

## Haematology and serum biochemical indices of broiler chickens fed black soldier fly larvae meal-based diets

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### Abstract

This study investigated the effect of the replacement of fish meal with black soldier fly larvae meal (BSFLM) 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 (5) dietary treatments, of three (3) replicates and ten (10) chicks per replicate in a Completely Randomized Design. The BSFLM was added at 0, 25, 50, 75 and 100% and designated diets I, II, III, IV and V, respectively. The feeding trial lasted for 42 days. At the end of the feeding trial, three (3) birds per replicate were selected and slaughtered for blood collection for the haematology and biochemical studies. Data were subjected to one-way analysis of variance. Results showed that for the haematology indices, only the packed cell volume, red blood cell, haemoglobin concentration, neutrophils, monocytes, and haematocrit and for the serum indices (aspartate aminotransferase) were significantly ( $P < 0.05$ ) influenced by the dietary treatments. Bird fed diet III had the highest packed cell volume (34.67%), highest red blood cell ( $3.3310^6/\mu\text{l}$ ) and highest haemoglobin concentration (11.53g/dl). Highest aspartate aminotransferase (4.88u/l) was recorded in bird fed diet IV, while lowest aspartate aminotransferase (1.42u/l) was recorded in bird fed diet I. Generally, there was no deleterious effects of the dietary treatments on the haematology and serum indices of the broiler chickens. Consequently, it was concluded that BSFLM could replace fish meal at 100% broiler chicken diet.

**Keywords:** Chicken, larvae meal, Fish meal, Blood profile

### 1.0 Introduction

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 fish meal, 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 a lesser impact on human consumption and readily available. Therefore, the need for alternative protein sources for livestock is becoming increasingly. Insects as a protein source have emerged as a viable substitute, an option recognized by various researchers (Awoniyi *et al.*, 2003). According to Makkar

*et al.* (2014), insects present a promising solution for addressing the worldwide need to discover affordable new protein sources. This is particularly true when considering their nutritional significance and their minimal space requirements for breeding. The utilization of unconventional feed components such as insects, microalgae, and surplus food materials in animal production holds the potential to enhance both efficiency and sustainability in farming practices (Madeira *et al.*, 2017; De Souza-Vilela *et al.*, 2019). Several research studies 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 on an individual. Thus, the use of blood parameters for nutritional assessments has been recommended (Adeyemo and Sani, 2013).

## 2.0 Materials and methods

### 2.1 Experimental sites

The experiment was carried out at the Poultry unit of the Teaching and Research Farm of The Federal University of Technology Akure, Nigeria. The University is located on (Latitude 7°8'N and Longitude

5°10'E) Akure, Nigeria (NIMET, 2014). The altitude is about 350.52m above sea level, the annual humidity is 75% and that of temperature is 27°C (Ashaolu and Adebayo, 2014).

### 2.2 Collection and processing of test ingredient

Black soldier fly larvae were harvested from an insect farm in Ilesha, Nigeria and was transported to the Federal University of Technology, Akure, Nigeria. The live larvae plus debris, were poured into buckets containing cold water and thoroughly washed before processing. During processing, the live larvae were poured into heated water of about 70 - 80°C and was stirred for 3–5 minutes in order to make the larvae inactive. Filtered inactive larvae were spread on nylon and solar-dried to minimum moisture content. Dried larvae were then milled to produce Black Soldier Fly Larvae Meal (BSFLM).

### 2.3 Experimental diets

Five experimental diets were formulated and the black soldier fly larvae meal was used as a substitute to fish meal at 0, 25, 50, 75 and 100% to constitute Diets I, II, III, IV and V, respectively. The diets were thoroughly mixed and used to feed the chicks from day 1 – day 21. The gross composition of the starter diet is presented in Tables 1. The

basal finisher diet formulated were fed to the birds from day 22 – day 42.

## 2.4 Experimental layout and feeding trial

A total number of one hundred and fifty (150) day-old broiler chicks of Cobb 500 strains was procured from a reputable Hatchery in Ibadan, Nigeria and were randomly allotted into five (5) dietary treatments of three (3) replicates per treatment and ten (10) chicks per replicate in a Completely Randomized Design (CRD). Their respective starter diets were fed *ad libitum* to the chicks from day 1 – day 21. Thereafter, the finisher diets were fed to the birds from day 22 – day 42. Throughout the experimental period, clean and fresh water was supplied to the chicks. Vaccination and drug administration program was collected from the hatchery and was strictly adhered to.

## 2.5 Slaughtering and blood sample collection

At the end of the experimental period, three (3) birds per replicate were randomly selected, stunned, slaughtered by severing the jugular vein, for blood collection for the haematological and serum indices studies. For haematology, blood samples were collected into sterilized bottles containing Ethylene Diamine Tetra-acetic Acid (EDTA). Blood samples for serum biochemical studies were collected into test tubes (without anticoagulant) and placed in test tube racks in the microscopy laboratory of

Animal production and health Department, FUTA. After some hours, the serum was obtained by centrifugation and serum samples were stored in a deep freezer prior to further study and blood samples for haematological study was collected into Ethylene-Diamine Tetra-Acetic Acid (EDTA) bottles via bleeding for haematological analysis. Another blood sample was collected into non - EDTA bottles and was allowed to coagulate for six (6) hours. The serum was separated into sterile universal bottles to further determine serum biochemical indices.

