



Housefly maggot meal complement soybean meal in a fish-free diet for hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchius longifilis* ♂): Effect on growth, body composition, blood biochemistry and antioxidant enzyme activity

Femi J. Fawole^{a,*}, Naseemashahul Shamna^b, Hairat A. Memudu^a,
Najeeb Abdullahi^a, Mohamed S. Hassaan^c, Oluyemi K. Gbadamosi^d

^a Department of Aquaculture and Fisheries, University of Ilorin, P.M.B 1515, Ilorin, Nigeria

^b ICAR-Central Institute of Fisheries Education, Mumbai - 400061, India.

^c Department of Animal Production, Fish Research Laboratory, Faculty of Agriculture at Moshtohor, Benha University, Benha 13736, Egypt

^d Department of Fisheries and Aquaculture, Federal University of Technology, P.M.B 704, Akure, Ondo State, Nigeria

ARTICLE INFO

Keywords:

Maggot meal
Growth
Fish welfare
Immunity
Hybrid catfish

ABSTRACT

A 45-day experiment was designed to study the complementary effect of maggot (*Musca domestica*) meal (MG) in a fishmeal-free soybean meal (SBM)-based diet on the production performance of African catfish hybrids. Four isoproteic (398.78 g/kg $\pm$ 3.23) and iso-energetic (20.23 kJ/g $\pm$ 0.15) diets were formulated as positive control (FM-based diet), soybean-based diets (FM-free) and soybean-based diets supplemented with 14 % MG and 21 % MG as complementary ingredients, denoted as FM, SBM, MG14 and MG21, respectively. A total of 180 healthy hybrid catfish (average initial body weight: 4.31 $\pm$ 0.04 g) were distinctly stocked into 12 trial tanks (60-L capacity) and fed to apparent satiation twice daily with their respective diets. At the end of the feeding trial, fish fed MG21 outperformed those fed other diets in final body weight (FBW), weight gain (WG), specific growth rate (SGR), and daily growth index (DGI) ($p < 0.05$). However, MG had no influence on feed intake (FI), feed conversion ratio (FCR), or nutrient utilization indices among the groups ($p > 0.05$). The MG21 supplementation improved the immunophysiological indices such as white blood cell, lymphocyte count, total protein, and globulin values compared to other groups, while haemoglobin, haematocrit, and red blood cell counts were not affected. Supplementation with MG at 21 % enhanced the antioxidant capacity of hybrid catfish with no detrimental effect on the serum alanine aminotransferase and alkaline phosphatase enzymes activities. However, the aspartate aminotransferase was found higher in MG14 and MG21 fed fish compared to other dietary groups. Based on the results obtained in this study, it can be concluded that maggot meal could be used as a complementary ingredient in place of fishmeal in soybean meal diets for the culture of hybrid African catfish.

Data Availability: The data in this article is available upon request from the corresponding author.

* Corresponding author.

E-mail addresses: fawole.fj@unilorin.edu.ng, phem247@yahoo.com (F.J. Fawole).

1. Introduction

Aquaculture production and demand for aquafeed ingredients have increased considerably in recent decades due to the growing population and an increase in the use of external feed inputs, respectively (Tacon and Metian, 2015; Fawole et al., 2020). Over the years, fishmeal (FM) has become the main protein source used in aquafeed manufacturing due to its high nutritional and palatability values. However, as global aquaculture consumptive demand is projected to double by 2050, the production of equivalent quantities of aquafeeds, as well as the replacement of FM in aquafeeds, is critical for the continued growth and sustainability of the aquaculture industry. Hence, it is important to identify feed ingredients and formulation techniques that are not reliant on fishmeal as the major protein source. In view of this, much technological advancement has been made to reduce the level of FM in aquafeed formulation, and this effort has greatly led to increased utilization of plant-derived protein ingredients in commercial fish feed manufacturing, especially for herbivorous and omnivorous fish species (Turchini et al., 2019; Chaklader et al., 2021a). Because of its favourable amino acid profile among all plant proteins, sustainable supply, and reasonable price, soybean meal (SBM) is presently the most widely used vegetable protein source in fish diets (Zhou et al., 2020).

Interestingly, research has shown that soybean meal could be used as a replacement for fishmeal in different freshwater fish species such as African catfish (Balogun and Ologhobo, 1989; Abdel-Warith et al., 2019), common carp (Urán et al., 2008), gibel carp (Liu et al., 2017), Nile tilapia (Abdel-Warith, Younis, 2013), channel catfish (Kaushik et al., 1995), etc. However, studies have indicated that the inclusion of SBM above 50 % in aquafeed causes amino acid imbalances and antinutrient toxicity, thus causing suppression of feed intake and poor growth; intestinal inflammation, and a shortage of nucleotides as compared to fishmeal (Fagbenro and Davies, 2001; Krogdahl et al., 2003; Deng et al., 2006; Venold et al., 2012; Zhou et al., 2020; Kumar et al., 2021). Recently, Abdel-Warith et al. (2019) reported that *Clarias gariepinus* fed 41 % full fat soybean meal grows favourably to those fed FM, but the higher inclusion level resulted in growth depression and negatively affected the proteolytic enzyme activity in the stomach. As the aquaculture industry continues to expand and FM production remains unsustainable, the inclusion of alternative ingredients would be the solution. Among the animal-based ingredients, insect meal has been reported as a good source of proteins that can be used as an alternative ingredient in place of fish meal or soybean meal in aquafeeds (Alfiko et al., 2021). Insects are the third generation of proteins in the world and have been promoted as a sustainable alternative to fishmeal with the potential to reduce farmer's reliance on imported feed resources, particularly in Africa (Fawole et al., 2020; Boroojerdi and Rajabzadeh, 2021). Recently, Hossain et al. (2021) reported that the addition of black soldier fly (*Hermetia illucens*) larvae (BSFL) meal to the soybean-based diet enhanced rainbow trout (*Oncorhynchus mykiss*) growth performance. Similarly, dietary BSFL supplementation in a high-soybean-based diet was also reported to improve innate immunity and prevent SBM-induced intestinal enteritis in rainbow trout (Kumar et al., 2021). This indicates that a combination of different alternative dietary protein sources to complement the conventional ones may have a beneficial effect on fish performance. In particular, studies have shown that combining insect meals with other animal-based by-products (e.g., poultry by-products) can improve the performance of fish fed diets devoid of fishmeal (Randazzo et al., 2021; Chaklader et al., 2021a, b). On the contrary, dietary addition of full-fat black soldier fly pre-pupae meal to a soybean meal diet fed to largemouth bass resulted in growth depression, poor feed intake, and inflammation and necrosis of the liver compared to those fed fishmeal or a soybean meal-based diet (Fischer et al., 2022). A similar report was made by Kroeckel et al. (2012), in which increasing levels of pre-pupae meal of black soldier fly led to a significant reduction in growth, feed intake, and nutrient utilization of turbot. The authors opine that the negative effect observed could be related to the high content of chitin and the lack of chitinase enzyme in the gut of the test fish (Kroeckel et al., 2012; Fischer et al., 2022).

