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Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias gariepinus*): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker

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ABSTRACT

A 60-day experiment was conducted to examine the effect of fishmeal substitution with black soldier fly larvae (BSFL) meal on the growth performance, nutrient utilization, haematology, serum biochemistry, and oxidative status of *Clarias gariepinus* fingerlings. Four diets were formulated to be isonitrogenous (418 g Kg^{-1} crude protein), isolipidic (95 g Kg^{-1} crude lipid) and isocaloric (20.14 MJ Kg^{-1} gross energy). The control diet was formulated using fishmeal (FM) as the principal protein source and was progressively substituted with *Hermetia illucens* (hereafter referred to as HP) larvae meal at 25%, 50%, and 75%, on equivalent protein basis, corresponding to dietary inclusion level of 57, 115, and 172 g Kg^{-1}, respectively. A total of 180 fingerlings of African catfish (*Clarias gariepinus*) was used for the trial and distributed randomly to twelve plastic tanks each containing 15 (4.0 ± 0.01 g/fish) fish. At the end of the trial, the fish fed HP 50% recorded the highest final body weight, weight gain, weight gain percentage, and specific growth rate compared to other groups ($p < .05$). The nutrient utilization indices such as feed conversion ratio, protein efficiency ratio, and protein productive value were found to be better in fish fed HP 50% compared with the control but no significant effect ($p > .05$) was noticed on the feed intake. A significantly higher whole-body protein was recorded in HP 50%, whereas lipid and ash contents showed no variation ($p > .05$). The blood parameters and differential leucocyte counts were not statistically different; however, serum total protein, globulin, glucose, triglycerides, and total bilirubin differed significantly among the dietary groups ($p < .05$). The liver function enzyme (AST and ALT) showed that the fish fed HP 50% recorded the lowest value. The results of the oxidative stress biomarker indicate that the fish fed HP-based diet had similar malondialdehyde (MDA) content with the control, but the superoxide dismutase and catalase enzyme activities were found to be higher in the control and HP 50%, respectively. Summarily, the results of the current study showed that African catfish can effectively utilize *Hermetia illucens* up to 172 g Kg^{-1} (75% FM replacement) without impairing growth, nutrient utilization, antioxidant, and health status of the fish.

1. Introduction

The world aquaculture output has grown tremendously in recent years and currently contributes over half (about 53%) of the total human-consumed food fish (FAO, 2018). This growth has been facilitated by increased feed-based intensive fish culture system. However, formulated feeds used in intensive aquaculture are among the most expensive animal feeds in the market, and this is due to the high cost of protein source ingredients used in the production of aquafeed. Of the protein source ingredient, fishmeal takes the largest share owing to its outstanding nutritional quality, which is compatible with the requirement of most cultured fish species. But, the sustainable supply of fishmeal for use in aquafeed has come under tremendous pressure due to increasing demand but limited supply thereof. Hence, continued dependency on these finite aquatic resources may not support the current growth been experienced in the aquaculture industry. Now, efforts are being made on replacing limited fish-based feed resources with plant-based materials. However, plant protein could not replace higher amount of fishmeal due to the presence of non-starch polysaccharide (NSP) and high fibre content, imbalance amino acid profile, and associated limitation (i.e anti-nutritional substances) (Francis et al., 2001; Sinha et al., 2011); therefore, there is an urgent need to explore other sustainable solutions.

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Insect has been adjudged as a suitable protein for animal feeding and comparable to other conventional protein source ingredient especially fishmeal and soybean meal in terms of amino acids composition, lipid, vitamins, and mineral (Henry et al., 2015). Insects form part of the diet of many fish species in nature (Tran et al., 2015), and some findings had shown the adaptability of cultured fish to insect protein with comparable performance to those fed conventional protein sources (Anvo et al., 2017; Li et al., 2017; Dietz and Liebert, 2018; Belghit et al., 2018, 2019). Among the promising insect species investigated so far, *Hermetia illucens* has been identified as a suitable candidate for sustainable mass production because of its omnivorous nature and ability to assimilate nutrients from a variety of organic wastes, and turn them into a high-quality nutrients (protein > 40% and lipid > 30%) for animal feed (St-Hilaire et al., 2007; Yu et al., 2009; Makkar et al., 2014; Henry et al., 2015).

The potential of *Hermetia illucens* larvae meal as an alternative protein source ingredient has been tested in different fish species. Belghit et al. (2019) reported that *H. illucens* larvae meal can replace fishmeal completely in the diet of Atlantic salmon without any negative impact on fish performance and digestibility. Similarly, Dietz and Liebert (2018) and Xiao et al. (2018) were able to substitute 48% and 50% of fishmeal and soy protein concentrate in the diet of yellow catfish and Nile tilapia, respectively. Although, studies regarding yellow catfish fed *H. illucens* has been done (Xiao et al., 2018), but little or no empirical data is available on African catfish, *Clarias gariepinus,* the most commercially preferred freshwater fish among farmers and consumers in Nigeria. Furthermore, the little investigation so far done on insect protein using African catfish (Fasakin et al., 2003; Alegbeleye et al., 2012; Anvo et al., 2017) basically evaluate the animal performance and nutrient digestibility and often neglect the blood profile, serum biochemistry and oxidative status. Thus, the goal of this study is to evaluate the dietary impact of *H. illucens* (black soldier fly) as a replacement for fishmeal on the growth performance, nutrient utilization, haematology, serum biochemistry, and oxidative stress response of African catfish, *Clarias gariepinus*.

