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# Haematology and serum biochemical indices of broiler chickens fed full-fat black soldier fly (*Hermetia illucens*) larvae meal-based diets as a fishmeal substitute

[1]Afam-Ibezim, E.M., [2]Ukonu, A.B., [1]Akinmtimi, A.H., [1]Onunkwo, D.N., [1]Anyaegbu, B.C. and [3]Akinsola, K.L.

1. Department of Animal Nutrition and Forage Science, Michael Okpara University of Agriculture, Umudike, Abia State.
2. Department of Animal Production and Livestock Management, Michael Okpara University of Agriculture, Umudike, Abia State.
3. Department of Animal Production and Health, Federal University Oye-Ekiti.

***Corresponding author: eberechiafamibezim@gmail.com, Phone number: 08063939797**

**Target audience: Animal Nutritionists, Producers, Farmers, Students.**

## Abstract

*This study investigated the effect of the replacement of fishmeal with full-fat black soldier fly meal (FFBSFLM) as an animal source protein in broiler chicken diets using haematology and serum biochemical indices as response criteria. A total of 150 Day-old chicks were assigned to five dietary treatments, of three replicates and ten chicks per replicate in a Completely Randomized Design. The FFBSFLM was added at 0, 25, 50, 75 and 100% and designated diets 1, 2, 3, 4 and 5 respectively. The feeding trial lasted for 56 days. At the end of the feeding trial, 3 birds per treatment were selected and slaughtered for blood collection for the haematology and biochemical studies. All data were subjected to analysis of variance. Results obtained for antinutrients revealed that, FFBSFLM is higher in haemaglutinin and trypsin inhibitor compared to tannin and phytate. Results showed that among the parameters measured for the haematological responses, only haemoglobin, lymphocytes and MCH were significantly ($P<0.05$) influenced by the dietary treatments, while for biochemical indices all parameters were significantly ($P<0.05$) affected except for globulin and low-density lipoprotein. Birds fed diet 4 recorded the highest PCV (32.67%), haemoglobin concentration (5.20g/dl) and highest red blood cell (19.33 x $10^6$/ µl), highest aspartate aminotransferase (205.30 U/I) was recorded in birds fed diet 4, while lowest aspartate aminotransferase (186.80 U/I) was recorded in birds fed diet 1. Due to high level of haemagglutinin in the larvae meal, further processing of the larvae meal is recommended before use to enhance the overall well-being of the broilers.*

**Keywords:** Broiler Chicken; Full-fat Black Soldier Fly Larvae Meal; Fish Meal; Haematology and Serum Biochemistry

## Description of Problem

Poultry production especially that of the broiler chickens remain one of the veritable ways of achieving sustainable and rapid production of high-quality protein to meet the increasing demands of the Nigerian teeming population (1, 2, 3) due to short generation interval of broiler chickens (4). Since feed is a major factor in poultry production, reducing feed cost is a priority for every poultry farmer and animal nutritionist. Dietary protein, a fundamental nutritional element, is commonly sourced from animal-derived products. There is a growing scarcity and

expense of conventional animal protein sources like fishmeal, due to its competition for usage between human and animal and also due to the fact that it is usually being imported into Nigeria by feed millers. This presents a significant challenge, prompting numerous investigations into sustainable protein alternatives that have lesser impact on human consumption and readily available. Insects as a protein source have emerged as a viable substitute, an option recognized by various researchers (5). The black soldier fly larvae (BSFL) is a non-pest with a more extended larvae period that allows for higher bio-accumulation through a wide variety of organic degradation, because of the economic feasibility of manufacturing, BSFL's organic bio-conversion can be positively incorporated to the livestock feed industry (6). The utilization of unconventional feed components like insects, microalgae, and surplus food materials in animal production holds the potential to enhance both efficiency and sustainability in farming practices (7, 8). Series of researches had investigated the effects of various feedstuffs on the haematology and serum biochemistry of livestock and concluded that feedstuffs including alternative sources affect the physiology of animal. The blood contains several metabolites which provide useful information on nutritional status of an individual. Thus, the use of blood parameters for nutritional assessments has been recommended (9). The objective of this study was to determine the effect of black soldier fly larvae meal as an alternative protein source to fishmeal in broiler chicken diets using blood chemistry as measures of assessment.