Similarly, housefly maggot (*Musca domestica*) meal (HMM) has been reported to be a cost-effective and ecologically sustainable insect species with high nutritional value (crude protein range between 42 and 60 g/kg, Makkar et al., 2014) and has been shown to be a suitable alternative to conventional protein sources in freshwater aquaculture. Specifically, Kolawole and Ugwumba (2018) demonstrated that maggot meal could be used to partially substitute FM in the diet of African catfish. Maggot (*Musca domestica*) larvae supplementation improves the gut microbiota and promotes growth and immunity in swamp eels, *Monopterus albus* (Xiang et al., 2020). Fasakin et al. (2003) also discovered that defatted HMM can completely replace FM in the diet of African catfish without impairing growth. Conversely, total replacement of FM with HMM in Nile tilapia resulted in growth depression and poor feed utilization (Wang et al., 2017). Although earlier studies have delineated the possibility of replacing FM with maggot meal in different fish species, the majority of the studies have focused mainly on the effect of HMM as a single alternative ingredient. However, little is known regarding their complementary effect in plant protein-based diets free of fishmeal, especially for hybrid African catfish. For instance, in *Oncorhynchus mykiss*, Randazzo et al. (2021), reported that black soldier fly larvae meal and poultry byproduct meal (PBM), singly or in combination, could be used to improve the growth performance of fish fed fishmeal-free diets. Therefore, this study hypothesized that a proper blend of SBM and maggot meal (MG) with other ingredients may represent a novel approach for the formulation of aquafeed free of FM for freshwater fish species. Based on this, the present study examined the complementary effects of MG meal in an SBM-based diet for the African catfish hybrid (*Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂) deprived of fishmeal. The study assessed growth and nutrient utilization indices, whole-body composition, immuno-physiological parameters, liver health, and antioxidant status of the African catfish hybrid.

2. Materials and methods

2.1. Production of maggot meal and chemical analysis

An equiproportional mixture of brewery spent grain and piggery manure was used as the substrate for the maggot production, and

the collected cow blood was used as an attractant for houseflies to deposit their eggs for larvae production. The deposited eggs were cultured for a period of 6–8 days following the procedure described by Mafwila Kinkela et al. (2019). The collected maggots were then rinsed and immersed in lukewarm water (40–60 °C). Subsequently, it was dried in a hot air oven at 60 °C, ground into powder, and defatted with n-hexane using the Soxhlet extraction apparatus. Chemical composition and amino acid profile of defatted maggot meal were analyzed according to AOAC (2005) procedure (Table 1). The brewery spent grain was collected from Nigerian brewery in Ibadan, Oyo State, Nigeria, while the pig manure and cow blood were collected from the University of Ilorin Piggery Farm and the abattoir in Ilorin metropolis, respectively.

2.2. Amino acid analysis of the major ingredient

A BioChrom 30 + amino acid analyzer was used to analyse the amino acid content of three major ingredients (FM, SBM, and MG) at the ICAR-Central Institute of Fisheries Education, Mumbai, India, as described by Kader et al. (2010). In a nutshell, a sample containing about 100 mg of protein was placed in a hydrolysis tube, and 1 mL of hydrochloric acid was added. The atmosphere inside the hydrolysis tube was then evacuated using a vacuum pump and N₂ gas. After degassing the hydrolysis tubes, they were placed in a heating block preheated to 110 °C for 24 h. The hydrolyzed sample was then transferred into a test tube with 9 mL of BioChrom Loading Buffer using a 3 mL syringe and stainless-steel needle. Then, a 2 mL diluted sample was taken out, and using NaOH, the pH was adjusted to between 2.0 and 2.2. A total of 2 mL of the pH adjusted sample was filtered (25 mm syringe filter) into a labelled 1.5 mL sample vial. The pH adjusted samples were then prepared for analysis by using 100 µL of filtered sample, 800 µL of filtered Biochrom loading buffer, and 100 µL of filtered 2500 mol/L norleucine standard. Finally, the sample was vortexed and run on the BioChrom 30 + for amino acid analysis.

2.3. Experimental diets formulation and preparation

Four experimental diets were formulated with FM and SBM serving as the main protein sources in the positive and negative control, respectively. The other diets were formulated by supplementing two levels of maggot meal (14 % and 21 %) in the SBM diet without FM (Table 2). The remaining feed material was obtained from a feed mill in Ilorin metropolis, finely ground, and homogeneously blended. Water was added to the mixture until the proper consistency was obtained. The diets were cold pelleted using a hand pelletizer fitted with a 2 mm die at the University of Ilorin, Department of Aquaculture and Fisheries (AQF), Nigeria. Feeds were dried before being packed into a Ziploc bag and kept in a refrigerator at 4 °C (Hisense™ 100). Prior to the start of the feeding trial, feed samples were taken for chemical analysis to ensure that the expected values were met, as shown in Table 2. The chemical composition and amino acid profile of the FM and SBM used in this study are shown in Table 1.

2.4. Experimental animal, design, and feeding protocol

The African catfish hybrid (*Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂) was obtained from Kay Fish hatchery, Oke-andi phase 2, Sango, Ilorin, Kwara State, and transported to the AQF department, University of Ilorin, where they were acclimated to laboratory

Table 1

Proximate and amino acid composition of fishmeal, soybean meal and defatted housefly maggot meal (g/100 g).

Parameters (g/100 g)	Fishmeal	Soybean meal	Housefly maggot meal
Crude protein	66.12	40.00	49.49
Crude lipid	9.12	12.04	10.31
Crude fibre	1.17	13.51	–
Ash	16.19	5.85	11.03
<i>Essential amino acids composition (g/100 g)</i>			
Arginine	5.10	3.99	4.12
Histidine	1.30	1.01	2.36
Leucine	nd	1.89	8.15
Isoleucine	5.69	3.59	4.16
Phenylalanine	2.57	2.22	3.81
Lysine	5.77	3.00	5.10
Methionine	2.13	0.30	2.10
Threonine	2.99	1.72	3.33
Valine	3.52	2.38	4.02
<i>Non-essential amino acids composition (g/100 g)</i>			
Alanine	2.31	1.29	4.36
Aspartic acid	2.95	nd	9.73
Glutamic acid	11.12	10.19	12.25
Tyrosine	1.97	1.41	2.50
Cysteine	0.33	0.33	1.57
Serine	2.38	2.28	3.67
Glycine	4.43	2.03	3.81
Proline	3.48	3.47	3.60

nd, not detected.

Table 2
Ingredients, proximate, and essential amino acid composition of the trial diets.

Ingredients (g/kg)	FM	SBM	SBM+MG14	SBM+MG21
Fishmeal	150	0	0	0
Soybean	0	300	300	300
MG meal	0	0	140	210
Poultry meal	200	210	200	165
Groundnut cake	350	290	154	150
Yellow maize	233	117	130	100
Vegetable oil	17	33	26	25
Vit-mineral premix	20	20	20	20
Vitamin C	10	10	10	10
Dicalcium phosphate	10	10	10	10
Binder (cassava flour)	10	10	10	10
<i>Proximate composition (g/kg)</i>				
Protein	390.4	397.1	404.8	402.8
Lipid	96.0	91.6	77.6	71.4
Ash	123.1	82.1	99.0	93.1
*NFE+ fibre	390.5	429.2	418.6	432.7
**Gross energy (Kj/g)	20.02	20.69	20.13	20.08
<i>Essential amino acids composition (g/kg)[#]</i>				
Arginine	30.36	32.69	33.43	34.31
Histidine	8.23	8.56	10.48	11.61
Isoleucine	18.98	20.25	23.93	25.87
Leucine	21.11	24.23	31.28	35.12
Lysine	25.44	24.25	27.44	29.63
Methionine	8.37	5.60	7.56	8.51
Phenylalanine	14.04	15.50	18.42	20.32
Threonine	15.72	15.30	17.52	18.86
Valine	24.12	24.81	26.80	28.46