2. Materials and methods

2.1. Diet preparation and experimental setup

Partially defatted *Hermetia illucens* larvae meal (crude protein: 426 g Kg^{-1} dry matter; crude lipid: 230 g Kg^{-1}) supplied by AgricProtein™ (Cape Town, South Africa), and the other ingredients purchased from Teejay Feed Industries, Ilorin, Nigeria, were used for the feed formulation. The insect meal was made from dried, heat-treated *Hermetia illucens* larvae and the composition is shown beneath Table 1. The producer provided no information on the culture procedure and feeding substrate used for the insect rearing. Four diets were formulated to be isonitrogenous (418 g Kg^{-1} crude protein), isolipidic (95 g Kg^{-1} crude lipid) and isocaloric (20.14 MJ Kg^{-1} gross energy). The control diet was prepared using fishmeal (FM) as the principal protein source and was progressively substituted with *Hermetia illucens* larvae meal at 25%, 50% and 75%, on equivalent protein basis, corresponding to dietary inclusion level of 57, 115, and 172 g Kg^{-1}, respectively (Table 1). The ingredients were measured and weighed based on their proportion, mixed thoroughly to ensure homogenous blend, and water (200 mL Kg^{-1}) was added. The dough was passed through a 2 mm die attached to a compressed pelletizer to produce a sinking pellet, which was air-dried and packed into a labelled, airtight container prior to use. Proximate analysis of the formulated diets was carried out to ensure that the expected values were achieved (AOAC, 2005) and are presented in Table 1.

The feeding trial was conducted at the Department of Aquaculture and Fisheries, University of Ilorin (8.4912° N, 4.5950° E), Nigeria. The fingerlings of African catfish (*Clarias gariepinus*) used for the experiment were sourced from a local fish hatchery (Members of Standard Fish Alliance, Ilorin, Nigeria) and were acclimated into the experimental rearing system for several weeks prior to the start of the feeding trial. Twelve (12) rectangular plastic tanks of size 45 × 32 × 23 cm were used for the feeding trial. Each tank was filled with bore-well water and connected to an air pump (pressure > 0.03 Mpa; output:70 L/min) to ensure good aeration. One hundred and eighty fish (average weight 4.00 ± 0.01 g/fish) were separated into 4 distinct groups in triplicate with each replicate tank containing 15 fish and fed with their respective diet for 60 days. The fish were hand-fed to apparent satiation with the test diets twice a day (0900 and 1600 h). The water quality variables were observed weekly (pH, 7.5 ± 0.02 & dissolved oxygen, 5.10 ± 0.01 mg L^{-1}), except temperature which was taken daily (26.61 ± 0.01 °C). The fish were maintained under natural photoperiod and observed dead fish were removed daily. Prior to the commencement of the experiment, 10 fish were sampled for initial body composition following the AOAC (2005) procedure.

Table 1
Ingredient and proximate composition of the experimental diets fed to African catfish (as-fed basis).

	Diets			
Ingredient (g Kg^{-1})	FM	HP 25%	HP 50%	HP 75%
Fishmeal	150	112.5	75	37.5
[a]*Hermetia illucens* larvae meal	0	57.2	114.5	171.8
Soybean meal	360	360	360	360
Poultry meal	220	220	220	220
Wheat bran	40	40	40	40
Maize	140	130.3	121.5	112.7
Vegetable oil	50	40	29	18
Fish premix	20	20	20	20
Vitamin C	10	10	10	10
Starch (Binder)	10	10	10	10
Total	1000	1000	1000	1000
Proximate composition (g Kg^{-1})				
Dry matter	895.59	908.68	894.68	906.59
Crude protein	424.03	417.02	420.66	410.84
Crude lipid	96.33	94.01	92.96	97.71
Ash	76.11	78.35	85.58	82.88
NFE	359.46	366.39	357.60	361.28
GE (MJ Kg^{-1})	20.30	20.16	20.05	20.07

Fish Premix: Vitamin A; 20,000,000 l,U, Vitamin D_3; 4,000,000 l,U, Vitamin E; 200,000, l,U, Vitamin K; 1200 mgr, Vitamin B_1; 10,000 mgr, Vitamin B_2; 30,000 mgr, Vitamin B_6; 19,000 mgr, Vitamin B_{12}; 1000 mgr, Vitamin C; 150 g, Niacin; 200,000 mgr, Folic acid; 5000 mgr; Panth acid; 50,000 mgr, Biotin; 400 mgr, Antioxidant; 125 g, Choline Chloride; 400 g, Manganese; 30 g, Zinc; 40 g, Iron; 40 g, Copper; 4 g, Iodine: 5 g, Selenium; 0.2 mgr, Calcium; 600 g, Lysine; 100 g, Methionine; 100 g (source; Henan Lvyuan Pharmaceutical Company Limited).

[a] *Hermetia illucens* larvae meal (Agriprotein Pvt., South Africa): Crude protein (42.6%), crude lipid (23%), Ash (10.5%), Crude fibre (10.8%), NFE (4.9%). Essential amino acid composition (g/100 g DM): arginine 1.29; histidine 1.68; isoleucine 2.36; leucine 3.43; lysine 3.91; phenylalanine 2.07; methionine 1.08; threonine 1.84; tryptophan 0.97; valine 2.94. HP: *Hermetia illucens* protein, FM: Fishmeal, NFE: Nitrogen free extract, GE: Gross energy = [23.9 × CP (g/kg) + 39.8 × CL (g/kg) + 17.6 × NFE (g/kg)]/1,000, FM: 100% fishmeal.

