 0.05) related to BSF larvae meal utilization regarding the blood metabolites and electrolytes between the control and trial diet. The creatinine levels ranged between 70.00-91.33 umol/L while the urea ranged between 3.33-5.2 mmols/L. The values for sodium, potassium, and chloride were 139.67-145.67, 3.57-3.9 and 98.33-104 mmols/L respectively. There was numerical increase of creatinine with increase of BSFL meal in the trial diet. The ratio of sodium to potassium ranged between 36.15–40.52 which was slightly higher than the normal 36.11 indicating that the birds were not going through stress.

Table 4. Liver enzymes and protein profiles of broiler chickens fed graded levels of black soldier fly larvae meal at finisher phase.

Parameters	T1	T2	T3	T4	T5	SEM±	P-Value
	0%	0.75%	15%	22.5	30%		
AST U/L	182.67±8.03	128.47±32.30	152.56±17.15	136.56±20.96	176.66±8.74	9.35	0.277 NS
ALT U/L	132.48±31.29	183.07±5.25	228.84±20.86	174.64±34.24	178.65±8.7	11.99	0.146 NS
AST: ALT	1.15±0.08	0.70±0.18	0.73±0.04	0.91±0.35	0.98±0.33	0.108	0.365 NS
ALP U/L	423.55±12.2	585.74±66.99	399.46±14.47	594.17±40.30	574.10±59.85	28.43	0.190 NS
GGT U/L	120.44±12.04	116.43±22.62	132.48±18.39	150.55±33.17	174.64±35.41	13.88	0.727 NS
T. protein (g/dl)	6.7±0.45	7.0±0.79	6.37±0.67	6.83±0.37	7.87±0.33	0.25	0.437 NS
Albumin g/dl	3.6±0.32	3.53±0.22	3.67±0.09	4.23±0.29	4.83±0.47	0.18	0.056 NS
Globulin g/dl	3.1±0.21	3.43±0.58	2.70±0.64	2.60±0.30	3.10±0.12	0.18	0.642 NS
ALB: GLO	1.17±0.10	1.08±0.14	1.54±0.40	1.67±0.23	1.50±0.19	0.11	0.380 NS
T. bilirubin g/dl	0.800±0.06	0.800±0.15	0.800±0.15	0.733±0.07	0.633±0.03	0.044	0.750 NS

AST= aspartate aminotransferase, ALT= alanine aminotransferase, ALP= alkaline phosphatase, GGT= gamma glutamyl-transferase T=total, U/L= units per litre, g/dl= grammes per decilitre

Table 5:Serum metabolites and electrolytes profiles of broiler chicken fed graded levels of black soldier fly larvae meal at finisher phase

Parameter	Treatments (BSFL meal inclusion level %)						
	T1	T2	T3	T4	T5	SEM±	P-Value
	0%	7.5%	15%	22.5%	30%		
Urea in blood mmol/L	3.33±0.98	4.27±0.93	5.20±1.27	4.80±1.27	3.27±0.24	0.422	0.699
Sodium mmol/L	141.7±0.88	144.00±0.58	145.67±3.92	144.33±0.88	139.67±1.67	0.949	0.298

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Potassium mmol/L	3.90±0.15	3.57±0.15	3.8±0.2	3.80±0.21	3.90±0.30	0.085	0.787
Sodium: Potassium	36.45±1.57	40.52±1.84	38.50±1.94	38.25±2.42	36.15±4.19	0.87	0.567
Creatinine µmol/L	73.33±4.91	87.33± 4.84	70.00± 6.03	87.67± 11.92	91.33± 9.33	3.77	0.284
Chloride mmol/L	104.7±0.13	102.00±2.08	99.33±1.86	102.33±1.76	98.33±1.33	0.864	0.096

DISCUSSION

Studies have shown that serum profiles of chicken can be used to assess their health and nutritional status hence evaluate the suitability of feed ingredient for their feeding application (Attia *et al.*, 2017). This study delved into the impact of total substitution of soy bean meal with BSF larvae meal on health of broiler chicken evaluated in terms biochemical indices and accumulation of abdominal fat.