## 2.6 Data analysis

Data collected were subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 22 to determine treatment effects and where means with significant differences were observed. Duncan Multiple range Test (DUNCAN, 1995) was employed to separate the means at  $P < 0.05$  level of significance.

## 3.0 Results

### 3.1 Haematological studies

The haematology of broiler chickens fed black soldier fly larvae meal-based diets is presented in Table 2. Packed cell volume, red blood cell, haemoglobin, neutrophils, monocytes and haematocrit were significantly ( $P < 0.05$ ) influenced by the dietary treatments. The highest packed cell volume (34.67%), highest red blood cell

( $3.34 \times 10^6 \text{mm}^{-3}$ ) and highest haemoglobin concentration (11.53g/100ml) were recorded in bird fed 50% BSFLM, while the lowest packed cell volume (26.17%), red blood cell ( $2.66 \times 10^6 \text{mm}^{-3}$ ) and haemoglobin concentration (9.80g/100ml) were recorded in bird fed 0% BSFLM. Highest values of erythrocytes sedimentation rate (3.5mm/hr), mean cell haemoglobin concentration (45.47%) were observed in bird fed 0% BSFLM. Lymphocyte value varies from 57.18 - 59.75%, basophils; 3.23 - 3.50% and eosinophils; 1.27 - 1.50%.

### 3.2 Biochemical indices

Table 3 shows the serum biochemical indices of broilers fed black soldier fly larvae meal-based diets. Aspartate aminotransferase was significantly ( $P < 0.05$ ) influenced by the dietary treatments. The highest aspartate aminotransferase (4.88u/l) was recorded in bird fed 75% BSFLM, while lowest aspartate aminotransferase (1.42u/l) was recorded in bird fed 0% BSFLM. Highest total protein (2.99mg/dl) was observed in bird fed 0% BSFLM, while lowest total protein (2.49mg/dl) was observed in bird fed diet 50% BSFLM. Lowest albumin (0.69mg/dl), lowest alkaline phosphate (7.36u/l) and lowest cholesterol (2.69mg/dl) were recorded in bird fed diet 75% BSFLM. Globulin varies; 1.71 – 2.24mg/dl, alanine aminotransferase;

1.17 – 2.43u/l, creatinine; 54.63 – 62.38mg/dl and glucose; 46.29 – 102.74mg/dl.

### 4.0 Discussions

Blood is very important in the body of animals as it plays important roles in defending the animal body against diseases, maintain water balance in the body and prevents excess loss of blood during injuries via blood clotting and all these are made possible as a result of its constituents (Awojobi and Opiah, 2000). The packed cell volume values (26.17 -34.67%) obtained from this study fell within the range of 25.00 – 45.00% for chickens as reported by Ahamefule *et al.* (2006). Packed cell volume values below normal range are an indication of anaemia (Radostis *et al.*, 1994) and poor quality of protein of the diets (Awoniyi *et al.*, 2000). Thus, PCV values obtained in this study could suggest that the nutritional quality of black soldier fly larvae meal in the diets compared favourably with the fish meal that was used as sole animal protein in the control diet (Diet I). In this study, dietary treatments affected the number of red blood cell which might be due to the changes in diets, which altered the synthesis and release of these cells from bone marrow as reported by Abdel-Rahman *et al.*, (2013). Red blood cell indicates a higher nutrient supply in the body system of the chickens

(Alabi *et al.*, 2013). In addition, the monocyte levels for all treatments were within the reference range. The role of the monocyte is to fight foreign materials that enter the bloodstream. The value for total protein (2.49 – 2.99 mg/dl) obtained in this study was lower than the normal ranges (5.00 -7.00 mg/dl) as reported by Robert, *et al.*, (2003). Studies by Marono *et al.* (2017), indicated a positive effect for black soldier fly larvae meal in the blood profile of layer chickens; these findings are in line with the current study as it is also reported by Loponte *et al.* (2017). In contrast to observations in the present study, Dabbou *et al.* (2018) and Kinasih *et al.* (2018) suggested that feeding birds a black soldier fly larvae diet did not affect their blood profile. There is a direct relationship between protein intake and blood urea levels (Szabo *et al.*, 2005) observed in diets I and IV.

## Conclusion

From this study, substituting fish meal with black soldier fly larvae in broiler chicken diets did not affect the birds negatively neither did it pose any deleterious effect on the health status of the birds, therefore it could be concluded that feeding broiler chickens with black soldier fly larvae meal at 25 to 100% in replacement for fish meal could be adopted by broiler farmers.