2.2. Sampling procedure

At the end of the feeding trial, fish were unfed for 24 h and batched weighed for the determination of final body weight, and other growth performance indices were calculated. For the blood and serum collection, 2 fish per tank (n = 6) were randomly selected and anaesthetized with 100 mg L^{-1} clove oil (Fernandes et al., 2017). The blood sample was collected from the fish via the caudal vein using a 1 mL hypodermic syringe and transferred to an EDTA-coated tube for the haematological investigation. Fresh blood was drawn from another 3 fish per tank (n = 9) without anti-coagulant, kept in ice for 4 h, serum separated by

centrifugation (3000 x*g* for 10 min at 4 °C) and stored in −20 °C for subsequent biochemical analysis. Additional 3 fish per tank were sampled for the whole-body composition and analysed as per the AOAC (2005) procedure.

2.3. Haematology and serum biochemical analysis

The haemoglobin, erythrocyte count, total leucocyte count, and differential leucocyte proportions were determined according to standard methods (Bain et al., 2017). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as previously described by Haney et al. (1992). The serum samples were used for the determination of total protein, albumin, glucose, triglyceride, cholesterol, total bilirubin, creatinine, total antioxidant, aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase, superoxide dismutase, catalase, and malondialdehyde using a commercial diagnostic kit and according to the manufacturers protocol (Randox Laboratories, United Kingdom).

2.4. Calculations and statistical analysis

The formula's documented below were used for the evaluation of growth performance, nutrient utilization and body indexes among the various experimental groups.

Weight gain (WG) = final body weight (g) − initial body wight (g).

Percentage Weight Gain (*WG%*)=[(final body weight (g) − initial body weight (g)) / initial body weight (g)] × 100

Specific growth rate (SGR,%/day) = [(ln final body weight (g)–ln initial body weight (g)) /number of feeding days] × 100

Feed intake (FI; g/fish) = total dry feed given (g)/number of fish

Feed conversion ratio (FCR) = [feed consumption (g)/body weight gain (g)]

Protein efficiency ratio (PER) = [net weight gain (g)/protein fed (g)]

Protein productive value (PPV) = 100 × [(final body weight × final body protein content) − (initial body weight × initial body protein content)] /(protein content in diet × total feed intake)

Hepatosomatic index (HSI) = 100 × wet weight of liver (g)/whole body weight of fish (g)

Viscerosomatic index (VSI) = 100 × wet weight of visceral (g)/whole body weight of fish (g)

Dressing percentage (DP) = [total weight without gut and gonad (g)/fish body weight (g)] × 100

Survival (%) = [(Total number of fish harvested/total number of fish stocked) × 100]

All data were checked for normality using the Kolmogorov–Smirnov test, and homogeneity of variances among different treatments was tested using the Barlett's test. Then, data were subjected to one-way analysis of variance to test for the significant difference in the means; where means differ significantly, Duncan's multiple range test was used to separate the means. The analysis was performed using the IBM SPSS (Statistical Package for Social Sciences) version 20. The confidence level was set at $p < .05$ and values were expressed as Means ± pooled standard error of the mean.

Table 2
Growth performance, nutrient utilization and body indexes of African catfish (*Clarias gariepinus*) fed *Hermetia illucens* larvae meal.

	Diets					
Variables	FM	HP 25%	HP 50%	HP 75%	SEM	*p*-value
Initial body weight (g)	3.97	4.00	4.01	4.00	0.01	0.797
Final body weight (g)	14.79^{a}	16.05^{a}	19.84^{b}	16.74^{a}	0.64	0.005
Weight gain (g)	10.82^{a}	12.05^{a}	15.83^{b}	12.74^{a}	0.63	0.005
Weight gain %	272.42^{a}	301.59^{a}	394.40^{b}	318.77^{a}	15.34	0.005
SGR (%)	2.19^{a}	2.32^{a}	2.66^{b}	2.39^{a}	0.06	0.006
FI (g feed per fish)	20.09	21.38	23.26	21.01	0.47	0.086
PER	1.29^{a}	1.35^{a}	1.62^{b}	1.46^{ab}	0.05	0.030
FCR	1.86^{b}	1.78^{b}	1.48^{a}	1.65^{ab}	0.05	0.039
PPV	22.67^{a}	23.93^{a}	28.99^{b}	24.98^{a}	0.85	0.018
Survival (%)	80.00	80.00	60.00	80.00	3.68	0.116
Body indexes						
HSI	0.86	1.00	1.02	0.99	0.06	0.834
VSI	3.78	4.95	4.38	5.30	0.25	0.148
DP	84.51	86.96	87.92	87.69	0.91	0.590

Mean values with different superscript in the same row are significantly different ($p < .05$). SEM – Pooled Standard error of the mean. HP: *Hermetia illucens* protein, FM: Fishmeal, SGR: Specific growth rate, FI: Feed intake, PER: Protein efficiency ratio, FCR: Feed conversion ratio, PPV: Protein productive value, HSI: Hepatosomatic index, VSI: Viscerosomatic index, DP: Dressing percentage.

