Preliminary assessment of black soldier fly (Hermetia illucens) larval meal in the diet of African catfish (Clarias gariepinus): Impact on growth, body index, and hematological parameters

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Preliminary Assessment of Black Soldier Fly (Hermetia illucens) Larval Meal in the Diet of African catfish (Clarias gariepinus): Impact on Growth, Body Index and Haematological Parameters

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Abstract

A study was conducted to evaluate the effect of black soldier fly (Hermetia illucens) larval meal (BSF) in the practical diets for African catfish (Clarias gariepinus). Four diets were formulated to contain levels of fishmeal replacement at 0%, 25%, 50% and 100% with BSF. Three hundred and sixty fingerlings (2.67±0.0 g $fish^{-1}$) were randomly distributed into 12 tanks (30L capacity) containing 30 fish each, and fed with respective diet to apparent satiation. After six weeks of feeding, growth performance and nutrient utilization indices of the groups fed 25% and 50% BSF diets were not significantly different ($P>0.05$) from the group fed the fishmeal-based control diet. However, further increase in the replacement of fishmeal with BSF (100%) led to a significant ($P<0.05$) reduction in the growth, feed intake, and protein efficiency ratio of the catfish whereas, the body index and survival were not affected. The haematological parameters among the BSF fed groups showed no significant variation ($P>0.05$) compared with the control group. From the current study, it was clear that fishmeal could be replaced with black soldier fly larvae meal up to 50% without affecting the growth performance, nutrient utilization, survival, and welfare of C. gariepinus fingerlings.

Introduction

Aquaculture production (110.2 million tonnes as at 2016) facilitated in part by the availability of aquafeed, has been responsible for the significant growth in the supply of fish for human consumption (FAO, 2018). Fishmeal is considered as an important and essential protein source for use in the manufacture of aquafeeds. This is mainly due to its high quality protein content, excellent amino and fatty acids profile, high nutrient digestibility, general lack of anti-nutritional factors, palatability, component concentration and other attributes which contribute to feed intake, health and immune function in fish (Drew et al., 2007, Gatlin et al., 2007, Tacon and Metian, 2013). However, the finite nature, seasonal variation as well as rising cost of fishmeal is a limiting factor that will continue to affect the continuous supply of fishmeal to support the growth of aquaculture production (Golden et al., 2016). As the growing aquaculture industry cannot continue to rely on the finite stocks of wild-caught fish, the identification, development and the use of sustainable and alternate protein source to fishmeal remains a high priority.

Insects have been adjudged to be an eco-friendly, renewable and sustainable alternate protein source with an appealing quantity, quality and acceptable nutritional composition for the production of aquafeeds (Sánchez-Muros et al., 2014, Henry et al., 2015). Protein meal from insects is highly nutritious with high fat, protein and mineral contents depending on the species (Rumpold and Schlüter, 2013). It is easily digestible and has been reported to have high protein efficiency ratio in addition to been relatively cheaper than conventional protein sources (Ssepuuya et al., 2017). Larvae of insects such as locusts (Locusta migratoria), grasshoppers (Zonocerus variegatus), termites (Macrotermes spp.), yellow mealworms (Tenebrio molitor), Asiatic rhinoceros beetles (Oryctes rhinoceros), domesticated silkworms (Bombyx mori), housefly (Musca domestica), mosquitoes (Culex pipiens) and black soldier fly (Hermetia illucens) have been tested and found suitable as alternate protein source in the diets of farmed fish species (Alegbeleye et al., 2012; Aniebo et al., 2009; Fasakin et al., 2003; Gasco et al., 2016; Henry et al., 2015; Ipinmoroti et al., 2019; Kroeckel et al., 2012; Magalhães et al., 2017; Ogunji et al., 2007; Piccolo et al., 2017; Sogbesan & Ugwumba, 2008; Wang et al., 2017; Xiao et al., 2018).