Vitamin-mineral premix supply the following to the diet: Vit A, 1500,000 i.u/kg; Vit B1, 800 mg/kg; Vit B2, 1600 mg/kg; Vit B6, 1500 mg/kg; Vit B12, 4 mg/kg; Vit D3, 3000000 i.u/kg; Vit E, 200,000 mg/kg; Vit C, 240 g/kg; folic acid, 300 mg/kg; niacin 5970 mg/kg; biotin, 40 mg/kg; pantothenic acid, 4000 mg/kg; copper, 800 mg/kg; iodine, 191 mg/kg; iron, 1200 mg/kg; manganese 20323 mg/kg; selenium, 60 mg/kg; zinc, 15,996 mg/kg; choline, 87,000 mg/kg. MG; Housefly maggot. *Nitrogen-free extracts (NFE) = 100 – (crude lipid + crude ash + crude protein). ** Calculated gross energy was calculated as 23.9, 39.8, and 17.6 kJ/g for protein, lipid, and carbohydrates, respectively. #Essential amino acid compositions of the experimental diets were calculated from the amino acid profiles of major ingredients (Table 1) and the National Research Council (2011).

conditions for 14 days before the feeding trial began and fed control diet. Before starting the feeding trial, the fish were fasted for 24 h and weighed. The feeding experiment was conducted at the Aquaculture technical experimental facility (8.47123° N, 4.66283° E) by using one hundred and eighty healthy hybrid catfish fingerlings (average initial body weight: 4.31 ± 0.04 g) randomly divided into four groups, with 15 fish stocked in each tank (60-L capacity). The experiment was carried out in a flow-through system that ensured an optimal total water volume replacement/h. Diets were assigned to each tank following a completely randomized design to account for any tank position effects, and fish were hand-fed to apparent satiation twice daily at 9:00 h and 17:00 h GMT for 45-days. The trial tank was filled with water and connected to an air compressor to ensure proper aeration. The tanks were cleaned daily, and any dead fish were removed and recorded. For the duration of the study, fish were weighed and counted every 2 weeks. To avoid the inclusion of consumed feed in the weight measurement, feeding was stopped for 24 h preceding each measurement.

2.5. Zootechnical parameters and body indices

Based on the initial and final weight and number of fish, zootechnical parameters such as weight gain, specific growth rate (SGR), daily growth index (DGI), feed intake (FI) and feed conversion ratio (FCR) were calculated using the formulae described below. The chemical composition of the whole body was ascertained at the start of the trial using a pooled sample of 20 fish and at the completion using a pooled sample of 6 fish/tank. Then the protein efficiency ratio (PER), protein productive value (PPV), and lipid productive value (LPV) were calculated. The viscerosomatic index (VSI), hepatosomatic index (HSI), and condition factor (CF) were calculated from selected and sacrificed fish using the standard formula.

Weight gain (WG, g) = final body weight (g) – initial body weight (g)

Specific growth rate (SGR, %/day) = $[(\ln \text{ final body weight (g)} - \ln \text{ initial body weight (g)}) / \text{duration of feeding}] \times 100$

Daily growth index (DGI, %) = $100 \times ((\text{FBW})^{1/3} - (\text{IBW})^{1/3}) / \text{duration of feeding}$

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

Feed conversion ratio (FCR) = $[\text{feed consumption (g)} / (\text{final biomass (g)} - \text{initial biomass (g)} + \text{dead fish weight (g)})]$

Protein efficiency ratio (PER) = $[\text{net weight gain (g)} / \text{protein fed (g)}]$

Protein productive value (PPV) = $100 [(final\ body\ weight \times final\ body\ protein\ content) - (initial\ body\ weight \times initial\ body\ protein\ content)] / (protein\ content\ in\ diet \times total\ feed\ intake)$

Lipid productive value (LPV) = $100 [(final\ body\ weight \times final\ body\ lipid\ content) - (initial\ body\ weight \times initial\ body\ lipid\ content)] / (lipid\ content\ in\ diet \times total\ feed\ intake)$

Hepatosomatic index (HSI, %) = $100 \times wet\ weight\ of\ liver\ (g) / whole\ body\ weight\ of\ fish\ (g)$

Viscerosomatic index (VSI, %) = $100 \times wet\ weight\ of\ visceral\ (g) / whole\ body\ weight\ of\ fish\ (g)$

Condition factor (CF, g/cm³) = $Body\ weight\ of\ fish\ (g) / (Body\ length\ of\ fish\ (cm))^3 \times 100$

2.6. Chemical analysis of feed and whole body composition

The chemical composition of the feed and the whole body was determined using the procedure of AOAC (2005). To determine the moisture, the samples were oven dried in a hot air oven at 103 °C for 12 h. The moisture-free samples were finely ground, and the crude protein was determined using Kjeldahl's method (total N $\times$ 6.25). The ether extract was determined by the Soxhlet extraction method, and the ash level was analyzed by incineration at 550 °C in a muffle furnace for 6 h.

2.7. Sampling and analytical methods

At the end of the 45-day experiment, nine (9) fish were chosen at random from each group, sedated with 100 mg of clove oil/L of ethanol to reduce handling stress, and blood was drawn from the fish's caudal vein with a 2 mL syringe. Blood collected was placed in an EDTA-treated sample tube for basic haematological analysis and differential count. Standard methods were used to determine haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), and differential count (Klontz, 1994; Bain et al., 2017). Fresh blood was obtained without the use of an anticoagulant from another three (3) fish per tank, transferred to a plain tube, and stored at 4 °C for 24 h. The serum sample was centrifuged (Eppendorf Centrifuge 5804 R) at 3000 $\times$ g for 10 min at 4 °C and refrigerated at - 20 °C until use. A diagnostic kit (Fortress Diagnostics Limited, UK) was used to analyze serum biochemical parameters such as total protein, albumin, aminotransferases (ALT & AST), and alkaline phosphatase (ALP). Superoxide dismutase (SOD) and catalase (CAT) enzyme activities were analyzed using a kit developed by Elabscience®, USA, while the method described by Siwicki (1995) was used to determine the total immunoglobulin concentration.

2.8. Statistical analysis

For statistical analysis, tank means were used as units of observation. Prior to single-factor ANOVA, all data were checked for normality and homogeneity of variance, and when significant distinctions were found, the Duncan Multiple Range Test (DMRT) was used to separate the means. At $p < 0.05$, differences were considered significant. SPSS for Windows was used for statistical analysis (SPSS Inc., 22.0, Chicago, IL, USA). The data is shown as mean $\pm$ standard error (mean $\pm$ SE).

Table 3

Growth, feed intake, and body indices of African catfish hybrid (*Clarias gariepinus* ♀ $\times$ *Heterobranchius longifilis* ♂) fed with various trial diets.

