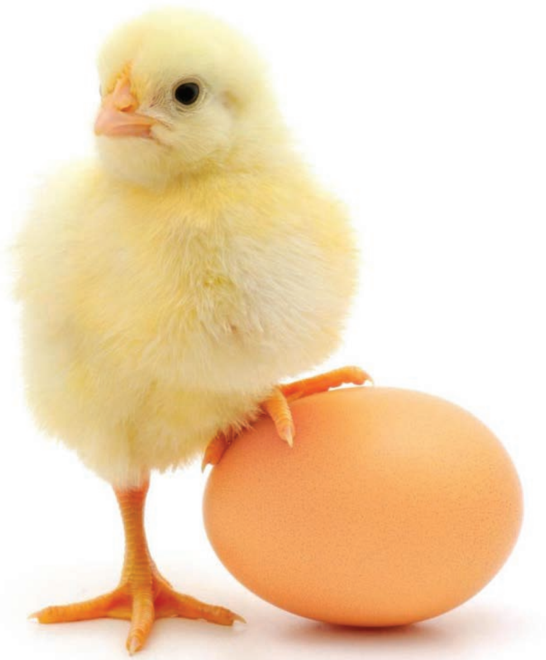
ISSN 1682-8356
ansinet.com/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS International Journal of Poultry Science
ISSN 1682-8356
DOI: 10.3923/ijps.2023.126.137

Research Article
Dietary Inclusion of Black Soldier Fly Larvae Reared on Hatchery Waste Affects Serum Biochemical and Haematological Parameters of Brahma Chickens

[1]Mube Kuietche Hervé, [2]B.J. Nke Bela, [3]Dzepe Daniel, [4]Vemo Bertin, [2]M.J. Manga, [1]Ebile Dayan, [1]A.S. Tatsinkou, [1]Kana Jean Raphael and [5]Chia Shaphan Yong

[1]Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222, Dschang, Cameroon
[2]Institut Agricole d'Obala, P.O. Box 233, Obala, Cameroon
[3]International Institute of Tropical Agriculture (IITA), Benin Station, IITA-Benin, 08 BP 0932 Tri Postal, Cotonou, Republic of Benin
[4]Department of Animal Science, Faculty of Agriculture and Veterinary Medicine, University of Buea, Buea, Cameroon
[5]Laboratory of Entomology, Wageningen University and Research, 6708 PB, Wageningen, Netherlands

Abstract
Background and Objective: To address the increasing pressure on the environment and feed costs in livestock, a sustainable production system with non-conventional feedstuff is an urgent priority. This study aimed to investigate the recycling potential of black soldier fly larvae (BSFL) on hatchery waste and the effect of larval biomass to serve as a protein ingredient in the diet of Brahma chicken. **Materials and Methods:** BSFL (4 day-old) were randomly distributed into three formulated diets D1 (66.33% of mango waste plus 33.33% of hatchery waste), D2 (66.33% of kitchen waste plus 33.33% of hatchery waste), D3 (chicken waste plus 33.33% of hatchery waste) in a completely randomized design. Larvae obtained from these substrates were further tested as protein sources in Brahma chicken feed. In a 12 week feeding trial, 54 chicks (21 days old) were randomly assigned to 3 treatments containing 0, 2.5 and 5% of black soldier fly larvae meal (BSFLM) as a replacement for fish meal. **Results:** Formulated diet significantly ($p<0.05$) affected larval biomass. The highest larval weight was recorded in D2. The Brahma chicken growth and carcass traits remained comparable across all the treatments. Incorporation of BSFLM in the diet decreased the level of red and white blood cells in Brahma chickens. The Brahma chicken intestinal microbial load was not affected by diet. As compared to the control group, the diet containing BSFLM had the lowest production cost. **Conclusion:** Black soldier fly larvae can be used as a source of proteins in the diet of Brahma chicken and can fully replace fishmeal with minimal effects on blood biochemistry and haematology.

Key words: Brahma chicken, insect larval meal, black soldier fly rearing, hatchery waste, blood parameters, production cost

Citation: Hervé, M.K., B.J.N. Bela, D. Daniel, V. Bertin and M.J. Manga *et al*., 2023. Dietary inclusion of black soldier fly larvae reared on hatchery waste affects serum biochemical and haematological parameters of Brahma chickens. Int. J. Poult. Sci., 22: 126-137.

Corresponding Author: Mube Kuietche Hervé, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222, Dschang, Cameroon

Copyright: © 2023 Mube Kuietche Hervé *et al*. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The ever-growing world population raises critical questions about our future ability to produce adequate food for all[1]. Earlier estimates postulate a 70% increase in global food production by 2050 to meet the additional need for food and feed[2]. Like other developing countries, the population growth rate in Cameroon is estimated at 2.54%[3]. In response to this population growth, the government of Cameroon has decided to intensify the production of short-life-cycle species like poultry and pigs[4]. This move by the government has led to a double increase in the production of poultry products in Cameroon in the last 20 years, representing about 55% of the livestock sector and contributing about 30% of the Gross Domestic Product (GDP). This is in line with the FAO's report entitled "The Shadow of Livestock" showing that the demand for livestock in the world will double in the next 50 years, i.e. from 229 million tons currently to 465 million tons[2]. However, the sector, which provides employment, nutrition and a source of livelihood to many, both in rural and urban settings is weakened by the high costs of feed, especially essential feed ingredients such as soy meal and fishmeal. Indeed, feed represents about 60-70% of the total production costs in the poultry sector. The increasing scarcity of protein ingredients is prompting the animal feed industry to explore sustainable alternatives to save the sector and above all to satisfy the entire production chain. Besides, it has been established that the growing demand for feed has potentially negative consequences on the environment in terms of greenhouse gas emissions and further implications for water, energy, land use and waste management[5,6]. The FAO advocates the use of insects for food and feed. Insects are a good source of energy, protein, fat, vitamins and minerals[7]. The breeding of insects is also more environmentally friendly, as insects produce fewer greenhouse gases and emit less ammonia than conventional livestock[8]. Indeed, insects have a higher feed/protein conversion ratio than cattle, pigs and poultry[9]. Thus, in recent years, there has been a significant increase in the number of studies and businesses associating the application of insect production with recycling and animal feed[10,11]. Of particular interest is *Hermetia illucens* commonly known as the black soldier fly (BSF). The larvae of BSF are rich in nutrients (lipids: 35% and proteins 55%) with a well-balanced essential amino acid profile[12,13]. BSF-based technology stands as an evergreen technology to boost food availability (animal protein and crop productivity) and contribute to a safe environment in line with the Sustainable Development Goals (SDGs) 2 (zero hunger) and 6 (clean water and sanitation)[14,15]. The larvae of BSF convert low-value organic waste such as coffee waste, distillery grain, vegetable waste, decaying fruit, urban organic waste, fish waste, human feces and animal manure into insect protein that can be used to feed chickens without negatively affecting their growth parameters[16,17].