Among other insects, black soldier fly (H. illucens) larval meal (BSF) has essential amino acids' pattern that is relatively similar to fishmeal (Henry et al., 2015) and the black soldier fly is an ideal candidate for sustainable mass production (Ssepuuya et al., 2017). The potential benefits of BSF have been demonstrated in many aquaculture species as a fishmeal replacement. Improved growth performance and immune indexes have been reported when BSF was used to replace fishmeal (at 48% inclusion level) in the diet of yellow catfish, Pelteobagrus fulvidraco (Xiao et al., 2018). In similar vein, there were no significant differences in the growth performance and nutrients utilisation of Jian carp, Cyprinus carpio var. Jian (Li et al., 2017, Zhou et al., 2018) and European seabass, Dicentrarchus labrax (Magalhães et al., 2017) fed fishmeal-based diets and BSF-based diets. The BSF has also been demonstrated as a valid alternate protein source capable of replacing up to 50% fishmeal in the diet of rainbow trout (Oncorhynchus mykiss) without impairing organosomatic indices nor fillet yield (Bruni et al., 2018). However, Kroeckel et al. (2012) reported reduced feed intake, reduced growth performance and feed conversion ratio in juvenile turbot (Psetta maxima) as the inclusion level of BSF increases.

Despite the potential of protein meal from black soldier fly larvae (BSF) for the manufacture of sustainable aquafeeds, little information exists on its effects and utilisation in African catfish (C. gariepinus), an important and dominant aquaculture species in sub-Sahara Africa. Though, meals from insects such as shea caterpillar (Cirina butyrospermi), housefly (Musca domestica) and variegated grasshopper (Zonocerus variegatus L.), have been included in the diets of African catfish (C. gariepinus) as alternate protein source (Fasakin et al., 2003, Aniebo et al., 2009, Alegbeleye et al., 2012, Olaleye, 2015, Anvo et al., 2017) but to the best understanding of the authors, no study has been reported on the dietary inclusion of BSF in the diet of African catfish (C. gariepinus). Furthermore, several studies done on insect protein basically neglect blood indices, a fundamental tool often used in assessing the welfare and health status of animal in response to diets (Jahanbakhshi et al. 2013; Fawole et al., 2017). Therefore, the objective of the study is to determine the effects of dietary inclusion level of black soldier fly (H. illucens) larval meal on the growth performance and haematological parameters of African catfish (C. gariepinus).

Materials and Methods

Experimental Design and Diets Preparation

The trial was carried out in a freshwater flow-through aquaculture system (2.5 L min^{-1} flow rate into the fish tank) of the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Nigeria. The flow-through system contains 12 rectangular tanks (30 L capacity each) and were supplied with freshwater from a deep well. Three hundred and sixty (360) African catfish (C. gariepinus) fingerlings of mean weight 2.67±0.0 g obtained from a reputable fish hatchery were randomly distributed (30 fish per tank) into the 12 tanks after two weeks of acclimatization. The photoperiod (~12 h: 12 h, light: dark) and water temperature (30.34±0.15 °C) was maintained at ambient condition throughout the experimental period.

Black soldier fly (H. illucens) larvae meal (MagMeal™ 50) was obtained from AgriProtein, South Africa and used for the study. The proximate and amino acids compositions of BSF larvae meal are presented in Table 1. Four diets were formulated as control (0% fishmeal replacement with MagMeal™ 50), 25% BSF (25% fishmeal replacement with MagMeal™ 50), 50% BSF (50% fishmeal replacement with MagMeal™ 50) and 100% BSF (100% fishmeal replacement with MagMeal™ 50) diets (Table 2). The feed ingredients were thoroughly mixed to form homogenous blend, moistened (200 mL kg^{-1}) and then cold pressed to produce 2mm pellets (flat die pelleting machine-CAPSFEED Nigeria). The diets were sundried and stored in airtight containers prior to use. Fish were fed twice a day (08.00 h and 16.00 h) to apparent satiation for six weeks. The proximate composition of the diet was analysed (Table 2) using AOAC protocols (AOAC, 1995). Briefly, the moisture content was determined by drying the samples in oven set to 105°C until constant weight was achieved, cooled in desiccator, and moisture content determined. For ash analysis, samples were weighed and placed in muffle furnace at 550°C for 8 h and ash content determined. The Soxhlet ether method was used for lipid analysis. The Kjeldahl method was used to determine the nitrogen content of the samples, and the crude protein content was determined. All samples were analysed in triplicate.

