GROWTH AND HAEMATOLOGICAL RESPONSE OF BROILER STARTER CHICKS, FED DIETS CONTAINING VARYING INCLUSIONS OF TOASTED BLACK SOLDIER FLY LARVAE (*HERMETIA ILLUCENS*) MEAL AS PARTIAL REPLACEMENT FOR SOYBEAN

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ABSTRACT
The larvae of the Black Soldier Fly (Hermetia illucens) have been extensively examined as a viable and sustainable protein source in poultry nutrition, primarily due to their remarkable potential and chemical composition suitable for various animal species. In broiler production, feed costs constitute approximately 75% of the overall production expenditures, rendering protein one of the costliest components in broiler diets. Consequently, there is a pressing need to explore the impact of Black Soldier Fly larvae (BSFL) meal in the diets of broiler chickens, with the objective of minimizing production costs as effectively as possible. This study was consequently conducted to assess the growth performance and haematological indices of broiler chickens fed diets containing varying inclusion levels of toasted Black Soldier Fly larvae (Hermetia illucens). A total of 200 day-old Cobb 500 broiler chicks procured from Zartech Company in Jos were used for this investigation. The birds were randomly distributed among five dietary treatments. Forty (40) birds were randomly allocated to each dietary treatment, with eight (8) birds per replicate in a completely randomized design. Toasted defatted Black Soldier Fly larvae meal (TDBSFLM) was incorporated at graded levels of 0%, 2%, 4%, 6%, and 8%, and were designated as T1, T2, T3, T4, and T5, respectively. The experimental diets and water were made available ad libitum during the starter phases. All management practices were meticulously adhered to, including brooding, vaccination, drug administration, and the maintenance of proper hygiene throughout the duration of the study. The results showed a significant ($p<0.05$) difference across all performance parameters measured among the various treatments during the starter phase. A linear increase was observed in the final weight (FW) and total weight gain of the birds as the inclusion of BSFLM increased during the starter phase. These performance parameters were significantly ($p<0.05$) higher in birds fed graded levels of BSFLM in comparison to those in the control group (T1). The feed conversion ratio exhibited a significant improvement in birds fed diets T5 (2.37) containing 8% BSFLM, as opposed to those in the other treatment groups. No significant ($p<0.05$) effects of the treatments were detected in the haematological parameters assessed. Thus, it was concluded that toasted defatted Black Soldier Fly larvae meal (TDBSFLM) at levels up to 8% could be integrated into broiler diets without inducing any detrimental effects on the growth performance and health status of the birds.

Keywords: Black soldier fly larvae, Broiler chicken, Inclusion levels, Growth performance, Blood profile

INTRODUCTION

Broiler feed amount to about 75% of the total cost in broiler production, thus rendering protein as the costliest feed omponent in broiler rations. Efforts aimed at mitigating the financial burden associated with protein in poultry feed are anticipated to yield considerable economic relief for both consumers and producers alike (Dar and Gowda, 2013; Yueng and Yee, 2002). At present, the protein sources derived from both animal and plant origins are insufficient to satisfy the projected future demands (Capper, 2013). This shortfall can be attributed to intense competition between human populations and livestock for traditional protein sources such as soybeans, groundnut cake, and fish meal. According to the findings of Fashina-Bombata & Balogun (1997), which compared the production costs of larvae and fish meal, it was determined that the cost associated with larvae meal production is approximately 20% lower than that of an equivalent quantity of fish meal. A similar investigation conducted by Ajani *et al.*

(2004) revealed an 18% and 20% reduction in costs when 50% and 100% of fish meal were substituted with larvae meal in Tilapia diets, respectively.

Given that black soldier fly larvae (BSFL) have been identified as a promising and sustainable protein source in poultry production (De Marco *et al.,* 2015) due to their advantageous chemical composition suitable for various animal species (Newton *et al.,* 2005b), there exists a pressing need to explore the implications of incorporating BSFL into the diets of broiler chickens in order to maintain protein prices at minimal levels. This study is designed to assess the growth performance, haematological parameters, and serum biochemistry of broiler chickens provided with diets containing varying levels of toasted black soldier fly larvae (*Hermetia illucens)* meal.

MATERIALS AND METHODS

Description of study area

The investigation was executed at the Poultry Unit of the Teaching and Research Farm within the Department of Animal Science at Taraba State University, Jalingo. The geographical locale of Jalingo is situated in the northeastern region of Nigeria. It is positioned between latitudes 8° 54' N to 9° 01' N and longitudes 11° 22' E to 11° 30' E. The annual temperature fluctuates between 39°C to 42°C, with an average precipitation of 8.30 mm observed in August and an average relative humidity of 62.9% (NOAA, 2023).