The production of chickens and related products generate tremendous amount of hatchery waste, which includes solid waste and wastewater. The solid hatchery waste comprises empty shells, infertile eggs, dead embryos, late hatchings and dead chickens and a viscous liquid from eggs and decaying tissue Glatz *et al.*[17]. Current disposal methods for solid hatchery waste include landfill, composting, rendering and incineration, which costs money and increases the total cost of poultry production each year[17,18]. The efficient management of these waste streams would require strategies that minimize costs and negative environmental impacts. Hatchery waste is rich in protein, moisture, fibre and energy[17,19]. Further development of these waste streams into animal feedstuffs or direct application into the soil as an organic fertiliser has great potential. However, direct soil applications could potentially pollute the environment, including groundwater[17,20]. Several methods of handling hatchery waste have been considered with both positive and negative implications. Recently, the use of insects such as the black soldier fly larvae in sustainable organic waste management has received growing attention globally but little is known about the use of this innovative technology in hatchery waste management. Therefore, this study aimed at investigating the potential of BSF larvae to recycle solid hatchery waste as well as use larvae reared on these waste streams in feed for chickens.

MATERIALS AND METHODS

Study area: The study was conducted at Obala Agricultural Institute School Farm in Bilone, located in the Center Region of Cameroon with 4°10′ North latitude and 11°31′ East longitude. At 542 m above sea level, Bilone has a mean temperature of 25°C. The general care and management of the animals followed the same guidelines as for organic farms. In this system, birds did not receive chemical medicine but were provided with a powder mixture of medicinal plants Moringa (25%), garlic (15%), thyme (25%), pepper (15%), dry aloe vera (10%), dry papaya seeds (10%). This mixture served as an antibiotic, internal deworming, hepato-protector and anticoccidial agent and was administered twice a week.

Insect colony: Black soldier fly larvae used in this study were obtained from a 5 years old domesticated colony maintained at 28°C, 60-70% RH and 12 hrs photoperiod. Male and female

BSF were housed in a cage (2 m×1 m) also referred to as a love cage with the sides, top and bottom and covered with a mosquito net material. Moist hatchery substrate in plastic containers was placed inside the cage to serve as an oviposition attractant. BSF eggs were collected on pieces of cardboard with flutes along the edges and placed on the surface of the oviposition attractant[21]. Eggs laid were allowed to hatch and feed on chicken feed for 4 days. This allowed larvae to grow and enabled their safe and easy collection and use in the experiments.

Substrates formulation for black soldier fly larvae: Hatchery waste including a mixture of unhatched eggs, egg shells and dead chicks were obtained from Obala Agricultural Institute School Farm hatchery. Three different experimental diets were prepared by mixing the hatchery waste with mango waste, kitchen waste and chicken manure to obtain the following larval diets:

- **D1:** 66.33% of mango waste and 33.33% of hatchery waste
- **D2:** 66.33% of kitchen waste and 33.33% of hatchery waste
- **D3:** 66.33% of chicken manure and 33.33% of hatchery waste

The incorporation was choice in order to maintain the same physical aspect of the substrate in each diet. The mango waste was consisted of rotten fruits without pits. Kitchen waste was consisted of boiled cassava root, cooked maize meal, vegetable and groundnut soup. Chicken waste was consisted of chicken manure from birds fed on a conventional diet (soya bean cake, maize, cotton seed cake and wheat bran). Hatchery waste was obtained after hatching ended. After formulation, each test substrate was maintained at 70-78% humidity by adding water as necessary.

Experimental design

Larval performance: One gram of 4 days old larvae were transferred into 150 g of diets each: D1, D2 and D3 in 3 tanks and allowed to feed. On days four and eight, 200 and 150 g of each diet, respectively were added to the respective tanks. Overall, each tank received a total of 500 g of the test diet at the end of the trial.

The larvae were harvested on day 12 and slaughtered by placing them in boiled water for 5 min. Thereafter, the slaughtered larvae were sun-dried and milled to obtain a black soldier fly larval meal for subsequent use in poultry diet formulation.

Chemical analysis of insect larval meal: Before its incorporation into the poultry diet, 50 g of the larval meal from each treatment was oven-dried for 48 h at 60°C. These samples were then transferred to the Laboratory of Animal Production and Nutrition and the Laboratory of Soil Sciences and Environmental Chemistry of the University of Dschang for proximate and mineral analysis respectively. The proximate analyses included dry matter, crude protein, crude fat, crude fibre and ash and were performed according to standard protocol provided by the Association of Official Analytical Chemists (AOAC)[22], while the mineral composition [Calcium (Ca), sodium (Na), potassium (K), phosphorus (P) and magnesium (Mg)] was determined according to the protocol described by Pauwels *et al.*[23].

Formulation of insect-based diets for laying hens: From a control ration containing fishmeal as the main source of protein, two other rations were formulated by replacing fishmeal with BSF larval meal (BSFLM) as shown in Table 1 and 2. The test diets were formulated according to the NRC[24] specifications for laying hens. At each phase (starter and grower-finisher), three experimental iso protein and isoenergetic rations were formulated. Diets were prepared by replacing the fish meal content in the conventional diet (control diet) with BSFLM at 2.5 and 5% (Table 1 and 2).

Poultry management: A total of 72 Brahma chicks (36 males and 36 females) aged 28 days and weighing on average 285 g were used in this study. Birds were randomly assigned

Table 1: Experimental diet during the starter phase

Ingredients	BSFLM0%	BSFLM2.5%	BSFLM5%
Maize	56	56	56
Soya beans cake	10	10	10
Wheat bran	8	8	8
Cotton seed cake	3	3	3
Groundnut seed cake	8	8	8
Palm seed cake	4	4	4
Borne meal	2	2	2
Fish meal	5	2.5	0
Premix*	4	4	4
BSFM	0	2.5	5
Total	100	100	100
Analyse nutritive values			
Crude proteins	21.058	21.045	21.032
Metabolizable energy	2788.74	2803.015	2817.29
Calcium	1.231	1.2183	1.2048
Phosphorous	0.8512	0.7899	0.7287
Sodium	0.0451	0.0451	0.0451
Lysine	1.121	1.0991	1.0769
Methionine	0.3886	0.3811	0.3811

*Premix 5%: Crude protein: 40%, Lysine: 3.3%, Methionine: 2.40%, Calcium: 8%, Phosphorous: 2.05% and Metabolisable energy: 2078 kcal kg^{-1}

