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Combined Substitution of Fishmeal and Fish Oil With Black Soldier Fly Larval Meal and Corn Oil: Effects on Growth, Hematology, Hematobiochemical, Amino and Fatty Acid Composition, and Fillet Nutritional Quality of Hybrid African Catfish *Clarias gariepinus* × *Heterobranchus longifilis*

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ABSTRACT

This study aimed to evaluate growth performance, amino acid and fatty acid composition of fillets, and blood biochemistry of hybrid catfish (*Heteroclarias*) cultured on Black Soldier Fly (BSF), *Hermetia illucens* larval-based diets. The experiment was conducted in a recirculating system, circular poly tanks (350 L), in a completely randomized design. Four isonitrogenous (400 g kg^{-1} crude protein) and isolipidic (140 g kg^{-1} crude fat) diets were formulated in which fishmeal (400 g kg^{-1}) was replaced at 0%, 25%, 50%, and 75% with defatted BSF larval meal and fish oil was completely replaced with corn oil in the test diets. 180 hybrid African catfish (12 weeks post-hatching) with an initial body weight of 200 ± 25 g were randomly distributed in the 12 experimental tanks (15 fish per tank, 45 fish per treatment) and were hand-fed at 3% body weight for 8 weeks. The findings showed that replacing 50% of fishmeal with BSF meal resulted in the highest growth performance (final weight, weight gain, specific growth rate [SGR], thermal growth coefficient [TGC]). However, at 75% level, growth performance and nutrient utilization (FCR, PER) significantly decline ($p < 0.05$). The dietary modification had no significant impact ($p > 0.05$) on organosomatic indices, proximate composition, amino acid profile, deposition, or retention of arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and sum n-3 polyunsaturated fatty acids (PUFA) in fish fillets. There were no significant differences in the hematological parameters ($p > 0.05$) across all treatments. Except for a reduction in globulin and cholesterol levels, all the plasma metabolites, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, remained stable ($p > 0.05$). Overall, the findings of this study suggest that BSF larval meal may partially replace dietary fishmeal up to 50% (200 g kg^{-1}), and corn oil may completely replace fish oil in a practical diet for hybrid African catfish without exerting adverse impacts on growth, feed conversion efficiency, fillet quality, health status, and physiological well-being.

1 | Introduction

Fish is an essential source of protein for humanity, accounting for at least 15% of the world's animal protein intake. Over 3.2 billion people, mainly in low-income countries, rely heavily on fish to meet their nutritional needs, reflecting its affordability, availability and accessibility [1]. Currently, as the leading source of seafood supply, aquaculture continues to play a crucial role in meeting demands for animal protein. Fishmeal and fish oil are the optimal protein and lipid components in fish feed formulation. However, the rising cost of these ingredients, along with concerns about their sustainability and ecological impacts on fisheries, has led to efforts to evaluate a wide range of relatively low-cost, sustainable ingredients that could partially or entirely replace fishmeal and fish oil [1, 2].

In recent decades, various insects (particularly larvae) have been increasingly utilized as attractive non-conventional protein and fat ingredient options in animal feed formulation and utilization [3–5]. Aquatic insects are a natural diet for fish. Insects are highly nutritious, fast-growing, and easy to produce using low-value organic products, requiring minimal water and land, and leaving no environmental carbon footprint [6–8]. Among the eight insects approved for use in aquafeed by the European Union Commission (EU regulation 2017/893-24/05/2017 and 2021/1925), the Black Soldier Fly (BSF) larvae are the most studied [9].

The nutritional composition of the larvae varies depending on their diet, life stage, rearing conditions and processing method [10, 11]. The larvae fed on poultry manure contained about 42% protein and 35% fats (dry matter basis) [8, 12]. The process of defatting may reduce the lipid content (to 9% or less) and consequently increase the protein content to ~60% DM [13–15]. BSF larvae have a well-balanced amino acid profile similar to that of fishmeal and are a rich source of vitamins and essential minerals [4, 16, 17]. Though rich in fat, the larvae have a limited ability to bioconvert short-chain polyunsaturated fatty acid (SC-PUFA) to long-chain (LC)-PUFA, resulting in generally low levels of n-3 LC-PUFA [18, 19]. Nonetheless, numerous studies have demonstrated that incorporating BSF larval meal into formulated diets promotes healthy fish growth, including that of African catfish *C. gariepinus* [20–22].