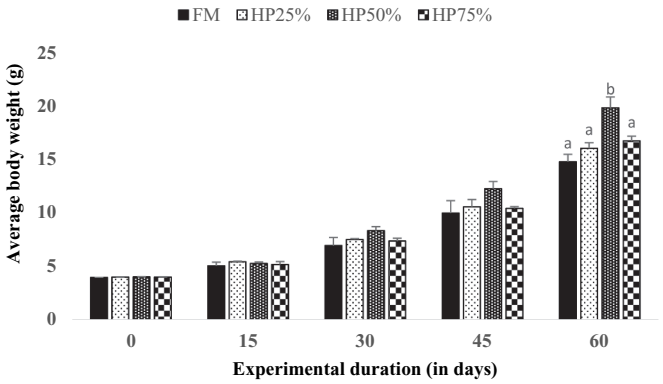


Fig. 1. Fortnightly weight increases of *Clarias gariepinus* fed different diets for 60 days. Values are represented as Mean ± S.E. of triplicate groups of fish. Values within the same period with different superscript letters are significantly different ($P < .05$).

3. Results

3.1. Growth performance, nutrient utilization and somatic indices

The effects of *Hermetia illucens* on the growth performance and other biological response indices are presented in Table 2. The fortnightly weight increases of the fish fed different diets are shown in Fig. 1. At the end of the 60 days trial, the fish fed HP 50% recorded the highest final body weight, weight gain, WG%, SGR, and PPV compared to other groups ($p < .05$). No significant difference was noticed in the feed intake and survival of all the fish fed the experimental diets. However, the highest protein efficiency ratio value was observed in fish fed with HP 50% which is similar to HP 75% but differ significantly from the control and HP 25%. The feed conversion ratio showed a reversed trend

Table 3
Whole body (wet basis) composition of African catfish (*Clarias gariepinus*) fed different experimental diets.

	Diets					
Variables	FM	HP 25%	HP 50%	HP 75%	SEM	p-value
Moisture	75.31[a]	75.54[a]	75.94[a]	76.72[b]	0.22	0.025
Crude protein	16.82[ab]	17.03[b]	17.30[c]	16.59[a]	0.10	0.005
Crude lipid	5.30	5.66	4.76	5.05	0.24	0.721
Ash	2.57	1.77	2.03	1.64	0.19	0.379

Mean value with different superscript in the same row are significantly different ($p < .05$). SEM – Pooled Standard error of the mean. HP: *Hermetia illucens* protein,

with the highest value recorded in the control and HP 25% compared to the lowest value found in HP 50% ($p < .05$). No dietary impacts on the hepatosomatic index (HSI), viscerosomatic index (VSI) and dressing percentage (DP) were observed among the groups.

3.2. Whole-body composition

The whole-body composition of different feeding groups is presented in Table 3. The crude protein contents of the fish fed HP 50% was significantly higher than that of HP 75% which recorded the lowest value like the control. There were no significant differences in the whole-body lipid and ash contents among the various groups, but the moisture content showed a significant variation with the highest value recorded in HP 75%.

3.3. Haematology and serum biochemical parameters

There were no significant differences detected in the packed cell volume, Hb, WBC, RBC, MCV, MCH, MCHC, neutrophil, lymphocytes, and monocytes values among the groups (Table 4). The serum total protein showed significant lower value in HP 50% compared to other groups (Table 5). The globulin and glucose contents of the fish fed HP 50% recorded the lowest values compared with HP 25% and the control which registered significantly higher value, respectively. A significantly higher triglyceride and total bilirubin were observed in *C. gariepinus* fed with the control diet, whereas HP 50% recorded the lowest value. The albumin, A/G ratio, cholesterol, total antioxidant, and creatinine values among the various dietary groups did not differ significantly. The aspartate aminotransferase (AST) enzyme activity showed that the group fed with the control diet registered the highest value while HP 50% recorded the lowest value. A similar trend was noticed in alanine aminotransferase (ALT) activity with the fish fed HP 50% showing significantly ($p < .05$) lowest activity in the serum. The alkaline phosphatase (ALP) enzyme activities among the groups fed HP-based diets were not different but varied significantly from the control.

Table 4
Haematological parameters of African catfish (*Clarias gariepinus*) fed *Hermetia illucen* larvae meal.

	Diets					
Variables	FM	HP 25%	HP 50%	HP 75%	SEM	p-value
PCV (%)	30.33	31.67	30.00	29.00	0.89	0.816
HB (g dL^{-1})	8.97	9.10	8.80	8.30	0.35	0.897
WBC (x$10^9 L^{-1}$)	11.87	8.73	7.97	8.77	1.13	0.689
RBC (x10^{12} L^{-1})	4.89	5.12	4.58	4.31	0.23	0.678
MCV (Fl)	62.33	61.67	65.33	68.00	1.42	0.408
MCH (pg)	18.40	17.73	19.37	19.33	0.29	0.101
MCHC (g dL^{-1})	29.50	28.67	29.27	28.33	0.35	0.690
Neutrophil (%)	35.33	29.33	36.67	48.00	3.51	0.328
Lymphocyte (%)	63.00	68.67	61.33	51.00	3.42	0.359
Monocyte (%)	1.67	1.33	2.00	0.67	0.26	0.341

Mean values with no superscript letter in the same row are not significantly different ($p > .05$). SEM – Pooled Standard error of the mean. HP: *Hermetia illucens* protein, PCV: Packed cells volume, WBC: White Blood Cell, HB: Haemoglobin, RBC: Red Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Concentration Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration,

3.4. Oxidative stress biomarker in the serum

The results of the superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) among the groups fed different experimental diets are depicted in Table 5. The SOD activity was found to be higher in the control compared to HP 25% and HP 75% which recorded the lowest activities ($p < .05$). A reverse trend was noticed in the catalase with the lowest activity recorded in fish fed control diet, while HP 50% and HP 75% registered the highest value. Nonetheless, no significant variation was observed in the MDA content among the groups.