Lipid profiles

Korver (2023) documented that modern broiler strains contain 15% to 20% fat but more than 85% of this fat is not physiologically required for body function. The excessive fat deposition is an unfavorable trait for producers and consumers because it is considered to be wasted dietary energy and a waste product with low economic value. It also reduces the carcass yield and affects meat acceptance by consumers (Korver, 2023). Fat content in meat has been considered to have a negative impact on human health and due to this, the consumer society has developed cholesterol phobia and become choosy by avoiding fatty meat Umemura *et al.*, 2019). The high sensitization of negative effect of cholesterol and LDL on cardiovascular health is an awakening call on any pre-dispositioning factors and more so eating habits.

The lipid profile results, detailed in Table 3, indicated that the inclusion of BSF larvae meal had a significant ($P < 0.05$) increase effect on abdominal fat, total cholesterol, triglycerides and LDL. However, HDL and the HDL:LDL ratio did not exhibit significant changes.

The abdominal fat content ranged from 20.0g (T1, 0% BSFL) to 61.50g (T5, 30% BSFL) and increased with increase in BSF larvae meal. Excessive subcutaneous and abdominal fat deposition in chickens was also reported by Abdalla *et al.* (2018). In general, fat deposition occurs in subcutaneous tissues, muscles, and the abdomen of chickens (Chen *et al.*, 2018). The accumulation of fat in broiler chickens is influenced by various factors, including genetic composition, nutritional factors, age and sex (Kang *et al.*, 2023). Broiler chicken have more fat than layers while chicken fed on high caloric and crude protein diets accumulate more fat than the ones fed the recommended levels. Older chicken contains higher fat content than the young ones. Female chicken accumulates more abdominal fat than male and overall tend to have more fat that males.

Contemporary nutritionists strive to produce a lean carcass. Strategies such as inhibiting the absorption of dietary fat and fatty acid synthesis, along with promoting fatty acid β-oxidation, can reduce abdominal fat deposition by decreasing the size and/or number of abdominal adipose cells. The role of dietary protein content is crucial in regulating lipid metabolism in broiler chickens. Srilatha *et al.* (2018) observed a significant increase in abdominal fat content when there was a reduced dietary crude protein levels from 23% to 20% (CP) during the starter phase and from 20% to 18% during the finisher phase. Similarly, Kamran *et al.* (2020) found that broiler chickens fed diets with low protein content (19.2%, 16.6%, and 15.5% CP) experienced an increase in total carcass fat deposition compared to those fed diets with standard protein content (22.9%, 19.9%, and 18.2% CP) recommended by the NRC in the starter, grower, and finisher phases, respectively. This is because energy is required to metabolize protein and so in case of imbalanced diets the excess carbohydrates is converted into fat. Conversely, increasing dietary protein levels to 26.6%, 23.5%, and 20.7% in the starter, grower, and finisher phases resulted in a reduction in total carcass fat deposition compared to formulated diets. In case of excess dietary protein, more energy is required for its metabolization therefore leaving less glucose to be converted and stored in form of fat.

Abdulla *et al.* (2019) demonstrated that an increase in dietary protein content led to a significant reduction in fatty acid synthase (FAS) mRNA expression in the livers of broiler chickens when compared with the control group. Additionally, Wang *et al.* (2020) found that providing broilers with a diet containing high crude protein (CP) levels suppressed the mRNA expression of hepatic malic enzyme, acetyl coenzyme carboxylase (ACC), and FAS in a comparison of low-protein diets. This indicated that the level of dietary protein directly influenced body fat deposition. Therefore, meeting the protein requirements of birds is essential for producing high-quality meat with low fat deposition. Wu *et al.* (2021) noted that dietary supplementation of L-methionine in broiler chickens resulted in a significant decrease in body fat content. The fat-lowering effect of dietary L-methionine may be linked to changes in lipogenesis and/or lipolysis.