Table 2: Experimental diet during the growing-finishing phase (61 à 100 days)

Ingrédients	FMSN0%	FMSN2.5%	FMSN5%
Maize	62	62	62
Soya bean cake	10	10	10
Wheat bran	8	8	8
Cotton seed cake	2	2	2
Groundnut seed cake	7	7	7
Palm seed cake	2	2	2
Borne meal	2	2	2
Fish meal	5	2.5	0
Premix	2	2	2
BSFM	0	2.5	5
Total	100	100	100
Analyse nutritive values			
Crude proteins	19.418	19.405	19.392
Metabolizable energy	2864.16	2878.15	2892.139
Calcium	1.0568	1.04357	1.0303
Phosphorous	0.7707	0.7106	0.6506
Sodium	0.0788	0.0788	0.0788
Lysine	1.0229	1.001	0.97929
Methionine	0.3494	0.342	0.3347

*Premix 5%: Crude protein: 40%, Lysine: 3.3%, Methionine: 2.40%, Calcium: 8%, Phosphorous: 2.05%, Metabolisable energy: 2078 kcal kg^{-1}

to three experimental groups (based on BSFLM incorporation rate) with four replicates of 6 birds each (24 birds per treatment). The number of animals used in this study was based on the available resources and ethical considerations (since the birds would be slaughtered at the end of the growing period). The control group received a commercial feed without BSFLM (BSFLM 0%) and the experimental groups (BSF groups) received the same commercial feed with 2.5 and 5% of black soldier fly larvae meal (BSFLM) as a replacement for fish meal. Birds were housed under identical conditions: same building, photoperiod (12 hrs of light and 12 hrs of dark), natural ventilation system and sawdust floor-bedding. The experiment lasted for 72 days. Feed was supplied *ad libitum* throughout the experiment.

Data collection

Black soldier fly larval growth performance and waste reduction: Larvae were weighed at 4 day intervals till the end (day 12) using a 0.01 g sensitivity balance of the Aroma brand to compare larval growth performance among diets. At the end of the larval phase, mortality was recorded. Residual waste was collected in each container and weighed using the same scale. The recycling parameters of BSFL evaluated include: waste reduction index, waste reduction rate and larval bioconversion rate[11], which were calculated as follows:

$$\text{Waste reduction index: WRI}(\%)=\frac{\text{Total waste reduction}}{\text{Time}}\times 100$$

$$\text{Waste reduction rate: WRR}(\%)=1-\frac{\text{Substrate residue}}{\text{Substrate added}}\times 100$$

$$\text{Bioconversion rate: BR }(\%)=\frac{\text{Fresh larvae weight}}{\text{Waste received}}\times 100$$

Growth, carcass and hematological characteristics of Brahma chickens: Body weight, body weight gain and feed intake of chickens were recorded weekly for each replicate. At the end of the 72 days, six birds per treatment were randomly selected and slaughtered (after 12 hrs of feed deprivation), weighed and eviscerated. Feed conversion ratio (FCR) was defined as the ratio of the amount of feed ingested throughout the rearing period to the body weight gained during that period. Dressing-out percentages were computed as the ratio between warm carcasses and live weights at slaughter. The length of the intestine was measured from the duodenal loop to the cloaca using a tape measure. The density of the intestine was calculated using the following equation:

$$\text{DI}(\text{g cm}^{-1})=\frac{\text{Intestine weight (g)}}{\text{Intestine length (cm)}}$$

During the slaughter process, 04 mL of blood was collected in two test tubes, one with EDTA and one without EDTA. Blood was transferred to the laboratory for hematological analysis. The hematological parameters mainly the white blood cell, red blood cell, platelet and hematocrit were analyzed using an automatic hemocytometer, Genius, model K-T 6180. Blood containing anticoagulant was centrifuged at 3000 rpm for 15 min, then the serum was collected and stored in a freezer at -20°C until analysis of aspartate aminotransferase, alanine aminotransferase, total protein, globulin, urea, triglycerides, creatinine and cholesterol as describe by Abdel-Fattah *et al.*[25].

Caecal microbiota: Faeces from each animal slaughtered during carcass evaluation were collected in sterile plastic tubes and directly submitted for microbiological analysis. The bacterial colonies sought were salmonella, enterobacteria, *Escherichia coli* and lactobacillus. The determination of the number of colonies involved a dilution with distilled water of 1 g of faeces in 9 mL, then 1 mL of the mixture was diluted 6 times consecutively in the tubes also containing 9 mL of distilled water to reduce the concentration of bacteria and to facilitate enumeration. From tubes 2, 4 and 6, 1 mL of the mixture was transferred into culture media previously heated to 60°C in a tight bath or an autoclave and cooled to 50°C. After the mixture had solidified, each jar was stored anaerobically (lactobacillus) or aerobically (salmonella, enterobacteria and *E. coli*) in an incubator at 37°C. The colony count was performed twice: the first count was 24 hrs after incubation and the second was 48 hrs after incubation. The number of colonies were determined using the jar which had the lowest number of spots, then this number was multiplied by the power indicated by the number of the jar (10^2, 10^4 or 10^6).

Profitability: The cost per kilogram of feed was estimated from the price of ingredients on the local market at the time of the study. The cost of feed consumption was obtained by multiplying the average consumption of the animals by the price of the corresponding kg of feed. The cost per kilogram of feed multiplied by consumption index was used to calculate feed cost per kilogram of live weight of chickens[26].

Data analysis: Data on feed intake, body weight, carcass characteristics, development of digestive organs, cost of production and blood biochemical parameters were subjected to one-way Analysis of Variance (ANOVA). When there was a significant difference between the treatments, the means were separated using Duncan's test at the 5% significance level. Statistical Package for Social Sciences (SPSS 21.0) was used for these analysis.

RESULTS

Recycling parameters of hatchery waste: Apart from the final live weight which was significantly ($p<0.05$) affected by diet, all other BSFL growth parameters were not affected by diet (Table 3). However, the association of hatchery waste and poultry feces (D2) recorded the highest biomass production.

The results of the reduction efficiency and the bioconversion rate are presented in Table 4. Except for feed intake and the reduction rate which were significantly affected by the type of diet, all other characteristics were comparable between the different substrates.

Consequently, feed intake recorded in D2 was significantly higher ($p>0.05$) than that of D1 and D3. This lowest waste reduction rate was recorded in D2 diet. On the other hand, the bioconversion rates and the reduction efficiency presented comparable results ($p>0.05$) in all groups.