4. Discussion

The present study was initiated to assess the potential of *H. illucens* larvae meal for *C. gariepinus* culture and its nutritive value. The growth response (FBW, WG, WG%, and SGR) and nutrient utilization (PER and PPV) indicators of the fish fed HP 25% and HP75% were higher than those fed control diet, but the difference was not significant. Although the fish fed higher inclusion level (172 g/kg HP) of *H. illucens* performed favourably well in comparison with those fed fishmeal-based diets, however, a significantly best performance was noticed in HP 50% group. This is consistent with the result of Xiao et al. (2018) who reported that *H. illucens* larvae meal protein could be used to replace 48% of fishmeal in the diet of yellow catfish without compromising the growth performance of the fish. Similarly, Anvo et al. (2017) showed that *Cirina butyrospermi* used in replacement for 50% of fish meal did not impair the growth performance and nutrient utilization of *Clarias gariepinus*. The similar feed intake recorded among the various groups signifies that the fish responded well to all the diets and no observable palatability-mediated feed rejection was noticed. Furthermore, the feed conversion ratio of 1.48 in fish fed HP 50% is indicative of better nutrient utilization and improved by 20% compared with the control (1.86). A complete replacement of fishmeal with *Gryllus bimaculatus* (cricket) and *Hermetia illucens* (black soldier fly) larvae meal at an inclusion level of 350 g Kg^{-1} and 147.5 g Kg^{-1} diets have also been reported in African catfish and Atlantic salmon, respectively (Taufek et al., 2016; Belghit et al., 2019). On the contrary, in the current study, a slight depression in growth was observed when the level of *H. illucens* increased above 50%, though not different from the control, and this could be associated with high fibre content (crude fibre content of *H. illucens* used is 10.8%) of which chitin forms a major part. Chitin, a component of non-starch polysaccharides, have been reported to induce growth depression in tilapia hybrid (*Oreochromis niloticus* × *O. aureaus*) fed diet containing 2% dietary chitin (Shiau and Yu, 1999). This is probably due to its fibre-like water-binding capacity characteristics and reduced enzyme mixing with the substrate, which interferes with nutrient digestibility and uptake (Tharanathan and Kiltur, 2003; Zhang et al., 2014; Gasco et al., 2016). Several other findings have also reported the inhibitory effect of chitin on nutrient digestion and absorption, hence, causing growth depression in some fish species (Ng et al., 2001; St-Hilaire et al., 2007; Kroeckel et al., 2012; Alegbeleye et al., 2012; Stamer et al., 2014; Anvo et al., 2017). The insignificant lowest survival rate recorded in HP 50% could not be traced to *H. illucens* inclusion because the fish fed HP 75% had higher survival similar to the control. However, it should be noted that most of the mortality in this group (HP 50%) occurred during the early stage (first 14 days) of the trial and this was the case for all the dietary groups (supplementary data).

Excessive lipid deposition could hamper liver health and function. In the current study, the HSI values of the fish fed *H. illucens*-based diets

Table 5
Serum biochemical and oxidative stress indices of the experimental fish fed *Hermetia illucens* larvae meal.

	Diets					
Variables	FM	HP 25%	HP 50%	HP 75%	SEM	p-value
Total protein (mg dL^{-1})	13.62^{b}	16.37^{b}	8.04^{a}	12.94^{b}	1.05	0.009
Albumin (mg dL^{-1})	2.84	2.44	1.91	2.71	0.14	0.069
Globulin (mg dL^{-1})	10.77^{b}	13.93^{b}	6.13^{a}	10.23^{ab}	1.01	0.019
A/G ratio	0.27	0.18	0.32	0.27	0.02	0.133
Triglyceride (mg dL^{-1})	184.00^{c}	115.02^{ab}	85.95^{a}	133.96^{b}	11.57	0.001
Cholesterol (mg dL^{-1})	4.98	4.85	4.44	5.79	0.10	0.111
Glucose (mg dL^{-1})	147.20^{c}	94.93^{b}	62.80^{a}	79.60^{ab}	9.91	< 0.001
Total antioxidant (mg dL^{-1})	110.30	112.03	110.43	113.17	0.65	0.379
Total bilirubin (mg dL^{-1})	6.15^{b}	4.49^{ab}	2.12^{a}	5.88^{b}	0.58	0.023
Creatinine (mg dL^{-1})	0.76	2.51	0.61	1.31	0.30	0.083
AST (U mL^{-1})	453.86^{c}	281.05^{b}	58.95^{a}	334.74^{b}	44.24	0.001
ALT (U mL^{-1})	467.30^{bc}	480.12^{c}	214.74^{a}	369.80^{b}	34.49	0.004
ALP (U mL^{-1})	186.45^{b}	111.20^{a}	139.69^{a}	139.84^{a}	9.11	0.001
Oxidative stress biomarker						
SOD (U mg^{-1} protein)	480.93^{c}	182.42^{a}	290.22^{b}	190.72^{a}	38.40	< 0.001
CAT (U mg^{-1} protein)	414.93^{a}	517.14^{a}	1938.13^{b}	1628.39^{b}	222.09	0.002
MDA (U mg^{-1} protein)	1.46	0.67	0.60	0.83	0.15	0.172