**Materials and Methods**

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State in the southern part of Nigeria which lies between latitude $3^0 33^0$ and $4^0 30^0$N longitude $7^0 10^0$ and $8^0 00^0$E. The area falls within the tropical rain forest zone, with an annual average rainfall of 2177mm, temperature range between $20^0$-$30^0$C, with relative humidity of 50-59% depending on season (10).

**Source and processing of Full-fat Black Soldier Fly Larvae (BSFL) Meal**

The Black soldier fly larvae were reared and supplied by a commercial company (The Fly Colony, Ilishan Remo, Ogun State) in 2 batches. The 2 batches of BSFL used for these experiments were produced under the same conditions: fed the same poultry manure as a substrate.

Larvae were harvested after 14 days (enough time for larvae to have reached mature stage). Harvested larvae were dried in an oven at $90^O$C (11) for about 12 hours and milled afterwards using JTC OmniBlend V Heavy duty professional blender TM-800 Model.

**Experimental Diets**

Five (5) experimental diets were formulated such that the control diet (Diet 1) did not contain full-fat black soldier fly larvae meal, while BSFL replaced fishmeal at levels of 25, 50, 75 and 100% in diets 2, 3, 4 and 5, respectively. The composition of the diets and calculated analyses are as shown in Table 1.

**Table 1: Percentage composition of Full-Fat Black Soldier Fly meal based Broiler Chickens Straight Diets**

| Ingredients (kg) | Diet 1 | DIET 2 | DIET 3 | DIET 4 | DIET 5 |
|---|---|---|---|---|---|
| Maize | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| Soybean meal | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| Groundnut cake | 14.0 | 14.0 | 14.0 | 14.0 | 14.0 |
| Palm Kernel Cake | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Wheat offal | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 |
| Fish meal | 2.0 | 1.5 | 1.0 | 0.5 | - |
| Full fat black soldier fly meal | _ | 0.5 | 1.0 | 1.5 | 2.0 |
| Bone meal | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Lysine | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Methionine | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Vitamin Mineral Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total (kg)** | **100** | **100** | **100** | **100** | **100** |
| **Calculated composition** | | | | | |
| Crude protein (%) | 22.05 | 21.96 | 21.86 | 21.77 | 21.67 |
| Energy (Kcal/kg ME) | 2882.40 | 2894.60 | 2906.80 | 2919.00 | 2931.20 |
| Crude fibre (%) | 3.90 | 3.93 | 3.96 | 3.99 | 4.02 |
| Ether extract (%) | 4.25 | 4.36 | 4.47 | 4.58 | 4.70 |
| Lysine | 1.18 | 1.17 | 1.16 | 1.15 | 1.14 |
| Methionine | 0.45 | 0.44 | 0.44 | 0.44 | 0.44 |

**Table 2: Proximate composition and Gross Energy values of Full-fat BSFLM**

| Parameters % | FFBSFLM |
|---|---|
| Dry matter | 96.29 ± 0.03 |
| Crude protein | 46.03 ± 0.08 |
| Ether extract | 29.91 ± 0.02 |
| Crude fibre | 6.80 ± 0.03 |
| Ash | 3.63 ± 0.01 |
| Nitrogen free extract | 9.93 ± 0.03 |
| Gross energy (kcal/g) | 5.89 ± 0.00 |

± Standard deviation

**Experimental Birds and Their Management**

A total of one hundred and fifty (150) day old Ross 308 broiler chicks were purchased from a reputable hatchery or distributor in the study area. The chicks were brooded together with a 60W bulb in a brooding pen for the first 7 days, thereafter, they were randomly divided into 5 treatments of 30 birds each. Each treatment was further replicated into 3 of 10 birds per replicate. The animal feeding trial lasted for 56 days. The birds were

managed in deep litter pens during the brooding and rearing phases. Wood shavings served as the litter material. Experimental feeds and clean water were given *ad-libitum*, kerosene stove and lantern served as heat source. Birds were vaccinated against Gumboro disease at $7^{th}$ and $18^{th}$ day of life while Newcastle disease vaccine was administered at $12^{th}$ day. Coccidiostats were also be administered to the birds during the experiment.