Growth performance of brahma chickens: The inclusion of BSFLM in feed did not impair (nor did it improve) the growth performance of Brahma chickens as none of their growth performance was significantly affected ($p>0.05$) (Table 5). However, with treatment BSFLM5, which contained the highest level of larvae meal (5%) in the diet, heavier birds were recorded.

Effects of BSFLM on carcass traits: Like growth parameters, carcass traits of Brahma chickens were not significantly affected ($p>0.05$) by the incorporation of black soldier fly larval meal in the diet (Table 6).

Effects of BSFLM on serum biochemical parameters of Brahma chickens: The serum biochemical parameters of Brahma chickens were affected ($p<0.05$) by the incorporation of BSFLM in the diet except for creatinine, urea and the albumin-globulin ratio (Table 7). In comparison to the other

Table 3: Growth characteristics of BSFL as affected by the nature of the substrate

	Types of substrates			
Characteristics	D1	D2	D3	p-value
Final weight (g)	0.16±0.02[a]	0.26±0.01[b]	0.15±0.01[a]	0.00
Mortality (%)	59.66±4.90[a]	46.15±15.29[a]	47.60±3.69[a]	0.24
Total larvae biomass (g)	37.94±8.15[a]	45.17±14.29[a]	26.86±2.56[a]	0.14

[a,b]Means with different superscript are significantly different at the threshold of $p<0.05$, D1: Kitchen waste and hatchery waste, D2: Poultry droppings and hatchery waste and D3: Mango waste and hatchery waste

Table 4: Effects of substrate type on waste reduction efficiency by BSFL

	Types of substrate			
Characteristics	D1	D2	D3	p-value
Feed intake (g)	328.15±3.37[b]	469.39±6.62[c]	306.53±11.89[a]	0.00
Waste reduction (%)	87.51±0.90[b]	72.21±1.02[a]	87.58±3.40[b]	0.00
Bioconversion rate (%)	10.12±2.17[a]	6.95±2.20[a]	7.67±0.73[a]	0.17
Reduction efficiency	11.55±2.38[a]	9.60±2.91[a]	8.77±0.86[a]	0.36

[a,b,c]Means with the different superscript are significantly different at the threshold of $p<0.05$, D1: Kitchen waste and hatchery waste, D2: Poultry droppings and hatchery waste and D3: Mango waste and hatchery waste

Table 5: Average weekly body weight, body weight gain, feed intake and feed conversion ratio of Brahma chicken fed BSFLM diets

		Rations			
Characteristics	Sex	BSFLM0	BSFLM2.5	BSFLM5	p-value
Feed intake (g)	M	9897.08±745.18	9677.50±509.38	10303.00±1051.99	0.41
	F	9897.08±745.18	9677.50±509.38	10303.00±1051.99	0.41
	MF	9897.08±710.50	9677.50±485.68	10215.70±1003.04	0.25
Body weight (g)	M	2408.33±279.67	2446.67±152.93	2570.00±179.56	0.41
	F	1821.67±360.74	1740.00±236.81	1932.00±163.92	0.52
	MF	2115.00±434.25	2093.33±415.11	2280.000±371.32	0.50
Weight gain (g)	M	1916.67±274.78	1953.33±191.28^a	2015.00±132.17^a	0.73
	F	1421.67±353.23	1323.33±224.38^a	1460.00±124.90^a	0.67
	MF	1669.17±397.32	1638.33±384.40^a	1762.73±314.61^a	0.71
Feed conversion ratio	M	5.21±0.38	4.97±0.27	5.14±0.70	0.70
	F	5.21±0.38	4.97±0.27	5.14±0.70	0.70
	MF	6.44±2.69	6.24±1.66	5.96±1.18	0.85

BSFLM: Black soldier fly larvae meal, M: Male, F: Female and MF: Male et female

Table 6: Carcass traits of Brahma chickens fed a diet containing different level of black soldier fly larvae meal

	Rations			
Carcass traits	BSFLM0%	BSFLM 2.5%	BSFLM 5%	p-value
Body weight (g)	2298.00±623.67^a	2051.50±481.54^a	2327.00±581.24^a	0.87
Head weight (g)	2.62±0.20^a	3.03±0.74^a	3.09±0.75^a	0.74
Feet weight (g)	3.69±1.17^a	3.68±0.94^a	4.35±0.21^a	0.71
Liver weight (g)	1.35±0.15^a	1.30±0.18^a	1.37±0.16^a	0.93
Heart weight (g)	0.44±0.05^a	0.45±0.13^a	0.48±0.04^a	0.85
Gizzard weight (g)	2.14±0.60^a	2.09±0.53^a	1.84±0.04^a	0.80
Intestine (g)	3.91±0.16^a	3.65±1.05^a	3.25±0.68^a	0.69
Abdominal fat (g)	3.35±1.57^a	2.01±2.84^a	2.11±2.98^a	0.85
Carcass yield (%)	77.49±0.54^a	77.58±3.26^a	78.24±2.00^a	0.94

BSFLM: Black soldier fly larvae meal

Table 7: Effects of BSFLM on the blood biochemical parameters of Brahma chickens

	Rations			
Biochemical parameters	BSFLM0%	BSFLM 2.5%	BSFLM 5%	p-value
Pro (g dL^{-1})	5.12±0.15^b	5.42±0.20^c	4.75±0.16^a	0.001
Alb (g dL^{-1})	3.46±0.04^a	3.57±0.08^b	3.35±0.07^a	0.004
Glo (g dL^{-1})	1.66±0.15^{ab}	1.86±0.27^b	1.40±0.17^a	0.034
T.Chol (mg dL^{-1})	104.28±11.85^b	101.89±9.06^{ab}	87.64±6.70^a	0.070
Crea (mg dL^{-1})	0.34±0.32^a	0.63±0.46^a	0.48±0.31^a	0.566
Urea (mg dL^{-1})	12.20±5.54^a	13.41±18.49^a	26.22±22.10^a	0.463
ALAT (U L^{-1})	15.63±2.19^a	23.94±3.60^b	22.66±2.00^b	0.004
ASAT (U L^{-1})	17.67±2.64^a	38.97±8.22^b	21.94±7.86^a	0.008
ALB GBL^{-1}	2.09±0.19^a	1.96±0.32^a	2.42±0.32^a	0.108

^{a,b,c}Means with the same superscript letters are not significantly different at 5% significance level, Pro: Total protein, Alb: Albumin, Glo: Globulins, Crea: Creatine, T. Chol: Total cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase and ALB/GBL: Albumin to globulins ratio

treatments, chickens fed on BSFLM2.5 ration had the highest levels of protein cholesterol, ALT, ASAT, Albumine and globuline (p<0.05).