Parameters	Dietary group				p-values
	FM	SBM	SBM+MG14	SBM+MG21	
FBW ¹ (g/fish)	9.53 $\pm$ 0.24 ^b	10.68 $\pm$ 0.25 ^b	10.09 $\pm$ 0.33 ^b	13.76 $\pm$ 1.14 ^a	0.005
WG ² (g/fish)	5.22 $\pm$ 0.27 ^b	6.35 $\pm$ 0.32 ^b	5.78 $\pm$ 0.34 ^b	9.42 $\pm$ 1.20 ^a	0.007
SGR (%) /day	1.76 $\pm$ 0.07 ^b	2.01 $\pm$ 0.09 ^b	1.89 $\pm$ 0.08 ^b	2.56 $\pm$ 0.22 ^a	0.012
DGI ³ (%)	3.87 $\pm$ 0.20 ^b	4.70 $\pm$ 0.23 ^b	4.29 $\pm$ 0.25 ^b	6.98 $\pm$ 0.89 ^a	0.007
FCR ⁴	1.56 $\pm$ 0.22	1.33 $\pm$ 0.05	1.26 $\pm$ 0.10	1.17 $\pm$ 0.21	0.493
PER ⁵	1.71 $\pm$ 0.22	2.10 $\pm$ 0.20	1.98 $\pm$ 0.15	2.25 $\pm$ 0.36	0.492
FI ⁶ (g /fish)	8.13 $\pm$ 1.22	8.76 $\pm$ 0.39	7.27 $\pm$ 0.59	8.80 $\pm$ 0.92	0.565
PPV ⁷	33.56 $\pm$ 5.14	40.51 $\pm$ 2.65	44.80 $\pm$ 4.32	42.04 $\pm$ 3.49	0.305
LPV ⁸	48.03 $\pm$ 5.87	63.07 $\pm$ 8.46	66.00 $\pm$ 5.18	67.33 $\pm$ 8.42	0.274
Survival (%)	93.33 $\pm$ 6.67	95.53 $\pm$ 2.23	91.10 $\pm$ 2.20	93.33 $\pm$ 3.84	0.900
HSI ⁹	1.16 $\pm$ 0.13	1.36 $\pm$ 0.29	0.81 $\pm$ 0.07	0.64 $\pm$ 0.15	0.074
VSI ¹⁰	6.89 $\pm$ 0.28	7.05 $\pm$ 0.52	6.24 $\pm$ 0.57	6.09 $\pm$ 0.55	0.474
CF ¹¹ (g/cm ³)	0.68 $\pm$ 0.04	0.66 $\pm$ 0.03	0.63 $\pm$ 0.04	0.62 $\pm$ 0.02	0.660

Data expressed as mean $\pm$ standard error, (n = 3). Row values with different superscripts denote significant differences ($p < 0.05$) between diets. FBW1, Final body weight; WG2, weight gain; SGR3, Specific growth rate; DGI4, Daily growth index, FCR5, Feed conversion ratio; PER5, Protein efficiency ratio; FI6, feed intake; PPV7, protein productive value; LPV8, lipid productive value; HSI9, hepatosomatic index; VSI10, Viscerosomatic index; CF11, condition factor; FM; fish meal, SBM; soybean meal, MG; Housefly maggot meal.

3. Results

3.1. Fish performance and whole-body composition

As presented in Table 3, the final body weight (FBW), weight gain (WG), specific growth (SGR) and daily growth index (DGI) of fish fed SBM diet complemented by 21 % MG outperformed those fed FM or SBM alone ($p < 0.05$), whereas the feed conversion ratio (FCR), feed intake (FI), protein productive value (PPV) and lipid productive value (LPV) between dietary groups were not influenced by supplementation with MG ($p > 0.05$). The survival rate and somatic indices (HSI and VSI) and condition factor (CF) among the different groups were not significantly different ($p > 0.05$). Feeding of MG did not influence the whole-body crude protein, lipid, and ash content ($p > 0.05$; Table 4).

3.2. Haematology parameters

Hematological parameters such as Hb, RBC, and Hct did not show any significant differences among dietary groups ($p > 0.05$; Table 5). However, MG supplementation in the SBM-based diet significantly ($p < 0.05$) enhanced the white blood cell and lymphocyte counts, with the highest value recorded in fish fed SBM+MG21. No significant variation was observed in neutrophil and monocyte counts ($p > 0.05$). The monocyte count was numerically higher in fish fed with SBM+MG compared to the lower value recorded in the negative control (SBM alone group).

3.3. Immuno-physiological indicators and antioxidant response

Table 6 shows the complementary effects of MG meal in SBM-based diets on the immuno-physiological and antioxidant status of hybrid catfish. Fish fed an SBM diet supplemented with MG14 and MG21 recorded the highest total protein compared to the lowest value observed in the SBM and FM groups ($p < 0.05$). A higher globulin value was recorded in MG21, which is similar to MG14 but differed significantly from the SBM fed fish ($p < 0.05$). The SBM-fed group had the lowest globulin values, but it was similar to the FM-fed fish. No significant difference ($p > 0.05$) in serum albumin and total immunoglobulin values was observed between the different groups. Dietary MG had no significant effect on ALT and ALP activities compared to other groups, but significantly higher AST activity was observed in fish fed MG14 and MG21 ($p < 0.05$).

Higher SOD activity was recorded in MG14, which was not different from the MG21 and FM groups but significantly different from the SBM-only fed fish ($p < 0.05$; Table 6). Catalase activity showed a reverse trend, with the highest activity seen in MG21, which is similar to the SBM-only fed group (Table 6). The lowest CAT activity was observed in the FM and MG14 groups, but it was similar to the SBM-only group. However, SOD and CAT activities were found to be consistently higher in fish fed MG21.

4. Discussion

The quality of a protein source ingredient is a reflection of its amino acid composition, the content of limiting amino acids and the digestibility of the ingredients. In the present study, the content of essential amino acids (EAAs), especially lysine, methionine, leucine, histidine, threonine, and valine, was found to be higher in MG meal than in SBM but comparable to that of FM. Although several studies on the replacement of FM with MG meal in Nile tilapia and African catfish have been done (Fasakin et al., 2003; Aniebo et al., 2009; Ogunji et al., 2008, 2021; Jimoh et al., 2022), to the best of our knowledge, no attempt has been made to evaluate the possible supplemental effect of MG meal in an SBM-based diet devoid of FM for freshwater fish especially hybrid catfish. Based on the results obtained from the current study, supplementation with 210 g/kg (21 %) of MG meal in an SBM-based diet significantly improved WG, DGI, and SGR values compared to those fed positive (FM) and negative (SBM) control diets. Similarly, Hossain et al. (2021) reported that the supplementation of BSFL meal improves FBW, WG (%), SGR, and restoration of some fatty acids in rainbow trout. Also, Randazzo et al. (2021) demonstrated that dietary BSFL meal and PBM, singly or in combination, considerably enhanced the growth of rainbow trout fed a fish meal-free diet. In another study, dietary addition of full-fat black soldier fly pre-pupae meal to a soybean meal diet fed to largemouth bass resulted in growth depression, poor feed intake, and inflammation and necrosis of the liver compared to those fed fishmeal or a soybean meal-based diet (Fischer et al., 2022). The observed increase in WG in fish fed MG 21 % represents 44.58 % and 32.59 % compared to fish fed positive and negative control diets, respectively, and this could be associated with the complementary effect of MG meal and the remarkable increase observed in the composition of EAA in the MG21 % diet and its

Table 4

Whole body (wet basis) composition of African catfish hybrid (*Clarias gariepinus* ♀ x *Heterobranchius longifilis* ♂) fed with various trial diets.

Parameters	Dietary group				p-values
	FM	SBM	SBM+MG14%	SBM+MG21 %	
CP ¹ , %	16.83 ± 0.34	17.23 ± 0.92	18.74 ± 0.57	17.07 ± 0.86	0.293
CF ² , %	4.82 ± 0.32	5.15 ± 0.38	4.63 ± 0.32	4.24 ± 0.33	0.353
Ash, %	4.41 ± 0.30	5.20 ± 0.26	5.09 ± 0.31	4.29 ± 0.25	0.111

Data expressed as mean ± standard error, (n = 3). Row values with no superscripts are not significantly different ($p > 0.05$). 1CP, crude protein; 2CF, crude lipid; FM, fish meal; SBM, soybean meal; MG, housefly maggot meal.

Table 5Haematology parameters and differential count of African catfish hybrid (*Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂) fed with various trial diets.

