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## 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 fishmeal 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. All data were subjected to analysis of variance. Results shows that among all the parameters measured for the haematology indices, only the packed cell volume, red blood cell, haemoglobin concentration, neutrophils, monocytes, and haematocrit and aspartate aminotransferase for the serum indices 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 (3.33106/µl) 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. In totality, there was no deleterious effects of the dietary treatments on the haematology and serum indices of the broiler chickens. Consequently, it could be concluded that BSFLM could replace fish meal at 100% in the broiler chicken diet.

Keywords: Broiler Chicken; Black Soldier Fly Larvae Meal; Fish Meal; Haematology and Serum

## 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 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 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 [1]. According to Makkar, *et al.* [2], 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 like insects, microalgae, and surplus food materials in animal production holds the potential to enhance both efficiency and sustainability in farming practices [3,4]. Series of 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 [5].

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## Materials and Methods 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 [6]. The altitude is about 350.52m above sea level, the annual humidity is 75% and that of temperature is 27°C [7].

#### **Collection and processing of test ingredient**

Black soldier fly larvae were harvested from a location 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 at about 70 - 80°C and was stirred for about 3-5 minutes in order to make the larvae inactive. Filtered inactive larvae was spread on nylon and was solar dried to minimum moisture content. Dried larvae was then milled to produce Black Soldier Fly Larvae Meal (BSFLM).

#### **Experimental diets**

Five experimental diets were produced in such a way that the black soldier fly larvae meal was used as a substitute to fish meal at 0, 25, 50, 75 and 100% and each diet and designated 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 Table 1. The basal finisher diet produced was used to feed the birds from day 22 - day 42.

Ingredients Diet I **Diet II Diet III Diet IV** Diet V 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 1.30 1.30 1.30 1.30 1.30 Calcium (%) Av. phosphorus (%) 0.52 0.50 0.50 0.50 0.43 Lysine (%) 1.31 1.31 1.31 1.30 1.30 0.48 0.48 0.48 0.47 Methionine (%) 0.47

**Table 1:** Feed ingredients and Gross composition of the experimental diets.

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## **Experimental layout and feeding trial**

A total number of two hundred (200) broiler day old chicks of Cobb 500 were procured from a reputable Hatchery in Ibadan, Nigeria, out of which One Hundred and Fifty (150) 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 feed diets were fed *ad libitum* to the chicks from day 1 - day 21. Thereafter, the finisher diets were fed to the started chicks *ad libitum* 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.

#### Slaughtering and blood sample collection

At the end of the experimental period, three (3) birds per replicate were randomly selected 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.

#### Data analysis

All 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 [8] of the same statistical package was employed to separate the means. The level of significance was set at P < 0.05.

#### Results

#### **Haematological studies**

The haematology of broiler chickens fed black soldier fly larvae meal-based diets is presented in Table 2. Among all parameters measured, packed cell volume, red blood cell, haemoglobin, neutrophils, monocytes and haematocrit were significantly (P < 0.05) influenced by the dietary treatments. Highest packed cell volume (34.67%), highest red blood cell (3.34×106mm-3) and highest haemoglobin concentration (11.53g/100ml) were recorded in bird fed diet III, while lowest packed cell volume (26.17%), lowest red blood cell (2.66×106mm<sup>-3</sup>) and lowest haemoglobin concentration (9.80g/100ml) were recorded in bird fed diet I. Highest values of erythrocytes sedimentation rate (3.5mm/hr), mean cell haemoglobin concentration (45.47%) were observed in bird fed diet I. Lymphocyte value varies from 57.18 - 59.75%, basophils; 3.23 - 3.50% and eosinophils; 1.27 - 1.50%.

| 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 <sup>b</sup>  | 30.67 <sup>ab</sup>  | 34.67ª             | 34.23ª               | 33.73ª              | 1.21  | 0.12    |
| Red blood cell (10 <sup>6</sup> /µl)    | 265.67 <sup>b</sup> | 279.83 <sup>ab</sup> | 333.67ª            | 309.33 <sup>ab</sup> | 331.31ª             | 10.11 | 0.09    |
| Haemoglobin concentration (g/100ml)     | 9.80 <sup>b</sup>   | 10.20 <sup>ab</sup>  | 11.53ª             | 11.37ª               | 11.21 <sup>ab</sup> | 0.25  | 0.06    |
| Lymphocyte (%)                          | 59.67               | 59.50                | 59.33              | 59.75                | 57.18               | 0.41  | 0.25    |
| Neutrophils (10 <sup>3</sup> μl)        | 24.67 <sup>b</sup>  | 24.00 <sup>b</sup>   | 24.33 <sup>b</sup> | 23.79 <sup>b</sup>   | 28.54ª              | 0.63  | 0.04    |
| Monocytes (10 <sup>3</sup> µl)          | 10.83 <sup>ab</sup> | 11.83ª               | 11.33ª             | 11.54ª               | 9.77 <sup>b</sup>   | 0.24  | 0.02    |
| Basophils (10³ μl)                      | 3.50                | 3.33                 | 3.50               | 3.46                 | 3.23                | 0.05  | 0.30    |
| Eosinophils (10 <sup>3</sup> μl)        | 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 (ρg of Hb)        | 37.00               | 37.00                | 35.00              | 37.00                | 34.00               | 0.01  | 0.45    |
| Mean cell volume (µ³)                   | 99.00               | 111.00               | 104.00             | 112.00               | 102.00              | 0.03  | 0.03    |
| Haematocrit (%)                         | 261.67 <sup>b</sup> | 306.67 <sup>ab</sup> | 346.67ª            | 342.29ª              | 337.29ª             | 12.13 | 0.04    |

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

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

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#### **Biochemical indices**

Table 3 shows the serum biochemical indices of broilers fed black soldier fly larvae meal-based diets. Among all parameters measured, only aspartate aminotransferase was significantly (P < 0.05) influenced by the dietary treatments. 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. Highest total protein (2.99mg/dl) was observed in bird fed diet I, while lowest total protein (2.49mg/dl) was observed in bird fed diet III. Lowest albumin (0.69mg/dl), lowest alkaline phosphate (7.36u/l) and lowest cholesterol (2.69mg/dl) were recorded in bird fed diet IV. 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.

| 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> | 4.88ª  | 3.92ª  | 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    |

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

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

#### 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 clothing and all these are made possible as a result of its constituents [9]. The packed cell volume values (26.17 -34.67%) obtained from this study falls within the range of 25.00 -45.00% for chickens as reported by Ahamefule., et al. [10]. Packed cell volume values below normal range is an indication of anaemia [11] and poor quality of protein of the diets (Awoniyi., *et al.* 2000). Thus with the values obtained in this study, it could be suggested 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.* [12]. Red blood cell indicate a higher nutrient supply in the body system of the chickens [13]. In addition, the monocyte levels for all treatments were generally based on the reference range. The role of the monocyte component is to fight foreign materials that enter the bloodstream. The value for total protein (2.49 - 2.99 mg/dl) obtained in this study is lower than the normal ranges (5.00 - 7.00 mg/dl) as reported by Robert., *et al.* [14]. Studies by Marono., *et al.* [15], 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.* [16]. In contrast to observations in the present study, Dabbou., *et al.* [17] and Kinasih., *et al.* [18] 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 [19] observed in diets I and IV.

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## 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 if could be concluded that feeding broiler chickens with black soldier fly larvae in replacement for fish meal could be adopted by broiler farmers.

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