Effects of BSFLM on the haematological parameters of Brahma chickens: Generally, birds receiving 2.5% BSFLM in the diet showed the lowest values for all the hematological parameters studied except for lymphocyte 2 and mean red blood cell volume where this treatment recorded the highest values (p<0.05) (Table 8).

Effect of BSFLM on intestinal microflora: None of the four different bacterial colonies studied in the cecum was significantly affected (p<0.05) by the incorporation level of BSFLM in the diet (Table 9). However, the *Lactobacillus* and *Enterobacteria* populations tended to increase with the level of BSFLM in the diet.

Effect of the incorporation rate of black soldier fly larvae on the feed cost: Economic parameters were not significantly affected (p>0.05) by the incorporation of BSFLM in the diet.

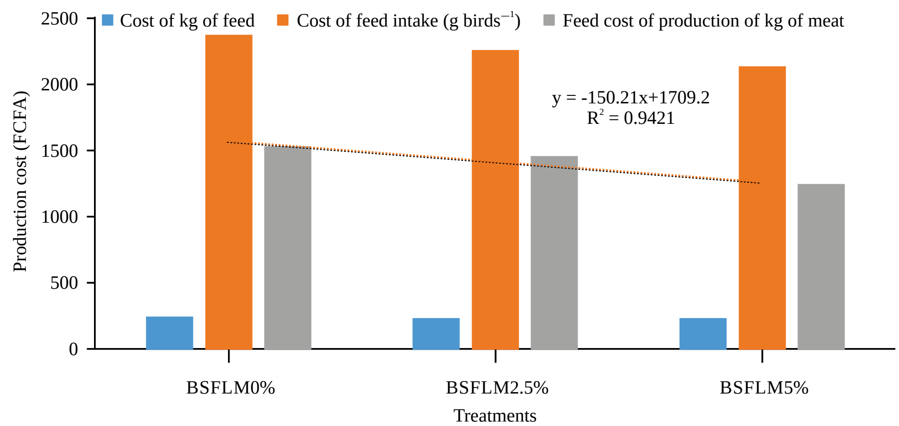


Fig. 1: Economic analysis of Brahma fed on diet containing various level of BSFLM

Table 8: Effects of BSFLM on the hematological parameters of Brahma chickens

	Rations			
Hematological parameters	BSFLM0%	BSFLM 2.5%	BSFLM 5%	p-value
White blood cells	67.20±7.92^{c}	29.00±1.41^{a}	46.20±1.98^{b}	0.010
Red cells	7.00±0.40^{b}	3.51±0.20^{a}	4.48±1.60^{ab}	0.040
Hemoglobin	23.80±1.98^{b}	9.80±1.98^{a}	14.00±3.96^{a}	0.030
Hematocrit	71.90±5.23^{b}	31.40±4.53^{a}	44.00±10.47^{a}	0.020
Mean corpuscular volume	111.50±4.95^{a}	124.00±9.9^{a}	113.50±4.95^{a}	0.300
Mean corpuscular hemoglobin content	33.95±0.92^{a}	28.20±7.21^{a}	31.65±2.33^{a}	0.500
Corpuscular hemoglobin concentration	30.40±0.57^{a}	23.10±7.78^{a}	27.95±0.92^{a}	0.380
Lymphocytes 1 (%)	23.80±5.94^{a}	15.18±1.67^{a}	19.60±3.96^{a}	0.270
Lymphocytes 2 (%)	35.79±3.78^{a}	51.65±2.33^{b}	42.28±6.75^{ab}	0.010
Monocytes 1 (%)	11.20±0.00^{c}	2.77±0.05^{a}	5.60±0.00^{b}	0.000
Monocytes 2 (%)	16.78±1.98^{b}	9.40±0.84^{a}	12.14±0.52^{a}	0.023
Granulocyte 1 (%)	32.20±1.98^{c}	11.06±0.21^{a}	21.00±1.98^{b}	0.002
Granulocyte 2 (%)	47.43±1.80^{a}	38.95±1.48^{a}	45.59±6.24^{a}	0.214

[a,b,c]Means with the same superscript letters are significantly different at 5% significant level

Table 9: Effects of BSFLM on bacterial colonies in Brahma chickens

	Rations			
Intestinal bacteria	FMSN0%	FMSN2.5%	FMSN5%	p-value
Enterobacteria	3.00*10±1.414^{a}	16.50*10±19.09^{a}	22.50*10±3.54^{a}	0.340
E. Coli	120.0*10±113.14^{a}	165.0*10±77.78^{a}	242.0*10±336.58^{a}	0.849
Salmonella spp.	11.50*10±9.192^{a}	9.00*10±8.485^{a}	9.00*10±5.657^{a}	0.937
Lactobacillus spp.	106.50*10±33.23^{a}	41.00*10±1.414^{a}	196.00*10±90.51^{a}	0.146

[a,b,c]Means with the same superscript letters are significantly different at 5% significant level

However, all the economic parameters tended to decrease with the incorporation of BSFLM in the diet. Hence, the diet containing the highest level of BSFLM recorded the lowest cost (Fig. 1).

DISCUSSION

This study evaluated the survival and growth of black soldier fly larvae reared on hatchery waste associated with three other organic waste streams (mango fruit waste, kitchen and poultry manures). Black soldier fly larvae have been shown to grow on a wide variety of substrates confirming that BSFL can upcycle low-value side streams into valuable biomass[11]. For this research, waste was selected based on its availability and non-competition with other forms of use. It was noted that the survival rate of BSFL was not significantly affected by the substrate combination (diet) tested. Waste reduction and bioconversion rates are the best indicators to determine the efficiency of the type of waste treated by BSFL[27,28]. Waste reduction is a value on a dry mass basis that is calculated from a difference between feed provided and residue then divided by feed provided during the experiment, whereas bioconversion rate is calculated by dividing larval weight gain (the final larval weight reduced by initial larval weight) by the total feed provided. The reduction rate recorded with hatchery waste associated with kitchen and mango waste were higher (87.5%) than that of chicken manure. This value was higher (65-75%) than those reported in previous studies using vegetables waste[29-31]. It can therefore be suggested that hatchery waste increases the rate of waste reduction by improving the digestibility of the substrates. This can be explained by a physicochemical and organoleptic modification of the substrates. In fact, bioconversion and waste reduction rate are affected by feed composition (amounts of digestible nutrients, pH and moisture content)[32,33].

The chicken manure associated with hatchery waste showed the highest larval weight. This value was higher than that reported by Mahamoudou[34], (0.18 g). This divergence can be explained by the chemical composition of our substrates which were richer in nutrients than those reported by Mahamoudou[34]. In order to manage these side streams and produce nutrient-rich larvae for livestock feed formulation, hatchery waste can be combined with other organic wastes to rear BSF larvae.