Growth, Feed Utilisation and Somatic Indices

At the end of the trial, growth performance, feed utilisation and somatic indices were assessed by final body weight (FBW), specific growth rate (SGR), metabolic growth rate (MGR), feed conversion ratio (FCR), protein efficiency ratio (PER). Three fish per tank (N=9) were randomly collected and euthanised with overdose of clove oil followed by brain destruction, after which they were sampled for liver weight, intestinal weight, and total length for the determination of hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor, respectively. All calculations were carried out using the formulae described below:

SGR (% day^{-1}) = 100 x (ln final body weight (g) − ln initial body weight (g))/feeding days

MGR (g $kg^{-0.8\ day-1}$) = (net weight gain in g)/ [{(initial body weight in g/1000)$^{0.8}$ + (final body weight in g/1000)$^{0.8}$}/2]/feeding days

FCR = feed intake (g) /wet weight gain (g)

PER = wet weight gain (g)/ protein ingested (g)

Condition factor = 100 × wet weight (g) / total length $(cm)^3$

HSI = 100 x (liver weight (g) / body weight (g))

VSI = 100 (visceral weight (g) / body weight (g))

Haematological Parameters

At the end of the feeding trial, three fish per tank (n = 9) were randomly selected for blood collection via the caudal arch using a 1 mL syringe which was previously rinsed with 10% ethylene diamine tetra acetic acid (EDTA) solution. The fish were anaesthetized with clove oil (100 mg L^{-1}) before blood collection. The collected blood was immediately transferred to an EDTA coated bottle and used for haematological analysis. Packed cells volume (PCV), haemoglobin, red blood cells (RBC), white blood cells (WBC) and differential leucocyte proportions were determined according to standard methods as described by Rawling et al. (2009).

Statistical Analysis

All data are presented as mean ± standard deviation. Data were analysed using one-way analysis of variance (ANOVA). Multiple comparisons were performed using Tukey post- hoc test. Differences were considered significant at a value of $P < 0.05$. The statistical analysis was carried out using SPSS for Windows (SPSS Inc., 24.0, Chicago, IL, USA).

Results

The growth performance, feed utilisation and somatic indices of African catfish (C. gariepinus) fed different experimental diets are shown in Table 3. There were no significant differences ($P > 0.05$) in the final body weight, specific growth rate, metabolic growth rate, feed conversion and protein efficiency ratios in fish fed the control (i.e. 100% fishmeal-based) diet and those fed diets containing 25% and 50% BSF fishmeal replacement. However, the fish fed diet containing 100% fishmeal replacement with BSF displayed significantly ($P < 0.05$) lower performance (in terms of their final body weight, specific growth rate, feed conversion and protein efficiency ratios) when compared with those fed 0%, 25% and 50% BSF diets.

The somatic indices (condition-factor, hepatosomatic and viscerosomatic indices) and survival of the African catfish (C. gariepinus) fed different experimental diets are shown in Table 3. The condition factors, hepatosomatic indices and survival among the various groups showed no differences ($P>0.05$) compared with fish fed the control (100 % fishmeal based) diet.

The haematological parameters of African catfish (C. gariepinus) fed the experimental diets are shown in Table 4. The haematological parameters such as haemoglobin, red blood cells counts, and packed cells volume, white blood cells counts, and differential leucocyte showed no significant difference ($P > 0.05$) between the fish fed the control (100% fishmeal-based) diet and those fed the BSF (fishmeal replacement) diets.

Discussion

This study is the first trial (to our understanding) of evaluation and assessment of black soldier fly larval meal (BSF) in the diet of African catfish (C. gariepinus), an important and dominant aquaculture species in sub-Sahara Africa. In this study, a partial replacement of fishmeal with BSF up to 50% level of inclusion would not impair the growth performance of African catfish (C. gariepinus). Similar findings have been reported in other aquaculture species fed diets supplemented with BSF (Li et al., 2017; Magalhães et al., 2017; Bruni et al., 2018; Xiao et al., 2018; Zhou et al., 2018). The growth performance observed could be attributed to essential amino acids pattern of BSF which is relatively similar to that of fish meal

(Henry et al., 2015), considered to be the gold standard protein with the best essential amino acids profile for fish (Tacon and Metian, 2013).