**Blood Chemistry Analysis**

Blood samples from three (3) birds randomly selected from each treatment at the $8^{th}$ week were used for the blood chemistry analysis. The blood collection was carried out using sterile needle to prick the jugular veins and drawing into syringes. Blood (3ml) samples were put in sample bottles treated with Ethylene di-amine tetra acetic acid (EDTA) to prevent coagulation, while those for serum biochemical determination were collected in non-heparinized capillary bottles. Serum was separated from the blood by centrifugation of blood at 4000rpm for 15 minutes and thereafter stored at $-20^0$C, and used later for blood chemistry analysis. Red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular concentration were determined as described by (12). The blood chemistry analysis was conducted according to the procedure of (13). The serum biochemical parameters determined are Total protein, Albumin, Globulin, Urea, Creatinine, Alanine Amino Transferase (ALT), Aspartate Amino transferase (AST) and Alkaline Phosphatase (ALP), (14).

**Determination of Anti-Nutrients**
**Haemagglutinin determination**

The haemagglutinin contents in the test ingredients were determined according to the method of (15)

**Procedure**

The method of (15) was followed in the determination of haemagglutinin content of the test ingredients. 0. 20g of defatted sample was weighed into a screw cap centrifuge tube. 10ml of 0.1M phosphate and 10ml of 0.85% Nacl was added. The mixture was shaken at room temperature for shir on a UDY shaker. The suspension after 18hrs was centrifuged at 1,500 rpm for 15minutes. The supernatant obtained was transferred into a separate clean screw cap centrifuge tube by decantation. The haemagglutinin inhibition activity was tested by preparing a set of haemagglutinin standard solutions by serial dilution % range 0 to 1.0ml of the stock haemagglutinin. Extract of sample were pipetted into a triplicate set of test tubes with each set for each level of haemagglutinin. Each sample and standard were treated with 0.9% strain of sample as well as standard solutions and read on a spectronic 21D spectrophotometer. The Haemagglutinin activity is calculated using the formula:
HU/mg= 98.56 (Absorbance of sample-absorb blank).

**Tannin determination**

The tannin in the test ingredients were determined according to the method of (16)

**Procedure**

The method of (16) were followed in the determination of tannin content of the test ingredients. 0.20g of sample was measured into a 50ml beaker 20ml of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80$^o$C for 1 hour.

It was shaken thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100ml volumetric flask. Water (20ml) was added, 2.5ml folin-Denis reagent and 10ml of 17% na2Co3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20min. The bluish – green color was observed to develop at the end of range 0-10ppm.
The absorbance of the Tannic acid standard solutions as well as samples were read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760nm.

Percentage Tannin was calculated using the formula;

$$\% \text{ Tannin} = \frac{\text{absorbance of sample X average gradient factor x Dilution factor}}{\text{Wt. of Sample x 10,000}}$$

**Phytate determination**
The phytate in the test ingredients were determined according to the method of (17)

**Procedure:** Two grams (2g) of each sample was weighed into 250ml conical flask. About 100mls of 2% Hydrochloric Acid was added to soak each sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter paper. 50ml of each filterate was placed in 0.50ml conical flask and 107mls distilled water was added in each case to give proper acidity. 10mls of 0.3% Ammonium Thiocyanate ($NH_4SCM$) solution was added into each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.00195g Iron per ml. The end point was slightly brownish-yellow which persisted for 5 minutes.

The % phytic acid was calculated using the formula:

$$\% \text{ Phytic Acid} = \frac{\text{Titre value x 0.00195 x 1.19 x 100 x 3.55}}{\text{Wt. of Sample}}$$

**Determination of trypsin inhibitor (TIA)**

**The caesin digestion method**
The trypsin inhibitor in the test ingredients was determined according to the method of (18).

**Procedure:** The method of Kakade *et al.* (1974) was followed in the determination of trypsin inhibitor of the test ingredients. 0.2g of defatted ground sample was weighed into a centrifuge tube. 10ml of 0.1M Phosphate buffer added and shook on a shaker at room temperature for 1hr. The suspension was centrifuged at 5000rpm in a centrifuge for 5min. The content was later filtered through a Whatman no 42 filter paper into a 250ml conical flask. 0.2, 0.4, 0.6, 0.8 and 1.0ml of filtrate were pipetted into a set of triplicate test-tubes (one set for each level of extract). The final volume is adjusted to 2ml by the addition of 0.1M phosphate buffer. These test-tubes were arranged into a water bath maintained at $37^0$C. A blank was prepared by adding 6ml of 5% TCA solution to one set of triplicate tubes. 2ml of 2% casein solution was added to all the tubes which was previously kept at $37^0$C to incubate for 20mins. The reaction of casein was stopped by the addition of 6ml of 5% TCA solution and this was allowed to proceed for 1hr at a room temperature. The mixture was later filtered at room temperature through a Whatman No 42 filter paper into 100ml conical flask. 0.2, 0.4, 0.6, 0.8 and 1.0ml of stock trypsin solution were also pipetted into a triplicate set of test-tubes (one set for each level of trypsin) as above and treated similarly as sample to the point of filteration.