Source of Black soldier fly larvae

A total of one hundred and thirty kilograms (130 kg) of desiccated Black Soldier Fly Larvae (BSFL) were procured from Afrimash Nigeria Limited located in Lagos.

Processing of Black Soldier Fly Larvae

Toasted Defatted Larvae

The toasting of Black Soldier Fly Larvae Meal (BSFLM) was conducted using a frying pan, with firewood serving as the primary energy source throughout the processing. A batch consisting of one hundred kilograms (100 kg) of larvae was toasted locally employing a modification of the methodology established by Amaefule and Nwagbara (2004) for the processing of BSFLM.

The larvae were vigorously and continuously stirred with a long metal spoon, and the toasting persisted until a greasy, oil-like substance became evident on the spoon. The toasting was subsequently halted, and the larvae were immediately crushed to reveal the fat and abdominal contents.
A manual pressing apparatus was employed to extract the free oil at a pressure of 250 bar and a temperature of 50°C for a duration of thirty minutes at the National Research Institute for Chemical Technology (NARICT) in Zaria. The toasted BSFLM was then milled and integrated into the formulated diets.

Chemical Analysis of Black Soldier Fly Larvae Meal

Black Soldier Fly Larvae Meal, serving as the test ingredient, was ground into smaller particulates. Samples were analysed at the National Animal Production Research Institute (NAPRI) Laboratory located in Zaria, Kaduna State, for proximate composition following the procedures delineated by the AOAC (2002).

Experimental Birds, design and management

A total of two hundred (200) day-old Cobb 500 broiler chicks, procured from Zartech Company in Jos, were used for this investigation. The birds were randomly assigned to five distinct dietary treatments. Forty (40) birds were randomly allocated to each dietary treatment, with eight (8) birds per replicate in a completely randomized design. Five (5) experimental diets were formulated, wherein milled toasted BSFL were incorporated into the dietary treatments at inclusion levels of 0%, 2.0%, 4.0%, 6.0%, and 8%. The diets were designated as T1 (control), T2, T3, T4, and T5, respectively.

Data collected

Data were systematically collected on a weekly basis on various parameters including total feed intake (TFI), overall weight gain, feed conversion ratio, and mortality rates. The growth performance of the birds was evaluated weekly The birds selected for the assessment of haematological parameters were fasted overnight prior to the collection of blood samples in the morning, as documented by Bush (1975). This procedure was implemented to mitigate any transient elevations in blood metabolite levels that could arise from feeding. At the point of slaughter, Ethylene Diamine Tetra-Acetic Acid (EDTA) sample containers were used to collect blood samples from the jugular vein of one bird per replicate across all treatment groups, based on the average final body weight of the

chickens at day 28. For the evaluation of haematological parameters, three (3) milliliters of blood samples were drawn from the designated birds into EDTA sample bottles. Blood smear were prepared for each bird using non-coagulated blood. The smears were stained employing Giemsa stains and May-Grunwald techniques to quantify the haematological parameters.

Table 1: Composition of broiler starter diets containing toasted Black soldier fly larvae meal (0-4 weeks)

	Treatments				
	T1 (Control) (0%)	**T2 (TDBSFLM) (2.0%)**	**T3 (TDBSFLM) (4.0%)**	**T4 (TDBSFLM) (6.0%)**	**T5 (TDBSFLM) (8.0%)**
Ingredients (%)					
Maize	47.10	47.10	47.10	47.10	47.10
Maize offal	10.00	10.00	10.00	10.00	10.00
Soybean meal	41.00	38.50	36.00	33.50	31.00
Toasted defatted BSFL	0.00	2.00	4.00	6.00	8.00
L-lysine HCL	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.20	0.20	0.20	0.20	0.20
Vit/min premix	0.26	0.26	0.26	0.26	0.26
Bone meal	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
Metabolizable energy (Kcal/kg)	2925.63	2937.17	2949.64	2960.39	2972.44
Crude protein	23.15	23.34	23.43	23.63	23.77
Crude fibre	4.65	4.73	4.88	5.01	5.13
Fat	3.41	3.51	3.62	3.73	3.80
Lysine	1.35	1.32	1.23	1.15	1.04
Methionine	0.52	0.51	0.47	0.46	0.44
Calcium	0.41	0.38	0.38	0.37	0.39
Phosphorus	0.31	0.31	0.31	0.30	0.29

Vit and mineral premix (per kilogram of diet): Vitamin. A 40,000 IU; Vitamin D_3 32,000 IU; Vitamin E 23,000mg; Vitamin K 1200mg; Vitamin $B_1$3000mg; vitamin B2 4000mg; Vitamin B_5 500mg; Vitamin B6 3000MG Pantothenic acid 7,500mg; Nicotinic acid 30,000mg; Riboflavin 600mg; Vit. B_{12} 160mg; Folic acid 700mg; Biotin 110mg; Cobalt 200mg; Iodine 400mg; Iron 25,000mg; Mg 5000mg; Selenium 20mg; copper 1200; Zinc 6,000mg.