Mean values with different superscript in the same row are significantly different ($p < .05$). SEM – Pooled Standard error of the mean. HP: *Hermetia illucens* protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, A/G: albumin/globulin.

were not different from the control, and this is the same for VSI, hence, no abnormal fat deposition as a result of feeding *H. illucens,* and that the liver functionality and intestinal physiological well-being of *C. gariepinus* were not hampered. This agrees with the results of Renna et al. (2017) and Xiao et al. (2018) who observed that the dietary *H. illucens* had no effect on the HSI and VSI of rainbow trout and yellow catfish, respectively. Differently, a significantly declining trend in HSI value was observed in rainbow trout as the level of *Tenebrio molitor* increases in the diet (Belforti et al., 2015). The evaluation of whole-body composition shows that the fish fed with HP 50% had higher protein content compared with the control, and this might be that more free amino acid was made available and utilized by the fish at equal proportion (50%FM:50%HP), which invariably led to a higher protein productive value and thus, enhanced muscle protein accretion. Furthermore, the insignificant differences observed for the body crude lipid content further explained the assertion that *H. illucens* would not cause excessive fat accumulation in *C. gariepinus*.

Analysis of blood parameters has been acknowledged as a valuable tool in evaluating the effect of ingredient replacement on fish health status in different study and species (Fagbenro et al., 2013; Adeparusi and Ajayi, 2004; Shamna et al., 2017; Fawole et al., 2017). The indifferences observed between the dietary groups in RBC, Hb, PCV, WBC, erythrocytic indexes (MCV, MCH, MCHC) and leukocytes differential counts signify the fish ability to utilize *Hermetia illucens* without compromising their physiological functions such as oxygen transportation to vital organs, the capability to defends the body against foreign materials, and health status negatively. Similarly, Ogunji et al. (2007) and Belghit et al. (2019) did not record any changes in the PCV and Hb when fishmeal was replaced with magmeal and BSFL meal in Nile tilapia and Atlantic salmon, respectively. Furthermore, the RBC, Hb, and PCV values recorded were within the range reported by other researchers for *C. gariepinus* (Taufek et al., 2016; Okore et al., 2016).

Increased level of serum total protein and globulin reflects the health condition and immune status in fish (Kumar et al., 2007; Fawole et al., 2016). Chitin found in insect meal has been implicated as immunopotentiator in animal, and this has been demonstrated in fish and shrimp (Gasco et al., 2018; Xiao et al., 2018; Motte et al., 2019). Although, in the current study no significant difference was found in the serum total protein and globulin between HP25%, control and HP75%, however, the fish fed low level of Hermetia protein (HP25%) showed a slight improvement, thus pointing at a possible immunostimulating effect of *H. illucens.* On the other hand, we observed a sudden decrease and increase in serum protein and globulin values in HP50% and HP 75%, respectively. The cause of the reduction in HP50% is not known and this requires additional study to better understand the effects of *H. illucens* on African catfish immune responses. Nevertheless, increasing the level of HP up to 50% significantly reduces the triglyceride content which might be due to the presence of chitin whose role in triglyceride hydrolysis has been reported (Zhang et al., 2008), thus improving lipid utilization. Conversely, such effects were not noticed in rainbow trout and Atlantic salmon (Li et al., 2017; Belghit et al., 2019). The attributed hypocholesterolemic effect of chitin reported in some study (Ji et al., 2015; Li et al., 2017) could not be substantiated in the present study as the cholesterol levels among the treatment groups were not different, similar to the report of Belghit et al. (2019) and Cardinaletti et al. (2019). Aminotransferase (AST and ALT) and ALP enzymes often leak into the blood when there is damage to the hepatic cell and or bile duct obstruction, and their increased level potent danger on the fish liver integrity. In the present study, the activities of these enzymes were found to be lower in HP fed fish compared with the control, and this signifies that the inclusion of *H. illucens* would not hamper the hepatic tissue health and integrity.

The SOD-CAT are important cellular antioxidant enzymes whose role in oxyradical reduction of superoxide anions into hydrogen peroxide and subsequent decomposition to oxygen and water has been elucidated in many studies (Nordberg and Arnér, 2001; Fawole et al., 2016; Taufek et al., 2016). In the current trial, the lower SOD activity observed in HP fed groups could be due to the accumulation of hydrogen peroxide (H_2O_2), thereby triggering the activity of catalase enzyme to decompose H_2O_2 and resulting in higher induction of CAT in HP groups compared with the control. Our observation is in line with the opinion of Wilhelm Filho et al. (2005) who stated that increasing concentration of hydrogen peroxide could be correlated with catalase activity. Taufek et al. (2016) and Ogunji et al. (2011) reported higher CAT activity in African catfish fed cricket and maggot meal-based diets, respectively. Similarly, Li et al. (2017) detected a higher level of CAT enzyme in the serum of Jian carp fed defatted black soldier fly larvae meal. Furthermore, although not significant, the lower malondialdehyde concentration, recorded in the HP fed groups indicate that the HP-based diets are less prone to peroxidation and contain lesser polyunsaturated fatty acid compared with the fishmeal (Elia et al., 2018). Summarily, the observed decrease in SOD and increasing CAT activity, and numerically lower MDA concentration in *C. gariepinus* is indicative of the

antioxidant potential of *H. illucens* and its immunostimulating effect since antioxidant enzymes are also used as immune response biomarkers in fish (Sagstad et al., 2007; Fawole et al., 2016).