Parameters	Dietary group				P-value
	FM	SBM	SBM+MG14	SBM+MG21	
HB (g dL ⁻¹)	5.71 ± 0.82	7.36 ± 0.63	6.77 ± 0.92	7.23 ± 1.11	0.568
RBC (x10 ¹² L ⁻¹)	2.57 ± 0.51	3.34 ± 0.33	3.08 ± 0.45	3.27 ± 0.58	0.678
Hct (%)	16.00 ± 3.00	20.00 ± 2.08	18.33 ± 2.19	20.00 ± 3.21	0.690
WBC (x10 ⁹ L ⁻¹)	6.90 ± 0.16 ^b	6.86 ± 0.21 ^b	6.28 ± 0.14 ^b	8.13 ± 0.37 ^a	0.003
MCV (fL)	62.43 ± 0.67	59.83 ± 0.64	60.07 ± 2.46	61.53 ± 1.33	0.577
MCH (pg)	22.58 ± 1.17	22.13 ± 0.88	22.13 ± 1.27	22.33 ± 1.04	0.990
MCHC (g dL ⁻¹)	36.20 ± 1.40	37.00 ± 1.35	36.80 ± 1.12	36.30 ± 1.57	0.969
Lymphocyte (%)	54.67 ± 0.88 ^b	53.67 ± 0.33 ^b	54.67 ± 1.76 ^b	58.67 ± 0.33 ^a	0.034
Neutrophil (%)	41.67 ± 1.67	42.33 ± 2.19	40.33 ± 1.20	36.33 ± 0.33	0.085
Monocyte (%)	3.00 ± 0.58	2.67 ± 0.67	4.00 ± 0.58	3.67 ± 0.67	0.460

Data expressed as mean ± standard error, (n = 3). Row values with different superscripts denote significant differences (p < 0.05) between diets. HB, haemoglobin; Hct, haematocrit (%); WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration. FM, fish meal; SBM, soybean meal; MG, housefly maggot meal.

Table 6Immuno-biochemical, aminotransferase and antioxidant enzyme activities of African catfish hybrid (*Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂) fed with various trial diets.

Parameters	Dietary group				p- value
	FM	SBM	SBM+MG14	SBM+MG21	
TP (g dL ⁻¹)	6.26 ± 0.01 ^b	5.72 ± 0.06 ^b	6.99 ± 0.24 ^a	7.00 ± 0.17 ^a	0.002
Albumin (g dL ⁻¹)	2.15 ± 0.10	1.81 ± 0.07	2.10 ± 0.17	1.98 ± 0.08	0.284
Globulin (g dL ⁻¹)	4.11 ± 0.11 ^{bc}	3.91 ± 0.02 ^c	4.89 ± 0.40 ^{ab}	5.03 ± 0.10 ^a	0.025
A/G ratio	0.52 ± 0.04	0.46 ± 0.02	0.44 ± 0.07	0.39 ± 0.01	0.289
TIG (g dL ⁻¹)	3.34 ± 0.45	2.86 ± 0.07	2.98 ± 0.30	2.82 ± 0.37	0.689
ALT (U L ⁻¹)	50.09 ± 4.55	35.34 ± 5.29	57.21 ± 1.36	61.76 ± 8.91	0.067
AST (U L ⁻¹)	18.87 ± 1.47 ^b	18.64 ± 2.77 ^b	37.04 ± 0.32 ^a	38.00 ± 5.32 ^a	0.017
ALP (U L ⁻¹)	58.06 ± 3.74	53.30 ± 1.29	56.34 ± 2.77	55.51 ± 1.51	0.626
SOD (U mL ⁻¹)	1.89 ± 0.21 ^a	1.15 ± 0.14 ^b	2.30 ± 0.07 ^a	1.90 ± 0.15 ^a	0.004
CAT (KU L ⁻¹)	27.13 ± 3.64 ^b	56.75 ± 7.35 ^{ab}	33.05 ± 7.14 ^b	73.02 ± 12.96 ^a	0.024

Data expressed as mean ± standard error, (n = 3). Row values with different superscripts denote significant differences (p < 0.05) between diets. TP, total protein; TIG, total immunoglobulin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CAT, catalase; SOD, superoxide dismutase; FM, fish meal; SBM, soybean meal; MG, housefly maggot meal.

physiological utilization by fish for protein synthesis. This is premised on the fact that a proper balance of amino acids must be available at the sites of protein synthesis in the muscle for continuous growth and development. Lysine and methionine are two of the most deficient amino acids in plant-based diets, and they are often supplemented in diets to compensate for imbalances in amino acids (Sardar et al., 2009; Teodósio et al., 2022). Due to the fact that these limiting amino acids are important in collagen synthesis (Teodósio et al., 2022; Wu et al., 2022) and regulate the expression of muscle growth-related genes in fish (Alami-Durante et al., 2018), their higher level in the MG21-based diet could have contributed to the improved somatic growth recorded. The results obtained in the current study are consistent with those of Fasakin et al. (2003), who demonstrated that feeding defatted oven-dried maggot meal as a sole protein source in place of fishmeal in the diet of *Clarias gariepinus* did show statistically similar growth and fish well-being in both treatments and control. Aniebo et al. (2009) further reiterated that maggot meal could be used to completely replace FM in the diet of *C. gariepinus*. Also, incorporation of BSFL meal with poultry byproduct meal allowed the total replacement of FM in *Lates calcarifer* without impairing growth whilst enhancing the gut health of the fish (Chaklader et al., 2021a). The statistical indifferences recorded in the PER, FCR, PPV, and LPV values among the groups suggest that the utilization of nutrients by the fish was not affected due to the supplementation with MG meal. Furthermore, the use of MG meal did not have any adverse effects on the survival rate of the fish.

The hepatosomatic index (HSI) is frequently used as a marker of adverse environmental or dietary impacts in fish (Pait and Nelson, 2003). The insignificant variations in the HSI and VSI between groups suggest that supplementation with MG meal, when included in diets, would not result in excess fat accumulation within the liver and whole body of hybrid catfish. This assertion is further supported by the result of whole-body lipids content. Furthermore, it was observed for the first time that hybrid catfish fed an SBM diet free of fishmeal could perform comparably to those fed a fishmeal-based diet. This is contrary to earlier reports for African catfish (Fagbenro and Davies, 2001; Abdel-Warith et al., 2019) and Nile tilapia (Abdel-Warith, Younis, 2013), where fish fed SBM diet recorded retarded growth compared with those fed fishmeal. It is opined that the reason might be in the different proportion of ingredients used in formulation, the composition of alternate protein blends, or species of fish. Nevertheless, this study has shown that African catfish hybrids can effectively use a soybean-based diet free of fishmeal without any impairment in growth and welfare, but this depends on dietary ingredient combination.