Corn oil is a highly dense, easily digestible oil rich in n-6 PUFA, particularly linoleic acid (LA), which constitutes about 58%–62% of the total fatty acid [23–25]. It contains tocopherols (Vitamin E) and phytosterols, which act as antioxidants and slow lipid peroxidation [24, 26, 27]. Compared to fish oil, corn oil is a cost-effective and sustainable lipid source. Although it is low in n-3 PUFA, some studies have shown that corn oil, either singly or as a blend of vegetable oils, may partially replace fish oil in diets for freshwater aquaculture species without negatively affecting production performance, as in Nile tilapia *Oreochromis niloticus* [23], grouper *Epinephelus malabaricus* [28], the gibel carp *Carassius auratus gibelio* [29], and tench *Tinca tinca* [30]. While vegetable oils such as soybean, sunflower, rapeseed, and linseed are commonly used in aquafeed, corn oil has been less extensively studied. In addition to its nutritional benefits, its relatively lower susceptibility to rancidity was another reason for its inclusion in this research.

The African catfish, *C. gariepinus* (Clariidae), is one of the most economically important aquaculture fish species globally due to its high fecundity, good feed conversion, disease resistance, high fillet quality, and good taste [31–33]. Between 2010 and 2020, global Clarias catfish production increased more than threefold, from 343,300 metric tonnes to 1.25 million metric tonnes, accounting for 2.5% of global inland finfish aquaculture production [34].

In recent decades, many catfish-producing nations (such as Hungary, which is the largest producer of African catfish in Europe) have been offering hybrid catfish, *Heteroclarias*, produced in hatcheries using oocytes from *Clarias gariepinus* and milt from *Heterobranchus longifilis* or vice versa. The resulting fry typically grows faster than the parental species. However, this hybrid has been reported to exhibit aggressive behavior, partly due to large variations in body sizes as they develop [35, 36].

Previous research has demonstrated that fish fillets produced from insect-based diets contain low levels of valuable omega-3 LC-PUFA (n-3 LC-PUFA), which is a major drawback as it may have implications for both fish species and human nutrition [37–40].

Although numerous studies have explored the use of BSF meal as a partial or complete replacement for fishmeal in various pure-bred fish species, including *C. gariepinus*, most focused on single-ingredient substitution while maintaining fish oil as the primary lipid source. In contrast, the present study evaluates a combined fishmeal-fish oil replacement in hybrid catfish, *Heteroclarias*. This approach reflects a more realistic, sustainable and economically viable strategy for feed formulation.

Therefore, the objective of this study was to quantitatively assess the species-specific response of *heteroclarias* to combined dietary substitutions of fishmeal and fish oil with BSF meal and corn oil, and their effects on growth, hematology, hematobiochemical, fatty and amino acid composition, and fillet quality.

To the best of the authors' knowledge, this study is the first study to simultaneously replace fishmeal and fish oil with BSF meal and corn oil, respectively, in diets for hybrid African catfish (*Heteroclarias*).

2 | Materials and Methods

2.1 | Animal Ethics Statement

All experimental procedures were approved by the Animal Welfare Committee of the University of Debrecen, Hungary (7/2025/DEMÁB) and complied with the European Union Directive (2010/63/EU) regarding animal experiments.

2.2 | Experimental Design

The experiment was performed at the Fish Biology Laboratory, Faculty of Agriculture, Food Science and Environmental Management, University of Debrecen, Hungary. The duration was 8 weeks. The hybrid African catfish used in this experiment were bred and raised in the Fish Biology Laboratory, University of Debrecen. The trial was conducted in a recirculating system, circular plastic tanks (350 L), in a completely randomized design.

The recirculation system consisted of two main parts: the rearing units and the filtration unit (mechanical and biological filters). Mechanical filtration was performed using sponges, while biological filtration was performed using plastic biofilters followed by UV sterilization.

Water quality parameters were monitored throughout the experiment following APHA [41] standards. Temperature, oxygen, pH, and conductivity were measured in situ, daily using a probe (HACH HQ30d) while the concentration of ammonia ($N\text{-}NH_3^+$), nitrite ($N\text{-}NO_2^-$), and nitrate ($N\text{-}NO_3^-$) were monitored ex situ, weekly using a spectrophotometer (HACH DR3900 spectrophotometer, Hach Company CO, Ames, Iowa, USA). The averages of water quality parameters measured during the experiment are as follows: Temperature 25.5 ± 1.5°C, DO = 5.3 ± 1 mg/L, pH = 7.5 ± 0.30, $NH_4^+ = 0.38 \pm 0.07$ mg/L, $NH_3^- = 0.2 \pm 0.1$ mg/L, $NO_2^- = 0.03 \pm 0.01$ mg/L.