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Table 1: Gross composition of the experimental diets (%)					
Ingredients	Diet I 0% BSFLM	Diet II 25% BSFLM	Diet III 50% BSFLM	Diet IV 75% BSFLM	Diet V 100% BSFLM
Maize	53.45	53.45	53.45	53.45	53.45
Soybean meal	16.00	16.00	16.00	16.00	16.00
Groundnut cake	20.00	20.00	20.00	20.00	20.00
Fish meal	5.00	3.75	2.50	1.25	0.00
Black Soldier Larvae Meal	0.00	1.25	2.50	3.75	5.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.10	0.10	0.10	0.10	0.10
Bone	1.00	1.00	1.00	1.00	1.00
Limestone	2.00	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25	0.25
Oil	1.50	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
Crude protein (%)	23.74	23.55	23.37	23.18	22.99
Metabolizable Energy (Kcal/kg)	3023.18	3037.43	3051.68	3065.93	3080.18
Calcium (%)	1.30	1.30	1.30	1.30	1.30
Av. phosphorus (%)	0.52	0.50	0.50	0.50	0.43
Lysine (%)	1.31	1.31	1.31	1.30	1.30
Methionine (%)	0.48	0.48	0.48	0.47	0.47

**Table 2:Haematological parameters of broiler chickens fed Black soldier fly larvae meal-based diets**

Parameters	Diet I	Diet II	Diet III	Diet IV	Diet V	SEM	P-Value
Erythrocyte Sedimentation Rate (mm/hr)	3.50	3.33	2.83	2.83	3.00	0.13	0.52
Packed cell volume (%)	26.17	30.67	34.67	34.23	33.73	1.21	0.12
Red blood cell ( $10^6/\mu\text{l}$ )	265.67	279.83	333.67	309.33	331.31	10.11	0.09
Haemoglobin concentration (g/100ml)	9.80	10.20	11.53	11.37	11.21	0.25	0.06
Lymphocyte (%)	59.67	59.50	59.33	59.75	57.18	0.41	0.25
Neutrophils ( $10^3 \mu\text{l}$ )	24.67 <sup>b</sup>	24.00 <sup>b</sup>	24.33 <sup>b</sup>	23.79 <sup>b</sup>	28.54 <sup>a</sup>	0.63	0.04
Monocytes ( $10^3 \mu\text{l}$ )	10.83 <sup>ab</sup>	11.83 <sup>a</sup>	11.33 <sup>a</sup>	11.54 <sup>a</sup>	9.77 <sup>b</sup>	0.24	0.02
Basophils ( $10^3 \mu\text{l}$ )	3.50	3.33	3.50	3.46	3.23	0.05	0.30
Eosinophils ( $10^3 \mu\text{l}$ )	1.33	1.33	1.50	1.46	1.27	0.10	0.10
Mean cell haemoglobin concentration (%)	45.47	33.25	33.26	33.21	33.25	2.44	0.17
Mean cell haemoglobin (pg of Hb)	37.00	37.00	35.00	37.00	34.00	0.01	0.45
Mean cell volume ( $\mu^3$ )	99.00 <sup>a</sup>	111.00 <sup>b</sup>	104.00 <sup>a</sup>	112.00 <sup>b</sup>	102.00 <sup>a</sup>	0.03	0.03
Haematocrit (%)	261.67 <sup>b</sup>	306.67 <sup>ab</sup>	346.67 <sup>a</sup>	342.29 <sup>a</sup>	337.29 <sup>a</sup>	12.13	0.04

*\*Mean values within rows with different superscripts are significantly different ( $P < 0.05$ )*

**Table 3: Serum parameters of broiler chickens fed Black soldier fly larvae meal-based diets**

Parameters	Diet I	Diet I	Diet I	Diet I	Diet I	SEM	P-Value
Albumin (mg/dl)	1.12	0.75	0.77	0.69	0.89	0.086	0.57
Globulin (mg/dl)	1.87	2.09	1.71	2.24	1.90	0.15	0.85
Total protein (mg/dl)	2.99	2.84	2.49	2.93	2.79	0.11	0.65
Alanine aminotransferase (u/l)	2.23	2.43	1.90	1.57	1.17	0.43	0.90
Aspartate aminotransferase (u/l)	1.42 <sup>b</sup>	3.33 <sup>ab</sup>	3.42 <sup>ab</sup>	4.88 <sup>a</sup>	3.92 <sup>a</sup>	0.37	0.04
Alkaline phosphate (u/l)	11.04	14.72	15.64	7.36	8.74	1.27	0.16
Urea (mg/dl)	29.99	21.77	9.33	24.33	15.50	3.79	0.50
Creatinine (mg/dl)	62.38	55.06	54.63	62.38	54.20	4.97	0.97
Glucose (mg/dl)	46.29	46.29	102.74	59.84	57.58	13.46	0.69
Triglycerides (mg/dl)	10.14	15.43	8.48	12.11	17.41	1.46	0.28
High density lipoprotein (mg/dl)	0.50	1.12	0.30	0.73	1.36	0.17	0.28
Low density lipoprotein (mg/dl)	2.32	2.54	2.71	2.11	1.41	0.32	0.76
Cholesterol (mg/dl)	3.01	3.21	3.43	2.69	3.62	0.36	0.94

*\*Mean values within rows with different superscripts are significantly different ( $P < 0.05$ )*