Haematological indices are one of the most commonly used tools to assess fish physiological status and health (Esmaeili, 2021;

Witeska et al., 2022). In the current study, no significant differences were observed in the blood profile, except for WBC, of fish regardless of the diet fed. Similarly, Ogunji et al. (2008) also found that magmeal fed to Nile tilapia in place of FM did not elicit any differences in the Hb and Hct of the fish. Replacement of FM with BSFL did not cause changes in the haematological indices in *C. gariepinus* (Fawole et al., 2020). On the contrary, Jimoh et al. (2022) reported that the replacement of FM with maggot larvae meal resulted in an improved Hb, RBC, and Hct in *C. gariepinus*. Increased WBC, lymphocytes, total protein, and globulin levels are thought to be associated with improved innate immunity in fish (Kaleeswaran et al., 2012; Kumar et al., 2007; Witeska et al., 2022). In the present study, high-level MG meal supplementation significantly improved the WBC, lymphocytes, total protein, and globulin levels, a paramount line of host defense in fish, and this is indicative of the immunomodulation effect of supplemental MG meal in an SBM-based diet. Further, the lower A/G ratio recorded in MG21, although not significant, signifies the abundance of globulin in this group. As the γ -globulin makes up the most abundant class of globulin and is required to maintain a healthy immune system in fish, it could be inferred that MG has the potential to stimulate nonspecific immunity in hybrid catfish. Pei et al. (2019) reported an immune enhancement in Asian swamp eel, *Monopterus albus*, fed maggot meal, and this could be due to the possible presence of peptides with antimicrobial properties in maggot meal, which could act as immune system stimulators. Hou et al. (2007) reported that extracts of housefly larvae showed higher antibacterial activity against *Shigella dysenteriae* in vitro, and this could be part of the reason for the growth and survival of housefly maggot larvae in the harsh environment where they thrive. In addition, Ido et al. (2015) stated that housefly pupae may possess unidentified bioactive components with an immune-stimulating function. However, studies in this area are scanty in fish, and this warrants further investigation in order to determine the extent to which maggot meal could improve immunity in fish and the bioactive molecule responsible. The inclusion of maggot meals was also reported to enhance serum total protein, lysozyme, alkaline and acid phosphatase activities, and reduce mortality in soft-shelled turtles, *Trionyx sinensis* (Cheng et al., 2017).

Superoxide dismutase and catalase play a crucial role in maintaining the body's oxy-radical balance and are essential for efficient performance of the fish immune system (Fawole et al., 2020; Jeong et al., 2021). Several studies have shown that insect meal has a remarkable antioxidant potential when included in a diet for fish (Xiao et al., 2018; Fawole et al., 2020; Chaklader et al., 2021b; Li et al., 2022). Similarly, in the present study, supplementation with MG meal generally improved the antioxidant enzyme response in hybrid catfish. Consistent with our observation, Jeong et al. (2021) found that dietary cricket, *Gryllus bimaculatus*, meal significantly improved the SOD and GPx activity in olive flounder. Furthermore, to maintain cellular homeostasis, CAT has been said to play a significant role in the reduction of hydrogen peroxide produced by the action of SOD into water and oxygen in order to prevent oxidative insult/stress (Ighodaro and Akinloye, 2018). Interestingly, the group fed an MG21-based diet showed a consistent higher activity of both SOD and CAT, suggesting that MG could work as a functional complementary ingredient to improve antioxidant capacity and nonspecific immune status in hybrid catfish. This is consistent with previous findings in which the use of magmeal and cricket meal in place of FM improved the liver CAT enzyme response in *C. gariepinus* (Ogunji et al., 2011; Taufek et al., 2016). Similarly, maggot meal supplementation significantly influences the activities of SOD, CAT, and GPx enzymes in black carp and Asian swamp eels compared to the non-supplemented control (Yixiang et al., 2013; Pei et al., 2019). ALT and AST are transaminase enzymes responsible for amino acid metabolism in fish (Fawole et al., 2018), but they could be found in the blood resulting from liver damage (Romano et al., 2016), and elevated levels may be detrimental to the health of hepatic tissue. In the current study, MG had no effect on the activities of serum ALT and ALP, but a higher AST level was observed in MG14 and MG21 compared to other groups. Nevertheless, it is important to emphasize that despite the higher level of AST in the MG-added groups, the absence of any other notable differences in ALT, ALP, and albumin could not lead us to conclude that the feeding of MG meal would be detrimental to the health of the fish liver. Furthermore, because AST is found in a variety of organs throughout the body (Giannini et al., 2005; Al-Busafi and Hilzenrat, 2013), the higher level observed in MG groups could indicate an injury to tissues other than the liver. However, more studies are needed in this area to better understand the hepatoprotective effect of MG meal in hybrid catfish.

5. Conclusion

The results of the present study showed that high levels of maggot meal combined with soybean meal in a fishmeal-free diet improved growth performance, haemato-immunological indices, and antioxidant response without any deleterious effect on the liver function enzymes of hybrid African catfish. For the first time, it was observed that hybrid catfish fed an SBM diet free of fishmeal could perform comparably to those fed a fishmeal-based diet without any impairment in growth. Based on the results obtained in the present study, which support the complementarity of nutrients for fulfilling the growth and well-being of fish, maggot meal could be used as a complementary protein ingredient in soybean meal-based diets devoid of fishmeal for African catfish hybrids.

CRedit authorship contribution statement

F.J.F conceived and designed the research project, formulated the diet, conducted the statistical analysis, and wrote the manuscript. H.A.M. and N.A. helped in planning the experiment and carried out the feeding trial, fish sampling, and laboratory analyses. The amino acid analysis was carried out by S.N. S.N., M.S.H., and O.K.G helped in the planning of the experiment. All of the authors who contributed read the draft, corrected it, and approved the final version of the manuscript for submission.

Declaration of Competing Interest

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.