BSFLM is a suitable ingredient in aquaculture and livestock feeds. BSFLM-based feed has comparable and in some cases, superior palatability to feeds containing fish meal[11,35]. Results of the present study agree with a previous study conducted by Chia *et al.*[35] who observed normal growth performance in pigs fed BSFLM-based diets. In the present study, the average feed intake of the chickens was not affected by the incorporation level of the BSFLM in the diet. However, the chickens fed on the highest level of BSFLM (5%) consumed more feed than those fed a lower level of BSFLM even when fish meal was fully by BSFLM. Although not significant, this trend aligns with the clearly significant trends observed in pigs with fishmeal replacement at 100% resulting in the highest growth performance[35]. Furthermore, Onsongo *et al.*[36] did not observe the growth improvement (or inhibition) in poultry fed with BSFLM-based feeds. It can be concluded that the incorporation of BSFLM and the inclusion levels did not decrease the palatability of the diets as the much higher replacement levels (up to 100%) were acceptable and well tolerated by the chickens. Acceptability and suitability of BSFLM-based feeds have also been recorded in broilers[37]. Rejection of feed due to texture, palatability or inclusion of BSFLM was not observed, which is in line with the findings of Ramos-Elorduy *et al.*[38] for broiler chickens fed with *Tenebrio molitor*. On the contrary, Mwaniki *et al.*[39] reported an improvement in feed intake for chicken feed containing 5% defatted BSF larvae as compared to the control diet (without BSF larval meal). These discrepancies could be due to the fact that the aforementioned studies used defatted larval meal while we incorporated full-fat BSF larval meal. It is well documented that increasing fat content in the diet leads to a reduction in the feed intake in chickens[40].

In the present study, it is observed that the incorporation of BSFLM in the chicken diet did not affect the body weight, body weight gain and consumption index. This result is in close agreement with Sumbule *et al.*[41] who also did not observe significant effect of BSFLM inclusion in the feed of laying hens. We can therefore assume that BSFLM provided adequate nutrient level for chickens' growth. In contrary, Marono *et al.*[42] reported an increase in the growth performance of laying hens when fed diet containing BSFLM. Divergence between these studies can be attributed to differences in bird breed and nutrient content of BSFLM reared on different substrates.

Blood parameters are used to investigate the health conditions and welfare of birds[43]. The Brahma serum parameters were significantly affected by the incorporation of BSFLM in the diet. The concentration of liver enzymes (AST and ALT) was higher in the diet containing BSFLM as compared to the control diet. This result seems to indicate the adverse effects of the dietary inclusion of BSF larvae on liver function. However, these values (15-24 UI L^{-1} for ASAT and 17-39 UI L^{-1} for ALAT) were within the normal range for the species[44]. This result is contrary to the results obtained previously in broilers by Dabbou *et al.*[45]. On the other hand, renal functions through urea and creatinine were not significantly affected by the incorporation of BSF in the diet, suggests that the BSFLM did not impair renal function in Brahma chicken. Similar results were reported by Gariglio *et al.*[46] in Muscovy ducks fed with BSF larval meal. This result is in agreement with the findings of Marono *et al.*[42] who assessed the effect of BSFLM in laying hens. This result can be explained by the bioactive compounds (e.g., chitin) found in BSFL. Although chitin is not associated with kidney function, some studies have reported that chitosan, produced by deacetylation of chitin, was able to improve kidney function in rats and humans with a decrease in serum creatinine levels[47].

The plasma protein levels were significantly higher ($p<0.05$) with birds fed the BSFLM 2.5 diet. This suggests a higher bioavailability of dietary protein in this group compared to the rest of the batches. However, blood cholesterol levels appeared to be reduced as BSFLM inclusion levels increased. A similar result was reported in laying hens by Marono *et al.*[42]. This result could be explained by the chelating effect of chitin. Chitosan, a chitin derivate, reduces fat absorption from the gastrointestinal tract by binding with anionic carboxyl groups of fatty and bile acids and interferes with the emulsification of neutral lipids (i.e., cholesterol and other sterols) by binding them with hydrophobic bonds and decreasing the serum total cholesterol levels by 5.8-42.6% and low-density lipoprotein levels by 15.1-35.1%[48].

Incorporation of BSFLM had significant effects on albumin and globulin with the highest numerical values obtained in birds fed the ration containing 2.5% maggot flour. According to the results, the incorporation of larvae increased the values of globulin and albumin. A study by Veldkamp *et al.*[49] showed that chitin in insects increases antimicrobial activity resulting in increased activity of albumin and globulin. The positive effects of BSFL on the health of chickens would be related to chitin as reported by Bovera *et al.*[50]. The high level of globulin and a low albumin globulin ratio as recorded with the BSFLM 2.5 indicates better disease resistance and adequate immune response in birds[51]. High disease resistance and improved immune response were also found in partridges receiving BSFL as a replacement for soybean meal[52].

The incorporation of BSFL meal had significant effects ($p<0.05$) on the haematological parameters of Brahma chickens. In contrast, Madibana *et al.*[53] found that BSFLM had no significant effects on ducks. This divergence between the results could be due to the differences in species of birds used in the two studies. However, our values remained within physiological limits for the species as reported by Madibana *et al.*[54].

Bacterial colonies of *Salmonella* spp., *E. coli* and *Lactobacillus* spp. were studied to demonstrate disease resistance in chickens fed with BSFL meal. The results did not reveal any significant effects of the use of BSFLM on these bacteria. Nevertheless, the highest numerical values of enterobacteria and lactobacteria were recorded with the BSFLM5. These results indicate that diets with larvae could promote the development of colonies of *Enterobacteria* and *Lactobacillus* spp. Moreover, it demonstrates that the incorporation of larvae meal potentially inhibits the growth of pathological bacterial colonies (*Salmonella* spp). This result corroborates with the study of Veldkamp *et al.*[49].

Diet containing the highest BSFLM (5%) exhibited the lowest cost of production. This can be explained by the low price of BSFLM as compared to fishmeal. The production cost of per Kg live meat decreased with the level of BSFLM in the diet. These results are consistent with those of the Fagbohoun[50] and Sumbule *et al.*[41] who indicated that the incorporation of BSFLM improved the profitability of chicken farms.

CONCLUSION

This study has demonstrated that bioconversion of hatchery waste by combining it with other organic waste streams is possible, which provides a practical and promising method for converting these waste streams into valuable insect biomass for use in animal feed production. The highest larval biomass (0.26 g fresh^{-1} larva) was obtained when BSF larvae were fed with hatchery waste combined with chicken manure. Most importantly, this study has demonstrated that BSFLM can be used to fully replace fishmeal in Brahma chicken feed without impairing their growth parameters and microbial populations. It is important to emphasize that the protein sources in livestock feeds are critical components of the total production and they need to be selected with care while ensuring sustainability of the process and affordability for resource-limited producers. Our findings are relevant for sustainable organic waste reduction and the production of nutrient-rich insect biomass for local chicken production with minimal biochemical and haematological effects on the test organisms.