However, the reduced growth performance observed in 100% BSF group could be associated with low feed intake and poor acceptability of the diet by the fish, hence, resulting in reduced protein and energy intake required for African catfish (C. gariepinus) growth. Low acceptability and intake due to reduced palatability have been reported when fish meal is substantially or completely replaced in fish diet (Day and Gonzalez, 2000; Fournier et al., 2004; Espe et al., 2007; Nagel et al., 2012a; Nagel et al., 2012b). This finding is similar to the observation of Kroeckel et al. (2012) who reported reduction in the dietary feed intake (at ≥33% inclusion level of black soldier fly, BSF) and growth performance of juvenile turbot (Psetta maxima) when fed diets supplemented with increasing level of BSF (at 49% level of inclusion) in replacement for fishmeal. Also, a relative decreased level of methionine in the diet of 100% BSF group could contribute to the growth decline observed as reported by Xiao et al. (2018) that low level of methionine or lysine could cause reduced feed efficiency and growth in fish, hence, dietary supplementation with methionine at higher inclusion level may be necessary. Furthermore, chitin, an indigestible unbranched polymer of N-acetylglucosamine, have been reported to hinder digestibility in fish (Kroeckel et al., 2012; Magalhães et al., 2017) thereby reducing nutrient absorption, and this could be part of the reason for the sharp decline in growth noticed in 100% BSF group.

The insignificant difference (P>0.05) observed in the values of the somatic indices among the various groups could possibly indicates that feeding BSF to African catfish (C. gariepinus) had no negative impact on the liver functionality and no excessive hepatic accumulation of fat or carbohydrate as the values were within the normal range (1-2%) (Piccolo et al., 2017). This observation is in accord with previous findings when fishmeal was replaced with black soldier fly larvae meal in the diets of yellow catfish (Xiao et al., 2018). Contrarily, Piccolo et al. (2017) and Zhou et al. (2018) observed an increasing trend in HSI value for gilthead sea bream (Sparus aurata) and Jian carp (Cyprinus carpio var. Jian) as the level of yellow mealworms (T. molitor) and black soldier fly (H. illucens) larval meals increases, respectively. Furthermore, the condition factor and survival among the various groups showed no differences (P>0.05) compared with the control group, thus, it could be said that feeding BSF to African catfish (C. gariepinus) would not adversely impair the liver and survival of African catfish (C. gariepinus).

Haematological indices are important tools in monitoring the health and physiological conditions of fish in relation to ingredient assessment and suitability of feed (Fagbenro et al., 2013; Fawole et al., 2017). For instance, any alteration or a decrease in the level of erythrocyte and haemoglobin would impair the oxygen carrying capacity of the blood, hence impact negatively on the fish health. A decreased level of haematological parameter with increasing addition of sesame meal was reported in Clarias gariepinus, and this was associated with the presence of antinutritional factors in the seed meal (Fagbenro et al., 2013). However, in the present study, all haematological parameters such as haemoglobin, red blood cells counts and packed cells volume (which are indicators of health status in fish) (NRC, 2011) as well parameters such as white blood cells counts and differential leucocyte, (important parameters in the non-specific immunity of fish) were not markedly different among the various groups. This possibly indicates that the dietary replacement of fishmeal with BSF (even at 100%) do not have deleterious effect on African catfish (C. gariepinus) health and welfare. Similar observation was made by Ogunji et al. (2007) in Nile tilapia fed maggot meal base diet.

In conclusion, this study indicates that up to 50% fishmeal can be replaced with black soldier fly (H. illucens) larval meal (BSF) without any adverse effect on the growth performance, survival and welfare of African catfish (C. gariepinus). Thus, black soldier fly (BSF) can be utilised as a good potential alternative to fishmeal in the diets of African catfish

(C. gariepinus). The authors do however recognise that digestibility trial would have been very informative for further assessment and evaluation of the suitability of BSF as an ingredient in the diet of African catfish (C. gariepinus). As the current study is more or less a preliminary one, further study is warranted to be more comprehensive and expanded in scope.