References

- Abdel-Warith, A.A., Younis, E.M., Al-Asgah, N.A., Mahboob, S., 2019. Effect of replacing fish meal by full fat soybean meal on growth performance, feed utilization and gastrointestinal enzymes in diets for African catfish *Clarias gariepinus*. *Braz. J. Biol.* 80, 535–543. <https://doi.org/10.1590/1519-6984.214763>.
- Abdel-Warith, A.W.A.M., Younis, E.M., 2013. Influence of dietary inclusion of full-fat soybean meal and amino acids supplementation on growth and digestive enzymes activity of Nile Tilapia, *Oreochromis niloticus*. *Turk. J. Fish. Aquat. Sci.* 13 (1), 69–77. https://doi.org/10.4194/1303-2712-v13_1_09.
- Alami-Durante, H., Bazin, D., Cluzeaud, M., Fontagné-Dicharry, S., Kaushik, S., Geurden, I., 2018. Effect of dietary methionine level on muscle growth mechanisms in juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 483, 273–285. <https://doi.org/10.1016/j.aquaculture.2017.10.030>.
- Al-Busafi, S.A., Hilzenrat, N., 2013. Mild hypertransaminasemia in primary care. *Int. Sch. Res. Not.*
- Alfiko, Y., Xie, D., Astuti, R.T., Wong, J., Wang, L., 2021. Insects as a feed ingredient for fish culture: status and trends. *Aquacult. Fish.* 7, 166–178.
- Anderson, D.P., Siwicki, A.K., 1995. Basic haematology and serology for fish health programs. In: Shariff, M., Arthur, J.R., Subasinghe, R.P. (Eds.), *Diseases in Asian Aquaculture II*. Asian Fisheries Society, Fish Health Section, Manila, Philippines, pp. 185–202.
- Aniebo, A.O., Erundu, E.S., Owen, O.J., 2009. Replacement of fish meal with maggot meal in African catfish (*Clarias gariepinus*) diets. *Rev. Cient. UDO Agric.* 9 (3), 666–671.
- AOAC (Association of Official Analytical Chemists), 2005. *Official Methods of Analysis of the Association of Official Analytical Chemists International*, 18th ed. AOAC International, Gaithersburg, USA.
- Bain, B., Bates, I., Laffan, M.A., 2017. *Dacie and Lewis. Practical Haematology*, 12th edition.. Elsevier, Amsterdam, Netherlands.
- Balogun, A.M., Ologhobo, A.D., 1989. Growth performance and nutrient utilization of fingerling *Clarias gariepinus* (Burchell) fed raw and cooked soybean diets. *Aquaculture* 76 (1–2), 119–126. [https://doi.org/10.1016/0044-8486\(89\)90256-1](https://doi.org/10.1016/0044-8486(89)90256-1).
- Borojerdi, N.N., Rajabzadeh, M., 2021. The Effect of Replacing Dried Meal Worm with Soybean Meal on Growth Performance and Carcass Characteristics of Broiler Chickens. P. 1–16. <https://doi.org/10.21203/rs.3.rs-782326/v1>.
- Chaklader, M.R., Howieson, J., Fotadar, R., 2021a. Growth, hepatic health, mucosal barrier status and immunity of juvenile barramundi, *Lates calcarifer* fed poultry by-product meal supplemented with full-fat or defatted *Hermetia illucens* larval meal. *Aquaculture* 543, 737026. <https://doi.org/10.1016/j.aquaculture.2021.737026>.
- Chaklader, M.R., Howieson, J., Foysal, M.J., Fotadar, R., 2021b. Transformation of fish waste protein to *Hermetia illucens* protein improves the efficacy of poultry by-products in the culture of juvenile barramundi, *Lates calcarifer*. *Sci. Total. Environ.* 796, 149045. <https://doi.org/10.1016/j.scitotenv.2021.149045>.
- Cheng, X., Huang, Q., Wang, F., Li, G., 2017. Effects of dietary housefly maggot protein on serum biochemical indices and nonspecific immunity in soft shelled turtle *Trionyx sinensis*. *Fish. Sci. (Dalian)* 36 (6), 768–772.
- Deng, J., Mai, K., Ai, Q., Zhang, W., Wang, X., Xu, W., Liufu, Z., 2006. Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* 258, 503–513. <https://doi.org/10.1016/j.aquaculture.2006.04.004>.
- Esmaili, M., 2021. Blood performance: a new formula for fish growth and health. *Biology* 10 (12), 1236. <https://doi.org/10.3390/biology10121236>.
- Fagbenro, O.A., Davies, S.J., 2001. Use of soybean flour (dehulled solvent-extracted soybean) as fish meal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): growth, feed utilization and digestibility. *J. Appl. Ichthyol.* 17 (2), 64–69. <https://doi.org/10.1046/j.1439-0426.2001.00252.x>.
- Fasakin, E.A., Balogun, A.M., Ajayi, O.O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clarid catfish *Clarias gariepinus* fingerlings. *Aquac. Res.* 34 (9), 733–738. <https://doi.org/10.1046/j.1365-2109.2003.00876.x>.
- Fawole, F.J., Sahu, N.P., Shamna, N., Phulia, V., Emikpe, B.O., Adeoye, A.A., Popoola, O.M., 2018. Effects of detoxified *Jatropha curcas* protein isolate on growth performance, nutrient digestibility and physio-metabolic response of Laboe rohita fingerlings. *Aquac. Nutr.* 24 (4), 1223–1233. <https://doi.org/10.1111/anu.12660>.
- Fawole, F.J., Adeoye, A.A., Tiamiyu, L.O., Ajala, K.I., Obadara, S.O., Ganiyu, I.O., 2020. Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias gariepinus*): Effects on growth, nutrient utilization, haematological response, and oxidative stress biomarker. *Aquaculture* 518, 734849. <https://doi.org/10.1016/j.aquaculture.2019.734849>.
- Fischer, H., Romano, N., Renukdas, N., Kumar, V., Sinha, A.K., 2022. Comparing black soldier fly (*Hermetia illucens*) larvae versus prepupae in the diets of largemouth bass, *Micropterus salmoides*: Effects on their growth, biochemical composition, histopathology, and gene expression. *Aquaculture* 546, 737323.
- Giannini, E.G., Testa, R., Savarino, V., 2005. Liver enzyme alteration: a guide for clinicians. *Cmaj* 172 (3), 367–379.
- Hossain, M.S., Fawole, F.J., Labh, S.N., Small, B.C., Overturf, K., Kumar, V., 2021. Insect meal inclusion as a novel feed ingredient in soy-based diets improves performance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 544, 737096. <https://doi.org/10.1016/j.aquaculture.2021.737096>.
- Hou, L., Shi, Y., Zhai, P., Le, G., 2007. Antibacterial activity and in vitro anti-tumor activity of the extract of the larvae of the housefly (*Musca domestica*). *J. Ethnopharmacol.* 111 (2), 227–231. <https://doi.org/10.1016/j.jep.2006.11.015>.
- Ido, A., Iwai, T., Ito, K., Ohta, T., Mizushige, T., Kishida, T., Miura, T., 2015. Dietary effects of housefly (*Musca domestica*) (Diptera: Muscidae) pupae on the growth performance and the resistance against bacterial pathogen in red sea bream (*Pagrus major*) (Perciformes: Sparidae). *Appl. Entomol. Zool.* 50 (2), 213–221. <https://doi.org/10.1007/s13355-015-0325-z>.
- Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alex. J. Med.* 54 (4), 287–293.
- Jeong, S.M., Khosravi, S., Mauliasari, I.R., Lee, B.J., You, S.G., Lee, S.M., 2021. Nutritional evaluation of cricket, *Gryllus bimaculatus*, meal as fish meal substitute for olive flounder, *Paralichthys olivaceus*, juveniles. *J. World Aquacult. Soc.* 52 (4), 859–880. <https://doi.org/10.1111/jwas.12790>.
- Jimoh, W., Ayelaja, A., Yusuf, Y., Lanre-Bhadmos, H.O., Ashaolu, E., Omiyale, A., 2022. Growth, haematology, nutrient retention and histology of african catfish, *clarias gariepinus* fingerlings fed larvae of *Musca domestica*. *Ife J. Agric.* 34 (1), 122–142.
- Kaleeswaran, B., Havelin, S., Ravikumar, S., 2012. Changes in biochemical, histological and specific immune parameters in *Catla catla* (Ham.) by *Cynodon dactylon* (L.). *J. King Saud. Univ. -Sci.* 24 (2), 139–152. <https://doi.org/10.1016/j.jksus.2010.10.001>.
- Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 133 (3–4), 257–274. [https://doi.org/10.1016/0044-8486\(94\)00403-B](https://doi.org/10.1016/0044-8486(94)00403-B).
- Klontz, G.W., 1994. Fish hematology. In: Stolen, J.S., Fletcher, C.T.C., Rowley, A.F., Kelikoff, T.C., Kaattari, S.L., Smith, S.A. (Eds.), *Techniques in Fish Immunology*. SOS Publications, Poland, pp. 121–132.
- Kolawole, A.A., Ugwumba, A.A.A., 2018. Economic evaluation of different culture enclosures for *Musca domestica* larval production and their utilization for *Clarias gariepinus* (Burchell, 1822) fingerlings diets. *Not. Sci. Biol.* 10 (4), 466–474. <https://doi.org/10.15835/nsb10410271>.
- Kroeckel, S., Harjes, A.G., 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, 345–352.
- Kroghdahl, A., Bakke-Mckellep, A., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquac. Nutr.* 9, 361–371. <https://doi.org/10.1046/j.1365-2095.2003.00264.x>.
- Kumar, V., Sahu, N.P., Pal, A.K., Kumar, S., 2007. Immunomodulation of Laboe rohita juveniles due to dietary gelatinized and non-gelatinized starch. *Fish. Shellfish Immunol.* 23 (2), 341–353.
- Kumar, V., Fawole, F.J., Romano, N., Hossain, M.S., Labh, S.N., Overturf, K., Small, B.C., 2021. Insect (black soldier fly, *Hermetia illucens*) meal supplementation prevents the soybean meal-induced intestinal enteritis in rainbow trout and health benefits of using insect oil. *Fish. Shellfish Immunol.* 109, 116–124. <https://doi.org/10.1016/j.fsi.2020.12.008>.