ACKNOWLEDGMENT

This study was partly funded by CLEVER CHICKEN.

REFERENCES

1. Fabio, A.D. and M.A. Rosen, 2020. An exploratory study of a new psychological instrument for evaluating sustainability: the sustainable development goals psychological inventory. Sustainability, Vol. 12, 10.3390/su12187617.

2. FAO., 2013. La contribution des insectes à la sécurité alimentaire, aux moyens de subsistance et à l'environnement. Document No. 13264F/1/04.13, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/3/i3264f/i3264f00.pdf.
3. INS, 2018. Annuaire statitisque du Cameroun. Institut National de Statistiques.
4. Ministere de L'Elevage, Des Peches ET Des Industries Animales, 2011. Document de strategie du sous-secteur de l'elevage, des pêches et des industries animales. Division des Etudes, des Statistiques et de la Coopération. https://faolex.fao.org/docs/pdf/cmr146473.pdf
5. van Huis, A., 2014. Insectes Comestibles: Perspectives Pour la Sécurité Alimentaire ET L'Alimentation Animale. FAO, France, Pages: 207.
6. Henchion, M., M. Hayes, A.M. Mullen, M. Fenelon and B. Tiwari, 2017. Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. Foods, Vol. 6. 10.3390/foods6070053.
7. Rumpold, B.A. and O.K. Schlüter, 2013. Nutritional composition and safety aspects of edible insects. Mol. Nutr. Food Res. 57: 802-823.
8. Oonincx, D.G.A.B., J.van Itterbeeck, M.J. W. Heetkamp, H.van den Brand, J.J.A. van Loon and A.van Huis, 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLoS One, Vol. 2010, 10.1371/journal.pone.0014445.
9. Oonincx, D.G.A.B., S. van Broekhoven, A. van Huis and J.J.A. van Loon, 2019. Feed conversion, survival and development and composition of four insect species on diets composed of food by-products. PLoS ONE, Vol. 14. 10.1371/journal.pone.0222043.
10. Sergiy, S., S. Eric and M. Alexander, 2019. Sustainable use of *Hermetia illucens* insect biomass for feed and food: Attributional and consequential life cycle assessment. Resour. Conserv. Recycl., 144: 285-296.
11. Dzepe, D., P. Nana, H.M. Kuietche, J.M. Kimpara, O. Magatsing, T. Tchuinkam and R. Djouaka, 2021. Feeding strategies for small-scale rearing black soldier fly larvae (*Hermetia illucens*) as organic waste recycler. SN Appl. Sci. Vol. 3. 10.1007/s42452-020-04039-5.
12. Magalhães, R., A. Sánchez-López, R.S. Leal, S. Martínez-Llorens, A. Oliva-Teles and H. Peres, 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture, 476: 79-85.
13. Gao, Z., W. Wang, X. Lu, F. Zhu, W. Liu, X. Wang and C. Lei, 2019. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. J. Cleaner Prod., 230: 974-980.
14. Mohd-Noor, S.N., C.Y. Wong, J.W. Lim, Y. Uemura and M.K. Lam *et al*., 2017. Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. Renewable Energy, 111: 646-654.
15. Rehman, K.U., C. Hollah, K. Wiesotzki, R.U. Rehman and A.U. Rehman, 2022. Black soldier fly, *Hermetia illucens* as a potential innovative and environmentally friendly tool for organic waste management: A mini-review. Waste Manag Res., 41: 81-97.
16. Amrul, N.F., I.K. Ahmad, N.E.A. Basri, F. Suja, N.A.A. Jalil and N.A. Azman, 2022. A review of organic waste treatment using black soldier fly (*Hermetia illucens*). Sustainability, Vol. 14, 10.3390/su14084565.
17. Glatz, P., Z. Miao and B. Rodda, 2011. Handling and treatment of poultry hatchery waste: A review. Sustainability, 3: 216-237.
18. Das, K.C., M.Y. Minkara, N.D. Melear and E.W. Tollner, 2014. Effect of poultry litter amendment on hatchery waste composting. J. Applied Poult. Res., 11: 282-290.
19. Sharara, H.H., H.Y. El-Hammady and H. Abd-El-Fattah, 1994. Nutritive value of some non-conventional by-products as poultry feed ingredients. Assiut J. Agric. Sci., 23: 349-366.
20. Tymczyna, L., A. Chmielowiec-Korzeniowska and L. Saba, 2000. Effect of a big pig farm on the physical and chemical properties of river and groundwater. Pol. J. Environ. Stud., 9: 97-102.
21. Chia, S.Y., C.M. Tanga, I.M. Osuga, S.A. Mohamed, F.M. Khamis *et al*., 2018. Effects of waste stream combinations from brewing industry on performance of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). PeerJ, Vol. 6. 10.7717/peerj.5885.
22. AOAC, 1995. Official Method of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA., pp: 1-18.
23. Pauwels, J.M., E. van Ranst, M. Verloo and A.M. Ze, 1992. Manuel de Laboratoire de Pédologie: Méthodes d'Analyse de Sols et de Plantes, Equipement, Gestion de Stocks de Verrerie et de Produits Chimiques. Publications Agricoles No. 28, Administration Générale de la Coopération au Développement (AGCD), Bruxelles, Belgium.
24. NRC., 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309048927, Pages: 155.
25. Abdel-Fattah, S.A., M.H. El-Sanhoury, N.M. El-Mednay and F. Abdel-Azeem, 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci., 7: 215-222.
26. Okosun, S.E. and S.A. Eguaoje, 2017. Growth performance, carcass response and cost benefit analysis of cockerel fed graded levels of cassava (*Manihot esculenta*) grit supplemented with Moringa (*Moringa oleifera*) leaf meal. Anim. Res. Int., 14: 2619-2628.