2.3 | Feed Formulation and Preparation

The BSF meal (defatted) used for this experiment was purchased from GRINSECT, Hódmezővásárhely, Hungary. The product label indicates that it containing crude fat 10%, crude protein 55.7% and moisture 4%. The corn oil (VFI GmbH, Wels, Australia) contains energy 3404 kJ/100 g, and fat 92 g/100 g (from which 12.8, 29, and 51 g are saturated, monounsaturated, and polyunsaturated, respectively). Fishmeal (herring, CP = 67%, fat 9%) and fish oil (cod) were obtained from Dobrcz, Poland. All other ingredients were purchased from local manufacturers/producers within Hungary.

Four isonitrogenous diets (400 g kg^{-1} crude protein) and isolipidic (140 g kg^{-1} crude fat) were formulated according to NRC [42]. The four diets were as follows: fishmeal-based without including BSF larval meal (Control diet), 16.0% (BSF 25), 26.0% (BSF 50), and 36.0% (BSF 75), where fishmeal was replaced at 0%, 25%, 50%, and 75% with BSF larval meal by weight, respectively. Fish oil was completely replaced with corn oil in the test diets. All ingredients were weighed and mixed in a commercial feed mixer (HECHT 2117, Budapest), adding water at 6% (of total weight) to obtain a homogenous dough mixture. Subsequently, the dough was pelletized at 4.5 mm using a pelletizer machine (BORMANN Pro BFP1100, Germany). The pellets were then dried in an air oven (ALPFRIGO CFD 700, UK) at 50°C for 24 h. The pellets were kept in an airtight bag and stored at a room temperature of 24°C until use.

2.4 | Stocking, Feeding, and Blood Sample Collection

Fish were acclimatized in the experimental system for 2 weeks prior to the start of the experiment, during which they were fed the same high-quality commercial diet (AQUA Garant, Austria). 180 hybrid African catfish (12 weeks post-hatching) with an initial body weight of 200 ± 25 g were randomly distributed in the 12 experimental tanks (45 fish per treatment, 15 fish per tank). Feed was administered by hand at a daily ration of 3% of biomass, three times daily, at 8:30, 13:30, and 18:30 h. Sampling was done weekly.

At the end of the experiment, all fish were individually weighed and measured for total length. Three fish from each tank were sacrificed to obtain organo-somatic indices (i.e., eviscerated, visceral, peritoneal fat, liver, spleen, fillet weights, intestinal length). Three fish were randomly selected from each tank (nine fish per treatment), slightly anesthetized using clove oil (3 mL /100 L), after which blood sample of 1 mL was drawn from the caudal vein into a heparinized vacuum container (BD Vacutainer tube 2 mL) using a single-use insulin syringe (1 mL) and needle (Sterican 0.5×0.4 mm). The blood samples were placed in an ice-box and immediately sent to the microbiology laboratory for a full blood count.

2.5 | Blood Biochemistry

Full blood count was done using URIT-3000Vet Plus Hematology Analyzer (Orvostechnika Ltd, Budapest) following the instructions of the kit manufacturer. A pre-diluent method was used where 20 µL of blood was pipetted into 1 mL dilution buffer. Readings were taken for red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume of red blood cell (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelets (PLT), white blood cells (WBC), granulocytes (GRAN), and lymphocytes (LYM). To obtain the plasma, the remaining blood samples were centrifuged at 3000 g for 15 min to separate the plasma from the cells. The supernatant was carefully poured into an Eppendorf tube and stored at −20°C for blood plasma analysis. Plasma analysis was carried out using an auto-analyzer (Lab-Analyse 10261, Orvos Technika Kft, Budapest) following the instructions of the manufacturer. The following parameters were analyzed: Triglyceride, albumin, total protein, globulin, urea, glucose, creatinine, phosphorus, cholesterol, lactate, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

2.6 | Histological Analysis

Liver samples were preserved in 4% formalin for later analysis. The samples were rinsed with tap water, transferred to 70% ethanol for dehydration, and then embedded in paraffin wax. Cross-sections 5 mm thick were made in a rotary microtome. Afterwards, the sections were stained with hematoxylin and eosin, sealed with neutral resin adhesive, and then mounted on glass slides for light microscopy reading. The microphotography was taken with a Nikon Coolpix 4300 digital camera coupled to an Olympus microscope (Tokyo, Japan, B941).

2.7 | Chemical Analysis

Chemical analysis was performed at the Chemistry Laboratory, University of Debrecen, Hungary, to determine the proximate composition, amino acid, and fatty acid profiles of the diets and fish fillets according to AOAC [43] standard methods. Moisture was achieved by drying and weighing (MSZ ISO 1442:2000), and protein was obtained by the Kjeldahl method (MSZ ISO 937:2002). Crude fat by acid–base hydrolysis, extraction and mass measurement (MSZ ISO 1443:2002), Ash by cremation, then mass measurement (MSZ ISO 5984:1992, withdrawn standards), Crude Fiber by acidic, alkaline extraction (Fibretec) (MSZ

EN ISO 6865:2001). Chitin content was determined by subtracting acid-detergent fiber (ADF) from acid-detergent lignin (ADL) following the method described in Hahn et al. [44]. The ADF and ADL were initially measured gravimetrically according to MSZ EN ISO 13906:2009.