Additionally, the amount and type of dietary fat influence the accumulation of fat in broilers. This increase could partly be attributed to the rise in dietary fat, as the BSFL meal had a 23% fat content compared to 8% in soybean meal. The elevated levels of abdominal fat the level of BSF larvae meal increased could also be attributed to an increase in saturated fatty acid which accounts for 52.5% – 67.9 % of the total fatty acids in BSF larvae (Liland *et al.*, 2017). Saturated fat tends to lead to more abdominal fat compared to unsaturated fat, as it inhibits the activity of FAS in the liver (Zou *et al.*, 2020). Fatty acid synthase (FAS) is a critical enzyme in the de novo lipogenesis pathway in the liver of chickens, and the activity of FAS in the liver determines the ability of chickens to synthesize fatty acid deposits in the body. Changes in lipogenesis and/or lipolysis are likely associated with the fat-

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lowering effect of dietary L-methionine. Studies indicate that dietary L-methionine supplementation regulates body fat content by reducing the activity of FAS (lipogenesis) and increasing the activity of hormone-sensitive lipase (HSL) (Wu *et al.*, 2021).

Lastly, Wu *et al.* (2021) demonstrated that feeding chickens a diet containing 70% of their energy requirements while limiting feed consumption to 80% of the full feed significantly reduced abdominal fat pad and subcutaneous fat thickness compared with the control.

Serum cholesterol levels are significantly influenced by genetic factors, feed, and medications. Cholesterol in the body originates from two sources; from the feed (exogenous cholesterol) and cholesterol produced by the body itself (endogenous cholesterol). Cholesterol derived from feed plays a crucial role as a primary sterol in the body, found in cell surface components and intracellular membranes (Aikpitanyi & Egweh, 2020).

In the current study, the total cholesterol in broilers exhibited a significant difference (p<0.05) and increased as the inclusion level of BSF larvae meal in the trial diets increased. The cholesterol levels ranged between 62.28-102 mg/dl and is comparable to 81-85mg/dl documented by Dabbou *et al.* (2018) from broiler chicken fed diets containing 5-15% partially defatted BSF larvae meal. The values are slightly lower than 108mg/dl documented by Marono *et al.* (2017) from layers chicken fed diets containing 17% of BSF larvae meal. This could be due genetic disposition of the layers in the refereed study.

However, the serum cholesterol values obtained in this study, were within the normal reference range of 52.00 mg/dl to 148.00 mg/dl (Aikpitanyi & Egweh, 2020). The highest level was recorded in T5, which contained 30% BSF larvae meal, indicating that high exogenous fat has an elevated impact on serum cholesterol, given that the BSF larvae meal used in this study had a crude fat content of 23.12% compared to soybean meal containing 4.94%.

Triglycerides (TRI) are synthesized in the liver from fatty acids. Triglycerides could also be synthesized from protein and glucose if their provision exceed the body's current needs. They are stored in adipose tissue (Odunitan-Wayas *et al.*, 2018). The serum triglyceride levels are influenced by diets, estrogen, fat formation, and diseases (Johnson & Stolzing, 2019). There was significant differences (P < 0.05) among treatments in the serum triglyceride levels of broiler chickens in the current study which ranged between 16.38mg/dl to 34.38mg/dl and increased as the inclusion level of BSF meal increased. The recommended normal level of triglycerides in chicken is ≤ 150 mg/dl (van der Heijden *et al.*, 2021). Therefore, the triglyceride levels in the present study were within the acceptable level.

Low-density lipoprotein (LDL), primarily transports cholesterol from the liver to body tissues and is positively influenced by cholesterol concentration in the serum. In the present study, the average serum LDL level ranged between 36.36 and 56.7 g/dL, with the highest level observed in birds fed the diet containing the 30% inclusion level of BSF larvae meal. A significant (P < 0.05) difference was observed among the trial diets, with a notable increase as the level of BSF larvae meal increased. However, all LDL levels in the diets were below 100 mg/dL, which is considered desirable.