27. Gold, M., C.M. Cassar, C. Zurbrügg, M. Kreuzer and S. Boulos *et al*., 2020. Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates. Waste Manage., 102: 319-329.
28. Siddiqui, S.A., B. Ristow, T. Rahayu, N.S. Putra and N.W. Yuwono *et al*., 2022. Black soldier fly larvae (BSFL) and their affinity for organic waste processing. Waste Manage., 140: 1-13.
29. Newton, G.L., D.C. Sheppard, D.W. Watson, G.J. Burtle, C.R. Dove, J.K. Tomberlin and E.E. Thelen, 2005. The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Proceedings of the Symposium State of the Science, Animal Manure and Waste Management, January 5-7, 2005 0-5.
30. Nakamura, S., R.T. Ichiki, M. Shimoda and S. Morioka, 2016. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing. Applied Entomol. Zool., 51: 161-166.
31. Devic, E., W. Leschen, F. Murray and D.C. Little, 2017. Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. Aquacult. Nutr., 24: 416-423.
32. Banks, I.J., W.T. Gibson and M.M. Cameron, 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Trop. Med. Int. Health, 19: 14-22.
33. Dortmans, B.M.A., S. Diener, B.M. Verstappen and C. Zurbrügg, 2017. Black Soldier Fly Biowaste Processing - A Step-by-Step Guide. 2nd Edn., Eawag – Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland Pages: 87.
34. Koné, M., 2020. Study of the Composition of Residual Plant Organic Matter on Growth Performance, Bioconversion Balances and Nutritional Quality of Black Soldier Fly Larvae. Laval University, Quebec City, Canada, Pages: 86.
35. Chia, S.Y., C.M. Tanga, I.M. Osuga, A.O. Alaru and D.M. Mwangi *et al*., 2019. Effect of dietary replacement of fishmeal by insect meal on growth performance, blood profiles and economics of growing pigs in Kenya. Animals, Vol. 9, 10.3390/ani9100705.
36. Onsongo, V.O., I.M. Osuga, C.K. Gachuiri, A.M. Wachira, D.M. Miano *et al*., 2018. Insects for income generation through animal feed: effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. J. Econ. Entomol., 111: 1966-1973.
37. Vilela, J.de S., N.M. Andronicos, M. Kolakshyapati, M. Hilliar and T.Z. Sibanda *et al*., 2021. Black soldier fly larvae in broiler diets improve broiler performance and modulate the immune system. Anim. Nutr., 7: 695-706.
38. Ramos-Elorduy, J., E.A. González, A.R. Hernández and J.M. Pino, 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol., 95: 214-220.
39. Mwaniki, Z., M. Neijat and E. Kiarie, 2018. Egg production and quality responses of adding up to 7.5% defatted black soldier fly larvae meal in a corn–soybean meal diet fed to Shaver White Leghorns from wk 19 to 27 of age. Poult. Sci., 97: 2829-2835.
40. Herve, M.K., K.J. Raphaël, T.C. D'Alex, Y.M.D. Doriane, T. Alexis and D.H. Fuelefack, 2015. Effect of the energy level of the ration on the laying performance of barred hens (*Gallus gallus*) villagers. Livest. Res. Rural Dev., Vol. 27.
41. Sumbule, E.K., M.K. Ambula, I.M. Osuga, J.G. Changeh and D.M. Mwangi *et al*., 2021. Cost-effectiveness of black soldier fly larvae meal as substitute of fishmeal in diets for layer chicks and growers. Sustainability, Vo. 13. 10.3390/su13116074.
42. Marono, S., R. Loponte, P. Lombardi, G. Vassalotti and M.E. Pero *et al*., 2017. Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. Poult. Sci., 96: 1783-1790.
43. Pozzo, L., G. Salamano, E. Mellia, M.S. Gennero and L. Doglione, 2013. Feeding a diet contaminated with ochratoxin A for chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 1. Effects on growth and slaughter performance, haematological and serum traits. J. Anim. Physiol. Anim. Nutr., 97: 13-22.
44. Cruz, C.E.B., E.R. Freitas, N.de M. Braz, R.P.R. Salles and I.N.G. da Silva, 2018. Blood parameters and enzymatic and oxidative activity in the liver of chickens fed with calcium anacardate. Rev. Ciênc. Agron., 49: 343-352.
45. Dabbou, S., F. Gai, I. Biasato, M.T. Capucchio and E. Biasibetti *et al*., 2018. Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. J. Anim. Sci. Biotechnol., Vol. 9, 10.1186/s40104-018-0266-9.
46. Gariglio, M., S. Dabbou, M. Crispo, I. Biasato and F. Gai *et al*., 2019. Effects of the dietary inclusion of partially defatted black soldier fly (*Hermetia illucens*) meal on the blood chemistry and tissue (spleen, liver, thymus and bursa of fabricius) histology of muscovy ducks (*Cairina moschata domestica*). Animals, Vol. 9, 10.3390/ani9060307.
47. Ahmed, F.A., M.S. Abdel-Lattife, A.S.A. El-Azeem, A.M. Hegazy and M.A. Algalaly, 2014. The Role of Chitosan and Wheat Germ as Antidiabetic Substances in Diabetic Rats. Res. J. Pharm. Biol. Chem. Sci., 5: 457-469.

48. Ylitalo, R., S. Lehtinen, E. Wuolijoki, P. Ylitalo and T. Lehtimäki, 2011. Cholesterol-lowering properties and safety of chitosan. Arzneim.-Forsch., 52: 1-7.
49. Veldkamp, T., L. Dong, A. Paul and C. Govers, 2022. Bioactive properties of insect products for monogastric animals-A review. J. Insects Food Feed, 8: 1027-1040.
50. Bovera, F., R. Loponte, M.E. Pero, M.I. Cutrignelli and S. Calabrò *et al.*, 2018. Laying performance, blood profiles, nutrient digestibility and inner organs traits of hens fed an insect meal from *Hermetia illucens* larvae. Res. Vet. Sci., 120: 86-93.
51. Griminger, P. and C.G. Scanes, 1986. Protein Metabolism. In: Avian Physiology, Sturkie, P.D. (Ed.). 4th Edn., Springer, New York, USA., pp: 326-345.
52. Loponte, R., S. Nizza, F. Bovera, N.D. Riu and K. Fliegerova *et al.*, 2017. Growth performance, blood profiles and carcass traits of barbary partridge (*Alectoris barbara*) fed two different insect larvae meals (*Tenebrio molitor* and *Hermetia illucens*). Res. Vet. Sci., 115: 183-188.
53. Madibana, M.J., M. Mwanza, B.R. Lewis, C.H. Fouché, R. Toefy and V. Mlambo, 2020. Black soldier fly larvae meal as a fishmeal substitute in juvenile dusky kob diets: Effect on feed utilization, growth performance and blood parameters. Sustainability, Vol. 12, 10.3390/su12229460.
54. Madibana, M.J., V. Mlambo, B. Lewis and C. Fouche, 2017. Effect of graded levels of dietary seaweed (*Ulva* sp.) on growth, hematological and serum biochemical parameters in dusky kob, *Argyrosomus japonicus*, sciaenidae. Egypt. J. Aquatic Res., 43: 249-254.