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Tables

Table 1. Proximate Composition (% DM) and Amino Acids Profile of Black Soldier Fly (Hermetia illucens) Larval Meal (g $100g^{-1}$ DM)

Variables	Black soldier fly larval meal
Crude protein	42.6
Crude fat	23.0
Moisture	8.2
Ash	10.5
Crude fibre	10.8
NFE	4.9
Energy KJ Kg^{-1}	5218
Calcium	3.34
Phosphorus	0.56
Indispensable amino acids (g $100g^{-1}$)	
Arginine	1.29
Histidine	1.68
Isoleucine	2.36
Leucine	3.43
Lysine	3.91
Methionine	1.08
Phenylalanine	2.07
Threonine	1.84
Tryptophan	0.97
Valine	2.94

Dispensable amino acids (g $100g^{-1}$)	
Alanine	3.78
Aspartic acid	4.16
Cysteine	2.43
Glutamic Acid	5.64
Glycine	2.63
Proline	2.82
Serine	2.23
Tyrosine	4.35

Source: AgriProtein, South Africa

Table 2. Formulation and composition of the experimental diets

Ingredients (g kg^{-1})	0% BSF	25% BSF	50% BSF	100% BSF
Fish meal (72% CP)	150.00	112.50	75.00	0.00
[a]MagMeal™ 50	0.00	37.50	75.00	150.00
Krill meal	10.00	10.00	10.00	10.00
Soybean meal (solvent extracted)	400.00	400.00	400.00	400.00
Poultry meal	212.00	221.00	230.00	247.90
Maize	152.90	148.90	144.90	140.00
Vegetable oil	50.00	45.00	40.00	27.00
[b]Vitamins minerals premix	20.00	20.00	20.00	20.00
[c]Antioxidant	0.10	0.10	0.10	0.10
Binder (cassava flour)	5.00	5.00	5.00	5.00
Total	1000	1000	1000	1000
Composition (% dry weight)				
Moisture	8.83	7.60	8.13	8.07
Crude protein	42.60	42.35	42.86	41.25
Lipid	13.19	12.86	9.89	8.70
Ash	7.11	7.48	7.66	7.69
[d]Calculated EAA content (g $100g^{-1}$ diet)				
Arginine	2.89	2.84	2.79	2.69
Histidine	1.15	1.17	1.18	1.20
Isoleucine	1.64	1.65	1.66	1.67
Leucine	3.00	2.99	2.97	2.95

Lysine	2.57	2.56	2.55	2.54
Methionine	0.75	0.73	0.71	0.68
Phenylalanine	1.79	1.79	1.79	1.79
Threonine	1.58	1.56	1.55	1.52
Valine	2.00	2.02	2.03	2.07

[a]MagMeal™ 50 - Larval meal of black soldier fly (H. illucens) produces by AgriProtein. [b]Vitamins minerals premix contains (per 2.5kg) 20,000,000IU vitamin A, 4,000,000IU vitamin D3, 200,000 vitamin E, 8,000mg vitamin K3, 20,500mg vitamin B1, 15,000 mg vitamin B2, 19,500 mg vitamin B6, 15mcg vitamin B12, 90,000 mg Nicotinic Acid, 40,000 mg Pantothenic Acid, 500 mg Folic Acid, 600,000 mcg Biotin, 40,000 mg Choline Chloride, 4,000 mg Iron, 500 mg Copper, 30,000 mg Manganese, 40,000 mg Zinc, 2,000 mg Iodine, 200 mcg Selenium, 300,000 mg coated Vitamin C, 50,000 mg Inositol, 750 mg Cobalt, 50,000 mg Lysine, 50,000 mg Methionine and 125,000 mg Antioxidant. [c]Antioxidant contains food grade sodium metabisulfite. [d]Calculated based on amino acid composition of Fishmeal, Krill meal, Poultry meal, Soybean meal and Maize (NRC, 2011).