The absorbance of the filtrates of both samples and standard trypsin solution were read on a spectrophotometer at a wavelength

of 280nm. The actual absorbance of the sample is the difference between absorbance of stock trypsin filtrate and that of sample filtrate. The absorbance of blank was also read. One trypsin inhibitor unit (TIU) is arbitrarily defined as an increase of 0.01 absorbance units at 280nm in 20mins per 10ml of the reaction mixture under the conditions mentioned herein.

Trypsin Inhibitor Unit for each sample was calculated using the formula:

$$\frac{\text{Change in absorbance of sample extract}}{0.01 \times \text{mg protein in sample}}$$

**Data analysis**

The data collected were subjected to analysis of variance (ANOVA) according to (19). The means were separated using the Duncan's New Multiple Range Test as described by (20).

**Results and Discussion**

**Antinutritional Factors of Full Fat Black Soldier Fly (*Hermetia illucens*) larvae meal**

The anti-nutritional factors contained in full fat BSFLM are as shown in Table 3. The table below reveals that, the larvae meal is higher in haemaglutinin and trypsin inhibitor compared to tannin and phytate.

**Table 3: Anti-nutritional factors of FFBSFLM**

| Parameters % | FFBSFLM |
|---|---|
| Haemagluttinin (HU/mg) | 39.74 ± 0.04 |
| Phytate | 0.09 ± 0.01 |
| Tannin | 0.12 ± 0.01 |
| Trypsin inhibitor (mg/g) | 11.68 ± 0.02 |

± Standard deviation

According to (7), the concentration of phytate in FFBSFLM typically ranges from 0.1-0.2%, which is higher than the value obtained in this study (0.09%). This implies that the use of BSFLM in this study will reduce the negative impact of phytate and reduce the cost of mitigating the negative effects of phytate which could be enzymatic treatment. According to (7), tannins in FFBSFLM are present in low concentrations, generally below 0.1% of dry matter, which is about the value obtained in this study (0.12%). Despite their relatively low levels, tannins can still negatively impact feed intake, palatability and nutrient absorption, especially in species sensitive to tannin's effect such as poultry and some fish species (21).

The binding of tannins to dietary proteins not only reduces protein digestibility, but also affects the activity of digestive enzymes such as trypsin and amylase, by forming tannin-enzyme complexes (21), processing techniques such as soaking, boiling and fermentation can reduce tannin levels and improve nutritional quality of BSFLM (22).

Values obtained for haemaglutinin in this study was 39.74HU/mg for FFBSFLM, normal range for haemagluttinin should be 7-15 HU/mg in broiler diets (23), according to (24), high levels of haemagglutinin in the diet leads to agglutination of red blood cells, inflammation of intestines, kidneys and thyroid, they can also impair nutrient absorption leading to infiltration of bacteria into the blood stream (25).

Values obtained for trypsin inhibitor in this study was 11.68mg/g for FFBSFLM which is above 5mg/g, the level above which protein digestibility and growth performance are affected according to (26). High levels of trypsin inhibitor in the diet leads to decreased protein utilization, increased pancreatic enzyme secretion, and in severe cases pancreatic hypertrophy and intestinal inflammation (27)

**Haematological indices of broiler chickens fed FFBSFLM based diets as a substitute to fishmeal**

The haematology of broiler birds fed full-fat black soldier fly larvae meal is presented in Table 4. There were significant ($P < 0.05$) differences for haemoglobin, mean corpuscular haemoglobin, and lymphocytes. There were no significant ($P > 0.05$) differences recorded for packed cell volume, white blood cell, red blood cell, mean cell volume, and MCHC. The PCV recorded in this study fell within the normal range (26 – 45.20%) as stipulated by (28).