Statistical analysis

All data obtained were subjected to analysis of variance in Statistical Analysis System (SAS, 2020). Significant differences among treatment means were separated using Duncan's multiple range in SAS (Duncan, 1955).

RESULTS

The growth performance indices of broiler chickens subjected to varying inclusion levels of toasted black soldier larvae meal during the starter phase are presented in Table 2. Notably, there were statistically significant ($p<0.05$) effects of the treatment on all parameters evaluated, including final weight (FW), total weight gain (TWG), total feed intake (TFI), daily feed intake (DFI), and feed conversion ratio (FCR). The FW and TWG of birds in treatment groups T4 and T5 exhibited higher ($p<0.05$) performance metrics (1957.85, 1906.85g), (69.92, 68.10g), (808.05, 804.01g), and (768.04, 762.03g) respectively, in comparison to the other treatment groups ($p<0.05$) during the starter phase. T4 and T5 significantly ($p<0.05$) increased TFI (1957.83 and 1906.84) and DFI (69.91 and 68.16), which were comparable to the birds in T3 (1849.43 and 66.04), yet significantly ($p<0.05$) lower than those observed in birds fed diets T1 and T2 respectively. Furthermore, birds on diet T5 exhibited a significantly ($p<0.05$) lower FCR (2.37) when compared to all other treatment groups. No case of mortality were recorded in treatment

groups T3, T4, and T5. In contrast, a significantly ($p<0.05$) lower mortality rate of 0.76 and 0.49% was documented in treatment T1 and T2 respectively.

The haematological indices of broiler chickens subjected to different inclusion levels of toasted BSFLM during the starter phase are presented in Table 3. There were no statistically significant differences ($p>0.05$) observed among the treatment groups. The majority of the measured haematological parameters were found to be comparable, with the exception of white blood cells (WBC). The birds fed T5 treatment displayed elevated values for WBC (138.38 g/l) when compared with other treatments, whereas the T1 group exhibited the lowest recorded values for WBC (26.85 g/l).

Table 2. Effect of diets containing different inclusion levels of toasted Black soldier fly larvae meal on growth performance of broilers starter chicks (0-4 weeks)

	Treatments					
Parameters	**T1 (TBSF0%)**	**T2 (TBSFL2%)**	**T3 (TBSFL4%)**	**T4 (TBSFL6%)**	**T5 (TBSFL8%)**	**SEM**
Initial weight (g/bird)	40.00	40.00	37.50	40.00	42.00	1.37
Final weight (g/bird)	546.00^{c}	714.21^{ab}	685.01^{b}	808.05^{a}	804.01^{a}	7.26
Total Weight gain (g/bird)	506.00^{c}	674.22^{ab}	647.52^{b}	768.04^{a}	762.03^{a}	67.26
Total Feed intake (g/bird)	1770.40^{b}	1790.23^{b}	1849.43^{ab}	1957.83^{a}	1906.84^{ab}	94.29
Daily Feed intake (g/bird/day)	64.26^{b}	63.90^{b}	66.04^{ab}	69.91^{a}	68.16^{ab}	3.36
Feed conversion Ratio	3.25^{a}	2.25^{bc}	2.69^{b}	2.42^{bc}	2.37^{c}	0.06
Mortality (%)	0.76	0.49	0.00	0.00	0.00	0.34

abc Means within the same row bearing the same letter are not significantly different ($p<0.05$), SEM = Standard Error of Means

The results of mean corpuscular Haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular Haemoglobin concentration (MCHC) in broiler chickens demonstrated no statistically significant differences ($P>0.05$) across the various dietary treatments.

Table 3: Table: 4.11 haematological indices of broiler chickens fed varying inclusions levels of toasted black soldier fly larvae.