Table 3. Growth, Feed Utilisation and Somatic Indices of C. gariepinus fed the Experimental Diets

	0% BSF	25% BSF	50% BSF	100% BSF	P – value
IBW (g $fish^{-1}$)	2.67±0.00	2.67±0.00	2.67±0.00	2.67±0.00	0.000
FBW (g $fish^{-1}$)	13.82±0.18[a]	10.54±1.34[a]	11.37±0.28[a]	5.47±0.32[b]	0.002
SGR (% day^{-1})	5.48±0.06[a]	4.55±0.60[a]	4.83±0.10[a]	2.39±0.27[b]	0.001
MGR (g $kg^{-0.8}$ day^{-1})	18.02±0.14[a]	14.93±1.42[a]	15.86±0.27[a]	7.69±0.64[b]	0.014
FI (g $fish^{-1}$)	13.16±1.43[a]	11.04±1.04[a]	11.74±0.42[a]	7.62±1.07[b]	0.001
FCR	1.22±0.1[a]	1.41±0.24[a]	1.29±0.05[a]	2.96±0.3[b]	0.002
PER	1.55±0.20[a]	1.28±0.29[a]	1.41±0.07[a]	0.41±0.08[b]	0.003
HSI	1.06±0.18	1.13±0.13	1.03±0.11	1.35±0.18	0.340
VSI	8.56±0.54	7.47±0.35	8.77±0.93	10.71±1.83	0.225
Condition factor	0.80±0.07	0.83±0.07	0.90±0.09	0.86±0.08	0.567
Survival (%)	83.33±3.33	81.67±1.67	88.33±5.00	76.67±3.33	0.905

Means in the same row with different superscripts are significantly different ($P < 0.05$). IBW, initial mean body weight; FBW, final mean body weight; SGR, specific growth rate; MGR, metabolic growth rate; FI, feed intake; FCR, feed conversion ratio; PER, protein efficient ratio; HSI, hepatosomatic index and VSI, viscera-somatic index

Table 4. Haematological Parameters of C. gariepinus fed the Experimental Diets

	0% BSF	25% BSF	50% BSF	100% BSF	P – value
Haemoglobin ($g\ dL^{-1}$)	10.18±1.23	10.20±1.36	10.42±1.79	10.40±0.81	0.905
RBC ($10^{12}\ L^{-1}$)	2.84±0.35	2.91±0.39	2.85±0.55	2.97±0.20	0.988
Haematocrit (%PCV)	30.50±3.73	31.83±3.44	31.17±5.61	30.67±2.29	0.877
WBC ($10^{9}\ L^{-1}$)	140.10±30.06	130.48±33.82	125.37±49.20	151.93±30.75	0.758
Neutrophil (%)	33.00±8.76	29.67±10.06	27.17±15.50	37.83±12.00	0.689
Lymphocytes (%)	62.50±8.08	65.50±9.36	69.67±16.98	59.00±11.65	0.703
Basophil (%)	1.00±0.00	2.00±0.00	1.00±0.00	1.25±0.43	0.344
Eosinophil (%)	2.80±1.47	2.50±1.50	1.60±0.49	1.50±0.50	0.276
Monocytes (%)	2.00±1.15	3.33±1.49	2.25±0.83	1.60±0.80	0.147
MCV (fL)	107.82±6.36	110.15±5.20	110.02±8.62	103.30±4.03	0.435
MCH (pg)	35.98±1.70	35.32±3.23	36.83±2.85	35.05±1.48	0.540
MCHC $g\ dL^{-1}$)	32.42±1.63	32.13±3.28	31.42±1.81	33.85±0.16	0.435

There are no significant differences (*P > 0.05*) across the treatments. RBC, red blood cells; PCV, packed cells volume; WBC, leucocytes; %, mean percentage of total leucocytes; MCV, mean corpuscular volume (haematocrit (%PCV) × 10) / RBC ($10^{9}\ L^{-1}$); MCH, mean corpuscular haemoglobin (haemoglobin ($g\ dL^{-1}$) × 10) / RBC ($10^{12}\ L^{-1}$); MCHC, mean corpuscular haemoglobin concentration (haemoglobin ($g\ dL^{-1}$) × 100) / haematocrit (%PCV)