Values obtained for haemoglobin showed that birds fed diet 4 (65%) had the highest mean value than other treatments with control Diet 1 recording the lowest value; the values obtained in this study is comparable with results obtained by (29) who reported a range of 3.93 – 4.18%. The haemoglobin recorded in this study fell below normal range (7-13g/dl) as stipulated by (28) these low values could be as a result of the high level of haemagglutinin recorded in the FFBSFLM (39.74HU/mg). Haemagglutinin causes agglutination or clumping of red blood cells, potentially leading to reduced nutrient absorption and reduced iron absorption (30).

Low haemoglobin levels in broilers, a condition known as anaemia, can significantly impact the health, growth and overall productivity leading to various issues including decreased weight gain, impaired immune function and increased susceptibility to diseases (31). The white blood cells in this study showed no treatment effect ($P > 0.05$). The primary function of WBC is to help the body of the chicken to fight infection and other diseases, this may suggest that there was no toxicity in the blood arising from the feed.

For lymphocytes, Diets 1, 2 and 3 were statistically similar while Diets 4 and 5 were significantly higher ($P < 0.05$) than Diets 2 and 3. Values obtained for lymphocytes in this study were higher than normal range (50-62%) as reported by (32). High lymphocyte counts are often indicative of an immune response potentially triggered by stress or infection, persistently high levels may suggest underlying health issues or the body's attempt to fight off pathogens (33).

Monocytes are immunoglobins that help the body fight infections, as observed in this study the monocyte values of the test diets were below normal range (1-4%) as postulated by (34) and (35). Low monocyte counts in broilers can indicate that the bird's immune system is compromised making them more susceptible to infections and diseases (36).

Mean Corpuscular Haemoglobin (MCH) is the average amount of haemoglobin per red blood cell in a sample of blood, a normal range for MCH according to (28) is 33-47pg. This study reveals that all the treatments were below normal range which could be a sign of hypochromic anaemia (when the red blood cells are paler than normal), these low MCH values recorded in this study could be as a result of high amount of haemagglutinin in FFBSFLM. In conclusion, values in this study for all parameters indicated hypochromic anaemia which could have been as a result of the effect of the high level of haemagglutinin in FFBSFLM.

**Table 4: Haematological indices of broiler chickens fed full-fat BSFLM**

| Parameters | Diet 1 (Control) (0%) | Diet 2 (25%) | Diet 3 (50%) | Diet 4 (75%) | Diet 5 (100%) | Normal range | SEM |
|---|---|---|---|---|---|---|---|
| Packed cell volume (%) | 31.00 | 28.67 | 26.67 | 32.67 | 32.33 | 25.0-45.0 | 1.56 |
| Haemoglobin (g/dl) | $4.27^{c}$ | $4.40^{bc}$ | $4.87^{abc}$ | $5.20^{a}$ | $4.93^{ab}$ | 7.0-13.0 | 0.46 |
| Red blood cell (x $10^6$/ µl) | 19.07 | 13.50 | 15.73 | 19.33 | 15.00 | 2.0-4.0 | 3.57 |
| White blood cell (x $10^6$/ µl) | 65.25 | 64.95 | 66.67 | 58.03 | 65.45 | 9-31.0 | 6.65 |
| Lymphocytes (%) | $73.67^{ab}$ | $63.67^{b}$ | $61.33^{b}$ | $89.33^{a}$ | $89.00^{a}$ | 45-80.0 | 15.18 |
| Monocytes (%) | 1.00 | 0.33 | 0.00 | 0.00 | 0.33 | 0-5.0 | 0.62 |
| MCV (µ) | 16.62 | 21.22 | 21.71 | 18.52 | 21.98 | 90-140 | 5.03 |
| MCH (pg) | $2.28^{b}$ | $3.26^{ab}$ | $3.69^{a}$ | $2.75^{ab}$ | $3.43^{ab}$ | 33-47 | 0.77 |
| MCHC (%) | 13.75 | 15.42 | 18.89 | 16.32 | 15.42 | 26-35 | 2.90 |

[a-b] Means treatment in a row with different superscripts are significantly different ($P$ <0.0.5).
SEM = Standard error of mean
MCV= Mean corpuscular volume
MCH= Mean corpuscular haemoglobin
MCHC= Mean corpuscular haemoglobin concentration
Normal range adopted from 28, 43 and 45

**Serum Biochemical indices of broiler chickens to FFBSFLM based diets as a substitute to fishmeal**

Table 5 shows the serum biochemical indices of broiler chickens fed full-fat black soldier fly larvae meal-based diets. The results indicated that there were significant (P <0.05) differences in values for total protein, albumin, urea, creatinine, Alanine transaminase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), triglycerides, globulin and HDL.