- Li, X., Qin, C., Fang, Z., Sun, X., Shi, H., Wang, Q., Zhao, H., 2022. Replacing dietary fish meal with defatted black soldier fly (*Hermetia illucens*) larvae meal affected growth, digestive physiology and muscle quality of tongue sole (*Cynoglossus semilaevis*). *Front. Physiol.* 621, 1–13. <https://doi.org/10.3389/fphys.2022.855957>.
- Liu, H., Jin, J., Zhu, X., Han, D., Yang, Y., Xie, S., 2017. Effect of substitution of dietary fish meal by soybean meal on different sizes of gibel carp (*Carassius auratus gibelio*): digestive enzyme gene expressions and activities, and intestinal and hepatic histology. *Aquac. Nutr.* 23 (1), 129–147. <https://doi.org/10.1111/anu.12375>.
- Mafwila Kinkela, P., Bwabwa, D., Nyongombe, N., Kambashi, B., Mafwila, J., Dochain, D., Rollin, X., 2019. Optimization of housefly larvae production on pig wastes and brewers' grains for integrated fish and pig farms in the tropics. *Livest. Res. Rural Dev.* 1–10.
- Makkar, H.P., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Tech.* 197, 1–33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C., Rennert, B., 2008. Housefly maggot meal (maggotmeal) as a protein source for *Oreochromis niloticus* (Linn.). *Asian Fish. Sci.* 21 (3), 319–331.
- Ogunji, J.O., Nimptsch, J., Wiegand, C., Schulz, C., Rennert, B., 2011. Effect of housefly maggot meal (maggotmeal) diets on catalase, and glutathione S-transferase in the liver and gills of carp *Cyprinus carpio* fingerling. *Inter. Aquat. Res.* 3 (1), 11–20. <https://doi.org/10.3390/su13020921>.
- Ogunji, J.O., Iheanacho, S.C., Mgbabu, C.C., Amaechi, N.C., Evulobi, O.O., 2021. Housefly maggot meal as a potent bioresource for fish feed to facilitate early gonadal development in *Clarias gariepinus* (Burchell, 1822). *Sustainability* 13 (2), 921. <https://doi.org/10.3390/su13020921>.
- Pait, A.S., Nelson, J.O., 2003. Vitellogenesis in male *Fundulus heteroclitus* (killifish) induced by selected estrogenic compounds. *Aquat. Toxicol.* 64 (3), 331–342. [https://doi.org/10.1016/S0166-445X\(03\)00060-2](https://doi.org/10.1016/S0166-445X(03)00060-2).
- Pei, M.T., Yang, C., Yang, D.Q., Yi, T.L., 2019. Effects of housefly maggot meal and earthworms on growth and immunity of the Asian Swamp Eel *Monopterus albus* (Zuiew). *Isr. J. Aquac. – Bamidgah* 71, 1–9.
- Randazzo, B., Zarantonello, M., Gioacchini, G., Cardinaletti, G., Belloni, A., Giorgini, E., Olivetto, I., 2021. Physiological response of rainbow trout (*Oncorhynchus mykiss*) to graded levels of *Hermetia illucens* or poultry by-product meals as single or combined substitute ingredients to dietary plant proteins. *Aquaculture* 538, 736550. <https://doi.org/10.1016/j.aquaculture.2021.736550>.
- Romano, N., Simon, W., Ebrahimi, M., Fadel, A.H., Chong, C.M., Kamarudin, M.S., 2016. Dietary sodium citrate improved oxidative stability in red hybrid tilapia (*Oreochromis* sp.) but reduced growth, health status, intestinal short chain fatty acids and induced liver damage. *Aquaculture* 458, 170–176. <https://doi.org/10.1016/j.aquaculture.2016.03.014>.
- Sardar, P., Abid, M., Randhawa, H.S., Prabhakar, S.K., 2009. Effect of dietary lysine and methionine supplementation on growth, nutrient utilization, carcass compositions and haemato-biochemical status in Indian Major Carp, Rohu (*Labeo rohita* H.) fed soy protein-based diet. *Aquac. Nutr.* 15 (4), 339–346. <https://doi.org/10.1111/j.1365-2095.2008.00598.x>.
- Tacon, A.G., Metian, M., 2015. Feed matters: satisfying the feed demand of aquaculture. *Rev. Fish. Sci. Aquac.* 23 (1), 1–10. <https://doi.org/10.1080/23308249.2014.987209>.
- 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. <https://doi.org/10.1007/s10695-016-0204-8>.
- Teodósio, R., Araújo, C., Conceição, L.E., Dias, J., Engrola, S., 2022. Amino acid metabolism in gilthead seabream is affected by the dietary protein to energy ratios. *Aquac. Nutr.* 2022. <https://doi.org/10.1155/2022/8230704>.
- Turchini, G.M., Trushenski, J.T., Glencross, B.D., 2019. Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds. *North Am. J. Aquac.* 81 (1), 13–39. <https://doi.org/10.1002/naaq.10067>.
- Uran, P.A., Gonçalves, A.A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M., 2008. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish. Shellfish Immunol.* 25 (6), 751–760. <https://doi.org/10.1016/j.fsi.2008.02.013>.
- Venold, F.F., Penn, M.H., Krogh, K., 2012. Severity of soybean meal induced distal intestinal inflammation, enterocyte proliferation rate, and fatty acid binding protein (Fabp2) level differ between strains of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 364, 281–292. <https://doi.org/10.1016/j.aquaculture.2012.08.035>.
- Wang, L., Li, J., Jin, J.N., Zhu, F., Roffeis, M., Zhang, X.Z., 2017. A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): growth performance, flesh quality, innate immunity and water environment. *Aquac. Nutr.* 23 (5), 983–993. <https://doi.org/10.1111/anu.12466>.
- Witeska, M., Kondera, E., Ługowska, K., Bojarski, B., 2022. Hematological methods in fish—Not only for beginners. *Aquaculture* 547, 737498. <https://doi.org/10.1016/j.aquaculture.2021.737498>.
- Wu, Y., Guo, X., Zhao, D., Xu, C., Sun, H., Yang, Q., Zhang, T., 2022. Effect of methionine supplementation on serum metabolism and the rumen bacterial community of sika deer (*Cervus nippon*). *Animals* 12 (15), 1950. <https://doi.org/10.3390/ani12151950>.
- Xiang, J., Qin, L., Zhao, D., Xiong, F., Wang, G., Zou, H., Wu, S., 2020. Growth performance, immunity and intestinal microbiota of swamp eel (*Monopterus albus*) fed a diet supplemented with house fly larvae (*Musca domestica*). *Aquac. Nutr.* 26 (3), 693–704. <https://doi.org/10.1111/anu.13029>.
- 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 (4), 1569–1577. <https://doi.org/10.1111/are.13611>.
- Yixiang, M., Jianhua, Y., Jinyun, Z., Xianping, Y., Xia, W., Chenglong, S., Pei, L., 2013. The influence of maggot meal and l-carnitine on growth, immunity, antioxidant indices and disease resistance of black carp (*Mylopharyngodon piceus*). *J. Chin. Cer. Oils Assoc.* 2 (2013), 1–9.
- Zhou, Z., Yao, W., Ye, B., Wu, X., Li, X., Dong, Y., 2020. Effects of replacing fishmeal protein with poultry by-product meal protein and soybean meal protein on growth, feed intake, feed utilization, gut and liver histology of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) juveniles. *Aquaculture* 516, 734503. <https://doi.org/10.1016/j.aquaculture.2019.734503>.