5. Conclusion

This study has shown that *Hermetia illucens* is a good substitute for fishmeal in the diet of African catfish. The growth and nutrient utilization indices indicate that the fish fed HP 75% had comparable performance with the control and possess good nutritional value without negatively affecting the nutritional profile of the fish. The haematophysiological indices, differential counts, liver function and antioxidant enzyme activities further showed that the addition of HP up to 75% is practically feasible without impairing the health status of the fish. Based on the current findings, *Hermetia illucens* has proven to be a suitable substitute for fishmeal and is therefore suggested for inclusion in the diet of *C. gariepinus*. Finally, for practical in-feed application, 50% *Hermetia illucens* in replacement for fishmeal is recommended as it is shown to yield better results than other groups in the current study.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.734849.

References

Adeparusi, E.O., Ajayi, A.D., 2004. Haematological characteristics of Nile tilapia (*Oreochromis niloticus*) fed differently processed lima bean (*Phaseolus lunatus* L.) diets. J. Res. Sci. Manage. 2 (1), 73–80.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K., Jimoh, W., 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish *Clarias gariepinus* (Burchell. 1822) fingerlings. Aquac. Res. 43 (3), 412–420.

Anvo, M.P.M., Aboua, B.R.D., Compaoré, I., Sissao, R., Zoungrana-Kaboré, C.Y., Kouamelan, E.P., Toguyéni, A., 2017. Fish meal replacement by Cirina butyrospermi caterpillar's meal in practical diets for *Clarias gariepinus* fingerlings. Aquac. Res. 48, 5243–5250.

AOAC (Association of Official Analytical Chemists), 2005. Official Methods of Analysis of the Association of Official Analytical Chemists International, 18th ed. AOAC International, Gaitherburg, USA.

Bain, B., Bates, I., Laffan, M.A., 2017. Dacie and Lewis, Practical Haematology, 12th edition. Elsevier, Amsterdam, Netherlands.

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou, S., Schiavone, A., Zoccarato, I., Gasco, L., 2015. *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets. Ital. J. Anim. Sci. 14, 670–675.

Belghit, I., Liland, N.S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Krogdahl, Å., Lock, E.-J., 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture 491, 72–81.

Belghit, I., Liland, N.S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å., Lock, E.-J., 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). Aquaculture 503, 609–619.

Cardinaletti, G., Randazzo, B., Messina, M., Zarantoniello, M., Giorgini, E., Zimbelli, A., Bruni, L., Parisi, G., Olivotto, I., Tulli, F., 2019. Effects of graded dietary inclusion level of full-fat *Hermetia illucens* Prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*). Animal 9 (251), 1–21. https://doi.org/10.3390/ani9050251.

Dietz, C., Liebert, F., 2018. Does graded substitution of soy protein concentrate by an insect meal respond on growth and N-utilization in Nile tilapia (*Oreochromis niloticus*)? Aquac. Rep. 12, 43–48.

Elia, A., Capucchio, M., Caldaroni, B., Magara, G., Dorr, A., Biasato, I., Biasibetti, E., Righetti, M., Pastorino, P., Prearo, M., Gai, F., Schiavone, A., Gasco, L., 2018. Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 496, 50–57.

Fagbenro, O.A., Adeparusi, E., Jimoh, W.A., 2013. Haematological profile of blood of African catfish (*Clarias gariepinus*, Burchell, 1822) fed sunflower and sesame meal based diets. J. Fish. Aquat. Sci. 8 (1), 80–86.

FAO, 2018. The State of World Fisheries and Aquaculture 2018. FAO Fisheries and Aquaculture Department, Rome, pp. 2–227. http://www.fao.org/state-of-fisheries-aquaculture/en/.

Fasakin, E.A., Balogun, A.M., Ajayi, O.O., 2003. Evaluation of full–fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquac. Res. 34, 733–738.

Fawole, F.J., Sahu, N.P., Pal, A.K., Ravindran, A., 2016. Haematoimmunological response of *Labeo rohita* (Hamilton) fingerlings fed leaf extracts and challenged by *Aeromonas hydrophila*. Aquac. Res. 47, 3788–3799.

Fawole, F.J., Sahu, N.P., Jain, K.K., Gupta, S., Rajendran, K.V., Shamna, N., Poojary, N., 2017. Haemato-biochemical, non-specific immunity, antioxidant capacity and histopathological changes in *Labeo rohita* fingerlings fed rubber protein isolate. Fish Physiol. Biochem. 43 (3), 677–690.

Fernandes, I.M., Bastos, Y.F., Barreto, D.S., Lourenço, L.S., Penha, J.M., 2017. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Braz. J. Biol. 77 (3), 444–450.

Francis, G., Makkar, H.P., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199 (3–4), 197–227.

Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, F., Mola, P., Chatzifotis, S., 2016. *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: growth performance, whole-body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34–45.

Gasco, L., Finke, M., van Huis, A., 2018. Can diets containing insect promote animal health? J. Insects Food Feed 4, 1–4.

Haney, D.C., Hursh, D.A., Mix, M.C., Winton, J.R., 1992. Physiological and haematological changes in chum salmon artificially infected with erythrocytic necrosis virus. J. Aquat. Anim. Health 4, 48–57.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203, 1–22.