High-Density Lipoprotein (HDL) levels were not significantly different between the diets (p > 0.05). However, the levels fell below the desirable range (< 40 mg/dL), indicating a potential risk of heart disease (Mohamed *et al.*, 2021). This could be attributed to factors such as the birds' lack of physical activity, high levels of dietary fat, and stress due to physical handling during weight parameter recording. High-Density Lipoprotein plays a crucial role in maintaining the balance of cholesterol, preventing its accumulation in cells. This equilibrium is regulated by the shedding of sterol from the membrane at a rate matching the number of cholesterol synthesis entering the liver (Sekhar *et al.*, 2020). The HDL is often referred to as "good cholesterol," and its primary function is to transport excess cholesterol, which is not being used, from peripheral tissues to the liver. The excess cholesterol serves as a precursor in the formation of bile salts and steroid hormones, with the remaining portion being excreted. The inclusion of antioxidants in the diet can contribute to the improvement of HDL levels.

Serological liver enzymes and protein indices

The serological enzymes and protein indices evaluated the liver function. Table 4 outlined the effects of BSF larvae meal inclusion on serological liver enzymes and protein profiles. The AST levels ranged from 128.47 U/L (T3, 15% BSFL) to 182.67 U/L (T1, 0% BSFL). The ALT levels ranged from 158.48 U/L (T1, 0% BSFL) to 208.36 U/L (T3, 15% BSFL). Total protein levels varied from 6.37 g/dL (T1, 0% BSFL) to 7.87 g/dL (T5, 30% BSFL). Albumin levels ranged from 3.53 g/dL (T2, 7.5% BSFL) to 4.83 g/dL (T5, 30% BSFL).

The liver function biomarkers evaluated in the present study included AST, ALT, GGT, and ALP, and did not show significant changes across dietary treatments (P > 0.05). The AST to ALT ratio was below 1 in all broiler chicken fed BSF larvae-based diets, indicating a potential positive influence on liver function. Numerical increases in total protein and albumin were observed with increasing BSF larvae meal inclusion levels but did not pose significant difference.

Liver function is assessed by measuring related biomarker enzymes, which are proteins in cells that act as catalysts for various bodily processes. Key enzymes involved in evaluating liver function include AST, ALT, ALP, and GGT, along with other indicators like total protein, albumin, and direct bilirubin. The normal ranges for the concentrations of these liver enzymes are typically defined as follows: AST: 70-220 U/L, ALP: 568-8831 U/L, ALT: 55.46 U/L (Wu *et al.*, 2021; Kamran *et al.*, 2020; Aikpitanyi *et al.*, 2020).

An increase in the concentration of these enzymes may indicate damaged or diseased cells, reflecting the status of liver function. The impact of BSF larvae meal inclusion on the liver function profile of broiler birds was examined in

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Table 4. While the results were similar across the trial diets (p>0.05), there was a noticeable numerical increase in GGT and albumin as the BSF larvae meal inclusion level increased. The ranges for AST, ALT, GGT, and ALP in this study were 128.47-182.67, 158.48-208.36, 399.46-676.47, and 116.43-174.64 U/L, respectively. The AST values fell within the normal range of 70-220 U/L but were below the 379.57 U/L reported by Dabbou *et al.,* 2018, at an inclusion level of 15% defatted BSF larvae meal in broiler finisher feed. The GGT and ALT values were lower than 539.7-587 U/L and 330.7 respectively, as reported by Khairiyah et al., 2022. The difference could be due to age, sex and strain (Arzour-Lakehal & Boudebza, 2021). Dabbou *et al* (2018) evaluated serum from male broilers of Ross 308 aged 35 days and Khairiyah evaluated male cobb 500 strain at 42 days while the current study involved mixed sex cobb 500 at 49 days of age.