	Treatments					
Parameters	**T1 (Control)**	**T2 (TBSFL2 .00%)**	**T3 (TBSFL4%)**	**T4 (TBSFL6%)**	**T5 (TBSFL8%)**	**SEM**
Packed cell volume (%)	26.52	27.01	26.01	27.45	25.48	2.97
White blood cell (g/L)	26.84	120.66	59.72	50.01	138.37	57.94
Red blood cell (L)	2.56	2.73	2.36	2.76	2.54	0.30
Haemoglobin (g/dL)	8.11	8.48	7.51	8.4	8.11	1.11
MCH (pg)	31.61	31.31	30.96	30.93	31.64	0.96
MCV (fL)	84.24	84.58	84.41	82.81	80.94	3.14
MCHC (g/dl)	37.51	37.03	36.71	37.43	39.18	1.14

abc = Means within the same row bearing the same letter are not significantly different ($p<0.05$), SEM = Standard Error of Means; MCH = Mean Corpuscular Haemoglobin; MCV= Mean Corpuscular Volume; MCHC= Mean Corpuscular Haemoglobin Concentration

DISCUSSION

The incorporation of Black Soldier Fly Larvae Meal (BSFLM) in the diet of broiler chickens, along with the increment in body weight gain, aligns with the findings of Anankware (2018), who reported that an increase in the proportion of BSFLM led to a increase in the live body weight of broiler compared to those provided with a diet without BSFLM. The body weight, total feed intake, and feed conversion ratio in the present investigation surpassed the outcomes reported by Hwangbo (2009) and Sandi (2022). The enhanced body weight and weight gain observed can be attributed to the increased amino acid composition in BSFLM (Sandi, 2022).

It has been documented that broiler chickens receiving diets with up to an 8% inclusion of BSFLM exhibited greater body weight than those on diets with less than a 5% inclusion level (Moula *et al.,* 2018). In a similar vein, Moula and Detilleux (2019) elucidated that the incorporation of BSFL up to 10% enhances the growth performance of broiler chickens without any detrimental effects on feed intake (FI) and feed conversion ratio (FCR).

Conversely, the findings of Biasato *et al.* (2020) regarding toasted defatted BSFL indicated that the inclusion of BSFLM exceeding 15% adversely impacted broiler performance and diminished both microbial diversity and the potential presence of beneficial bacteria. Additionally, other investigations have demonstrated that the incorporation of BSFL at levels ranging from 20% to 30% significantly reduced the body weight of broiler chickens by week 6 (Moula *et al.,* 2018).

In the present study, insignificant value of mortality was recorded among the birds fed a BSFLM diet. It is posited that the antioxidant properties of BSFLM, attributable to the presence of chitin, may confer beneficial effects by enhancing the immune response in chickens (Sanchez *et al.,* 2017). It has been substantiated that chitin present in BSFLM displays prebiotic characteristics as well as bacteriostatic effects on bacteria (Ngo and Kim, 2014).
The results obtained from this study indicated that the different inclusion levels of BSFLM showed no significant effects on any of the haematological indices assessed ($p>0.05$). This suggests that the inclusion of varying inclusion levels of BSFLM did not adversely affect the health status of the birds. The findings of the current study demonstrated that all measured haematological parameters remained within the normal physiological range for broiler chickens (Lumeij, 1997). The normative physiological ranges for haematological indices in broiler chickens are as follows: RBC: 2.02-3.5 x 10^6 µl, PCV: 22-35%, HGB: 7-13 g/dl, MCV (FL): 90-140, MCH (pg): 20-69, and MCHC (g/dl): 20.77-64 (Bounous and Stedman, 2000; Kokore *et al.,* 2021). Furthermore, no group in the current study exhibited any indications of toxicity.

The erythrocytes are responsible for the transportation of oxygen from the pulmonary system to the peripheral tissues and facilitate the elimination of carbon dioxide from the tissues back to the lungs via Haemoglobin (Kaminski *et al.,* 2014). The findings of this investigation indicated that varying levels of Black Soldier Fly Larvae Meal (BSFLM) did not exert any significant influence on the red blood cell (RBC) across all experimental conditions. The packed cell volume (PCV) remained constant throughout the study. Nevertheless, PCV serves as a metric for assessing sexual maturity in animals, which can be influenced by both sex and age (Elagib and Ahmed, 2011). In the present research, the white blood cell (WBC) counts in all treatment groups were found to exceed the standard haematological reference range. This elevation may be attributable to stress factors that potentially raised WBC levels during the handling of birds at the time of slaughter or during the transportation of blood samples to the laboratory. Other researchers have posited that an increased inclusion of BSFLM in dietary formulations may lead to elevated levels of minerals, including vitamin A. Vitamin A is recognized for its role in enhancing immune responses, which may assist in mitigating the proliferation of certain infections such as coccidiosis (Diaz-Gomez *et al.,* 2015). The elevated WBC counts observed in the current study were consistent with the results reported by Addass *et al*. (2012).

CONCLUSION

Based on the results obtained from this investigation, it was concluded that the incorporation of toasted defatted black soldier fly larva meal (TDBSFLM) at a level up to 8% into the diets of broilers does not

result in any detrimental effects on the growth performance or the overall health condition of broiler chickens.

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