Total protein in diets 3, 4 and 5 differed significantly (P < 0.05) from Diets 1 and 2, with diet 1 (Control) having the lowest value. Serum protein has been reported as a pointer to strong amino acid metabolism (37). Normal range for total protein according to (28) is 2.5-5.5g/dl, values for total protein recorded in this study was within normal range. This study therefore suggests an effective protein metabolism and utilization. The observations in total protein is in agreement with the observation of (8) who noted increased in protein levels with BSF supplementation, an indicative of enhanced liver function or improved nutritional status.

The values for albumin indicate that diets 3 and 4 differed significantly (P < 0.05) from diets 1 and 2 but are statistically similar to diet 5. The fact is that these values were within normal range as reported by (38). The increased values supports the findings of (39) who observed that increased levels of BSFL significantly boosted key immune markers and that of (40) who stated that increase in circulating immunoglobulins demonstrate stronger resistance to disease and better immune function in hens fed diet containing larvae. The observations in albumin is complemented by the observations of (8) who noted increased in albumin levels with BSF supplementation, an indicative of enhanced liver function or improved nutritional status. The non-significant treatment effect of serum globulin in this study was a sign of good health, intact immune state and normal physiological function of the internal organs.

(41) reported increased globulin to be implicated in liver damage, chronic infections and kidney dysfunction. Values for urea showed that diets 4 and 5 had the highest values and differed significantly ($P < 0.05$) from diets 1, 2 and 3. This showed that increasing full-fat BSFLM in the diets beyond 50% could lead to an increase in the urea level in the blood. Urea is an indicator of protein quality, therefore this high urea level could be traced to high chitin in the larvae, which binds protein, reduces its digestibility and hence its quality (42). However, the urea obtained in this study is within normal range as stipulated by (43); 6-30mg/dl. Values obtained in this study for creatinine showed that there was significant ($p < 0.05$) difference across the treatment groups with Diet 2 recording the lowest value and Diet 4 recording the highest value. However, the range of creatinine level in this study was within normal range (0.5-2mg/dl) as stipulated by (44).

**Table 5: Serum biochemical indices of broiler birds fed full-fat BSFLM**

| Parameters | Diet 1 (Control) (0%) | Diet 2 (25%) | Diet 3 (50%) | Diet 4 (75%) | Diet 5 (100%) | Normal Range | SEM |
|---|---|---|---|---|---|---|---|
| Total protein (g/dl) | 4.94$^{b}$ | 4.98$^{b}$ | 5.21$^{a}$ | 5.42$^{a}$ | 5.30$^{a}$ | 6-8 | 0.06 |
| Albumin (g/dl) | 3.20$^{c}$ | 3.22$^{bc}$ | 3.42$^{a}$ | 3.56$^{a}$ | 3.40$^{ab}$ | 1.8-3.3 | 0.04 |
| Globulin (g/dl) | 1.74 | 1.76 | 1.79 | 1.86 | 1.90 | 3.9-6 | 0.05 |
| Creatinine (mg/dl) | 0.33$^{ab}$ | 0.28$^{b}$ | 0.31$^{ab}$ | 0.37$^{a}$ | 0.35$^{ab}$ | 18-79 | 0.01 |
| Urea (mg/dl) | 20.50$^{b}$ | 15.10$^{c}$ | 19.20$^{b}$ | 22.60$^{a}$ | 24.30$^{a}$ | 6-30 | 0.87 |
| ALT (U/I) | 47.80$^{d}$ | 50.70$^{c}$ | 53.30$^{b}$ | 52.80$^{b}$ | 55.70$^{a}$ | 9-43 | 0.74 |
| AST (U/I) | 186.80$^{e}$ | 191.80$^{d}$ | 198.50$^{b}$ | 205.30$^{a}$ | 195.80$^{c}$ | 17-24 | 1.68 |
| ALP (U/I) | 95.70$^{c}$ | 89.70$^{d}$ | 98.50$^{ab}$ | 99.50$^{a}$ | 97.30$^{bc}$ | 92-294 | 0.96 |
| Glucose (mg/dl) | 195.00$^{d}$ | 200.00$^{d}$ | 211.00$^{c}$ | 222.00$^{b}$ | 230.00$^{a}$ | 180-250 | 3.55 |
| Total cholesterol (mg/dl) | 98.30$^{e}$ | 103.60$^{d}$ | 117.30$^{c}$ | 128.90$^{b}$ | 135.50$^{a}$ | 87-192 | 3.62 |
| HDL (mg/dl) | 68.60$^{e}$ | 73.50$^{d}$ | 87.30$^{c}$ | 98.90$^{b}$ | 103.80$^{a}$ | 35-70 | 3.68 |
| Triglycerides (mg/dl) | 39.80$^{b}$ | 38.50$^{b}$ | 38.70$^{b}$ | 44.40$^{a}$ | 45.90$^{a}$ | 20-120 | 0.84 |
| LDL (mg/dl) | 21.73 | 22.40 | 22.27 | 21.10 | 19.50 | 20-56 | 0.55 |