Total protein ranged between 6.37-7.87 g/dl, albumin between 3.53-4.23 g/dl, total bilirubin between 0.633-0.80, Alb:Glo between 1.08-1.67 g/dl, and globulin between 2.6 - 3.43 g/dl. The total protein and albumin values were higher than the normal range of 3.0-4.9 g/dl and 1.17-2.74 g/dl, respectively. These values also increased numerically but not significantly (P>0.05) as the BSF larvae meal level increased, while the total bilirubin decreased inversely but the values were similar across the trial diets. Total protein and albumin are two criteria useful to evaluate body condition of poultry (Piotrowska *et al.,* 2011).

Total protein (TP) comprises albumin and globulin, with globulin calculated as the difference between TP and ALB. The normal ranges for TP and ALB in bird's blood are typically 3.0-4.9 g/dl and 1.17-2.74 g/dl, respectively (Edeh *et al.,* 2023). In this study, all birds, regardless of their diet, had values above the normal range, ranging between 6.37-7.87 for total protein and 3.53-4.83 for albumin. Serum proteins are primarily synthesized in the liver and serve various essential functions in the body. These functions include maintaining blood volume through the colloidal osmotic effect, buffering blood pH, transporting hormones and drugs, participating in cell coagulation, catalyzing chemical reactions as enzymes, regulating metabolism through hormones, and contributing to the body's defense against foreign agents. In broilers, the typical range for serum total protein values is from 2.50 to 4.50 g/dl (Sproston & Ashworth, 2018). Plasma proteins play a key role in maintenance of body homeostasis. Elevated blood protein levels may indicate dehydration, excessive protein intake, or conditions such as myeloma cancer and viral infections. In this case, the high blood protein could be a result of excess protein intake to support the rapid body growth rate of broilers.

Albumin serves as the most favorable source of amino acids for protein synthesis. Albumin also plays a crucial role in regulating oncotic pressure by preventing blood leakage from vessels Additionally, it acts as a plasma carrier by non-specifically binding to several hydrophobic steroid hormones and serves as a transport protein for hemin and fatty acids (Zou *et al.,* 2020; Edeh *et al.,* 2023). Albumin plays a crucial role in maintaining the osmotic pressure necessary for the proper distribution of body fluids between intravascular compartments and body tissues. Low albumin levels may indicate malnutrition.

Globulin is calculated as the difference between TP and ALB. Certain globulins bind with hemoglobin, while others transport metals, such as iron, in the blood and contribute to the body's immune response by aiding in the fight against infection. The globulin level observed in this study was higher than the values reported by Mahrous *et al.* (2023), which were 1.43 for male and 1.4 for female broilers in the grower stage. High globulin concentrations and low albumin:globulin ratios indicate a better disease resistance and immune response in chicken. Kudair & Al-Hussary (2010) demonstrated an increase in globulin levels in broilers following vaccination against infectious bronchitis, Newcastle disease, and infectious bursal disease, with values ranging between 1.00-1.90 g/dl in non-vaccinated birds and 1.63-1.92 g/dl in vaccinated ones. In the current study, the elevated level of globulins may be influenced by the high total protein levels, exceeding the reference values, and further increased due to vaccination against Newcastle disease (NCD), infectious bronchitis (IB), and infectious bursal disease (IBD).

The Albumin: Globulin (A: G) ratio ranged between 1.08-1.67, considered normal compared to the reference range of 1 to 2. A reduced ratio may suggest hypoproteinemia, indicating acute or chronic inflammatory processes due to the elevation of globulins. A ratio less than 1 can also indicate malnutrition, kidney or liver disease, among other factors. In this study, the A:G ratio indicates a healthy flock.