Ji, H., Zhang, J.L., Huang, J.Q., Cheng, X.F., Liu, C., 2015. Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac. Res. 46 (5), 1209–1221.

Kroeckel, S., Harjes, A.G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C., 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364–365, 345–352.

Kumar, V., Sahu, N.P., Pal, A.K., Kumar, S., 2007. Immunomodulation of *Labeo rohita* juveniles due to dietary gelatinized and non-gelatinized starch. Fish Shellfish Immunol. 23, 341–353.

Li, S., Ji, H., Zhang, B., Zhou, J., Yu, H., 2017. Defatted black soldier fly (Hermetia illucens) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. Aquaculture 477, 62–70.

Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33.

Motte, C., Rios, A., Lefebvre, T., Do, H., Henry, M., Jintasataporn, O., 2019. Replacing fish meal with defatted insect meal (yellow mealworm *Tenebrio molitor*) improves the growth and immunity of Pacific white shrimp (*Litopenaeus vannamei*). Animals 9 (5), 258.

Ng, W.K., Liew, F.L., Ang, L.P., Wong, K.W., 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. Aquac. Res. 32, 273–280.

Nordberg, J., Arnér, E.S., 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic. Biol. Med. 31 (11), 1287–1312.

Ogunji, J.O., Kloas, W., Wirth, M., Neumann, N., Pietsch, C., 2007. Effect of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics of *Oreochromis niloticus* fingerlings. J. Anim. Physiol. Anim. Nutr. 92, 511–518.

Ogunji, J.O., Nimptsch, J., Wiegand, C., Schulz, C., Rennert, B., 2011. Effect of housefly maggot meal (magmeal) diets on catalase, and glutathione S-transferase in the liver and gills of carp *Cyprinus carpio* fingerling. Int. Aquat. Res. 3, 11–20.

Okore, O.O., Ekedo, C.M., Ubiaru, P.C., Uzodinma, K., 2016. Growth and haematological studies of African catfish (*Clarias gariepinus*) juveniles fed with housefly larva (*Musca dometica*) as feed supplement. Int. J. Agric. Earth Sci. 2 (3), 21–30.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I., Gasco, L., 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. J. Anim. Sci. Biotechnol. 8 (57), 1–13.

Sagstad, A., Sanden, M., Haugland, Ø., Hansen, A.C., Olsvik, P.A., Hemre, G.I., 2007. Evaluation of stress- and immune response biomarkers in Atlantic salmon *Salmo salar* L., fed different levels of genetically modified maize (Bt maize), compared with its near-isogenic parental line and a commercial suprex maize. J. Fish Dis. 30, 201–212.

Shamna, N., Sardar, P., Sahu, N.P., Phulia, V., Rajesh, M., Fawole, F.J., Pal, A.K., Angel, G., 2017. Haemato-immunological and physiological responses of *Labeo rohita* fingerlings to dietary fermented *Jatropha curcas* protein concentrate. Anim. Feed Sci. Tech. 232, 198–206.

Shiau, S.Y., Yu, Y.P., 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus x O. aureaus*. Aquaculture 179, 439–446.
Sinha, A.K., Kumar, V., Makkar, H.P., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition–A review. Food Chem. 127 (4), 1409–1426.
Stamer, A., Wesselss, S., Neidigk, R., Hoerstgen-Schwark, G., 2014. Black soldier fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets. In: Rahmann, G., Aksoy, U. (Eds.), Proceedings of the 4th ISOFAR Scientific Conference. "Building Organic Bridges", at the Organic World Congress 2014, 13-15 Oct., Istanbul, Turkey, pp. 1043–1045 (eprint ID 24223).
St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W., 2007. Fly pre-pupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquac. Soc. 38 (1), 59–67.
Taufek, N.M., Aspani, F., Muin, H., Raji, A.A., Razak, S.A., Alias, Z., 2016. The effect of dietary cricket meal (*Gryllus bimaculatus*) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (*Clarias gariepinus*). Fish Physiol. Biochem. 42 (4), 1143–1155.
Tharanathan, R.N., Kiltur, F.S., 2003. Chitin- the undisputed biomolecule of great potential. Crit. Rev. Food Sci. Nutr. 43, 61–87.
Tran, G., Heuzé, V., Makkar, H.P.S., 2015. Insects in fish diets. Anim. Front. 5 (2), 37–44.
Wilhelm Filho, D., Torres, M.A., Zaniboni-Filho, E., Pedrosa, R.C., 2005. Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongatus* (Valenciennes, 1847). Aquaculture 244, 349–357.
Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J., Zhang, J., 2018. Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*). Aquac. Res. 49, 1569–1577.
Yu, G.H., Chen, Y.H., Yu, Z.N., Cheng, P., 2009. Research progression on the larvae and prepupae of black soldier fly *Hermetia illucens* used as animal feedstuff. Chin. Bull. Ent. 46, 41–45 in Chinese.
Zhang, J., Liu, J., Li, L., Xia, W., 2008. Dietary chitosan improves hypercholesterolemia in rats fed high-fat diets. Nutr. Res. 28, 383–390.
Zhang, Y., Zhou, Z., Liu, Y., Cao, Y., He, S., Huo, F., Qin, C., Yao, B., Ringo, E., 2014. High-yield production of a chitinase from Aeromonas veronii B565 as a potential feed supplement for warm-water aquaculture. Appl. Microbiol. Biotechnol. 98, 1651–1662.