$^{a-b}$ Means treatment in a row with different superscripts are significantly different ($P < 0.0.5$).
SEM = Standard error of mean
ALT= Alanine transaminase
AST= Aspartate aminotransferase
ALP= Alkaline phosphatase
HDL= High density lipoprotein
LDL= Low density lipoprotein
Normal range adopted from 28, 43 and 45

Alkaline phosphatase, aspartate aminotransferase and alanine transaminase are liver enzymes used to assess the homeostatic response of the liver to metabolites (toxins) that pass through the portal blood. Normal range for ALT is 9-43U/I (43), for AST is 92-294U/I (45) and for ALP is 92-294U/I (46). Values obtained for AST in this study (186-205 U/I), were similar to those (0.5-2mg/dl) reported by (43); 0.5-2mg/dl. Values obtained for AST and ALP were within normal range, which were observed to increase as the level of FFBSFLM increased in the diets. This

observation is in agreement with (47) who reported increase in ALP with higher BSF levels. Elevation of serum AST and ALT can occur as a result of exposure to toxins leading to toxaemia, inflammation and alteration in permeability of hepatocellular membrane (48). These elevated values for AST and ALT which are indicators of liver damage could be as a result of high level of anti-nutrients like chitin in FFBSFLM (49).

Lipid profile in this study showed that Total Cholesterol, HDL and Triglycerides increased in the blood as FFBSFLM increased in the diet; this is in agreement with (50) and (51) who observed changes in lipid profiles, with different levels of BSF supplementation affecting HDL cholesterol and Total cholesterol. The normal physiological ranges of the following parameters for broiler chickens are; glucose; 180-250mg/dl (52); cholesterol; 125-200mg/dl (53); HDL; 26-65mg/dl (54); triglycerides; < 150mg/dl (55) and LDL; < 130mg/dl (56). All parameters measured in this study were within normal range except for HDL values which were higher than normal range. HDL (High Density Lipoprotein) is a type of cholesterol that is often referred to as 'good cholesterol'. It plays a role in transporting cholesterol away from the body tissues back to the liver for processing, which can help prevent buildup of cholesterol in blood vessels (54). These alterations could stem from the unique fatty acid composition in BSFL products, directly influencing lipid metabolism. In this study we observed a favourable indicator in lipid profile like increase in HDL which is a good cholesterol that help prevent artherosclerosis, however values for liver enzymes showed possibility of liver damage which may have been as a result of the presence of antinutrients like haemagglutinin and trypsin inhibitor in BSFLM.

## Conclusion

It could be concluded from the result that full-fat black soldier fly larvae meal is a potential replacement of fishmeal in broiler diets. However, high levels of antinutrients like haemagglutinin and trypsin inhibitor in the larvae meal could lead to anaemia, which can significantly impact the health, growth and overall productivity leading to various issues including decreased weight gain, impaired immune function and increased susceptibility to diseases, hence further processing of the larvae meal is recommended before use to reduce these antinutrients and improve the overall well-being of the broiler chickens.

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