Serological metabolites and electrolytes

The serological metabolites and electrolytes are biomarkers used to assess the kidney function. The functions of the kidney include excretion of waste products resulting from protein metabolism and muscle contraction. These biomarkers include creatinine, sodium, chloride, potassium, sodium: potassium ratio and urea and the results for the current study are presented in Table 4 and did not significantly differ across dietary treatments ($p > 0.05$). Overall, the results indicate that BSFL meal inclusion did not adversely affect kidney function or disturb electrolyte balance. (T5, 30% BSFL) to 5.2 mmols/L (T3, 15% BSFL). Sodium levels ranged from 139.67 mmols/L (T1, 0% BSFL) to 145.67 mmols/L (T3, 15% BSFL). Potassium levels ranged from 3.57 mmols/L (T2, 7.5% BSFL) to 3.9 mmols/L (T5, 30% BSFL). Chloride levels ranged from 98.33 mmols/L (T5, 30% BSFL) to 104.87 mmols/L (T1, 0% BSFL). There were no significant differences ($P > 0.05$) for the metabolites and electrolytes thus implying that BSF larva meal has similar effects with soy bean meal on the kidney functions of the broiler chicken. The ratio of sodium to potassium ranged between 36.15 - 40.52. The and although it was slightly elevated, it remained within the range suggesting minimal stress on the birds.

Creatinine is excreted by the kidney as a by-product of creatine-phosphate metabolism which is produced as a result of energy production by the skeletal muscles (Odunitan-Wayas *et al.,* 2018). Elevated creatinine level than normal is

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an indication of infection, injured kidneys or poor blood flow to the kidneys. On the other hand, low levels is an indication of malnutrition, reduced muscles or liver disease.

The creatinine values observed in the present study fell within the normal range, as reported by Akinsanmi (2020). These results emphasize the notion that the dietary treatments were of good protein quality, as both serum urea and creatinine are indicators of the protein quality in a ration.

In this study, the serum potassium and sodium levels were within the normal range of 1.7-4.2 and 139-155 mmols/L, respectively, according to Akinsanmi (2020) and Aikpitanyi & Egweh (2020). However, the serum chloride values were slightly below the reference range of 108-124 mmols/L. The lower chloride values could be attributed to a low dietary supply of chloride, leading to mild hypochloremia across all the diets. There was no significant difference ($p>0.05$) or numerical trend observed as the BSF larvae meal inclusion level increased in the diets.

CONCLUSIONS

Overall, the results of this study indicated that BSF larvae meal can be incorporated in broiler chicken diet at 30% and as the sole source of protein without adverse effect on their physiological/health performance. The liver function biomarkers did not show significant changes, implying that BSF larvae meal inclusion did not induce adverse effects on this vital organ. However, the lipid profile showed significant alterations by the increase in total cholesterol, triglycerides, and LDL levels. However, these parameters though significantly different did not indicate potential implications for meat quality and cardiovascular health for the meat consumer because the values were within the reference ranges.

The increase in the abdominal fat deposition is an unfavorable trait for producers and consumers because it is considered as wasted dietary energy and a waste product with low economic value, which also reduces the carcass yield and lower consumer acceptance for meat. However, this can be resolved by inclusion of substances which exhibit antilipidaemic and anticholesterolaemic properties in the diets containing BSF larvae meal while incorporation of antioxidants can contribute to the improvement of HDL levels. This new knowledge gained from the findings of this research is applicable to the broiler feed industry, and it has the potential to aid Kenyan and global policymakers in their decision making to approve the use of BSF meals in broiler chicken feed formulation and the level of inclusion while guarding the welfare of the meat consumers.

RECOMMENDATION

Although this study indicates safe use of full fat BSF larvae meal in broiler as a sole source of protein (30%), growth performance parameters should be put into consideration while deciding the level of inclusion for overall advantage of the broiler producer. Further research should be conducted using BSF larvae meal of different fat content to assess the effect on broiler health response and control of fat deposition using antilipidaemic and anticholesterolaemic agent/substance. It is also important to conduct feeding trials to evaluate the interactive effect of fat, CP and BSF larvae meal inclusion level on the lipid parameters of broiler chicken

ETHICAL CONSIDERATION

Procedures involving the handling of animals during the experiment were approved by the Egerton University Institutional Scientific and Ethics Review Committee, approval number ***EUISERC/APP/528/2025***.

ACKNOWLEDGMENT

This research was supported by the Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM).

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