Amino acids were determined by high-pressure ion exchange chromatography HPLC (MSZ ISO 13903:2005) using an AAA 500 amino acid analyser (INGOS Ltd, Praha, Czech Republic), with post-column derivation with ninhydrin and photometric detection at 210 and 254 nm. Amino acid standard mixture (INGOS Ltd, Praha, Czech Republic) was applied as a reference. The recovery was higher than 95%. The amino acids were expressed as a percentage of the original sample weight.

The composition of fatty acids was determined using the fatty acid methyl ester method (MSZ ISO 12966-4:2015). The fat sample (200–300 mg) was dissolved in 6 mL hexane and 4 mL 0.5M NaOH: MeOH. The solution was heated in an oven at $80 \pm 1°C$ for 10 min. After saponification, the sample was diluted with 5–10 mL of distilled water and the unsaponified materials were extracted with 2 mL of hexane. Following the extraction, the solution was acidified with 0.5 mL 6 M H_2SO_4 and the saponified fatty acids were extracted with 2 mL hexane. The purified fraction was treated with 2 mL 14% BF_3:MeOH at $80 \pm 1°C$ for 30 min in an oven. 2 mL saturated NaCl solution was used. The supernatant hexane phase was applied into a GC vial with dry Na-sulfate and applied to GC-FID (Varian GC 3800). Supelco 37-component FAME mix (Sigma–Aldrich) was used as a reference. Measurements were repeated four times with CV% <5%. Calculated results were expressed as a percentage of the fat content.

2.8 | Production Performance Indices

Growth performance, nutrient utilization, and organo-somatic indices were calculated as follows:

$$\text{Mean weight gain(g)} = (\text{Final mean weight} - \text{Initial mean weight}),$$

$$\text{Specific growth rate(SGR)} = \text{In}[(\text{mean final weight}) - \text{In}(\text{mean initial weight})]/[\text{Time(days)}] \times 100,$$

$$\text{Average daily weight gain(ADG)} = [\text{weight gain(g)}]/[\text{time(days)}],$$

$$\text{Relative growth rate(RGR)} = (\text{Mean final weight of fish}/\text{Mean initial weight of fish}) \times 100,$$

$$\text{Thermal growth coefficient(TGC)} = 1000 \times \left[(\text{final weight g})^{1/3} - (\text{Initial weight g})^{1/3} \right]/[\text{Temperature°C} \times \text{Days}],$$

$$\text{Feed conversion ratio(FCR)} = (\text{Weight of feed fed})/(\text{Weight gain of fish}),$$

$$\text{Protein efficiency ratio} = \text{Weight gain(g)}/\text{Crude protein fed(g)},$$

$$\text{Fulton conditioning factor(K)} = W/L^3 \times 100$$

(where W is the wet weight(g), and L is the standard length(cm),

$$\text{Daily feed intake(g)} = (\text{Total feed consumed}/\text{no. of fish}/\text{no. of days}),$$

$$\text{Hepatosomatic index(HSI)} = (\text{weight of liver})/(\text{body weight of fish}) \times 100,$$

$$\text{Spleen-somatic index(SSI)} = (\text{weight of spleen})/(\text{body weight of fish}) \times 100,$$

$$\text{Viscerosomatic index(VSI)} = (\text{weight of visceral})/(\text{body weight of fish}) \times 100,$$

$$\text{Fillet yield} = (\text{weight of fillet})/(\text{body weight of fish}) \times 100,$$

$$\text{Carcass yield} = (\text{eviscerated weight of fish})/(\text{body weight of fish}) \times 100,$$

$$\text{Relative gut length(RGL)} = \text{Length of fish intestine(cm)}/\text{total body length of fish(cm)} \times 100,$$

$$\text{Survival rate(\%)} = (\text{initial number of individuals}/\text{final number of individuals}) \times 100,$$

$$\text{Intraperitoneal fat index} = (\text{weight of perivisceral fat}/\text{body weight of fish}) \times 100,$$

$$\text{Atherogenic index} = (12{:}0 + (4 \times C14{:}0) + C16{:}0)/(\text{sum n-6} + \text{sum n-3} + \text{sum MUFA}),$$

$$\text{Thrombogenic index} = (C14{:}0 + C16{:}0 + C18{:}0)/((0.5 \times \text{sum MUFA}) + (0.5 \times \text{sum n-6}) + (3 \times \text{total n-3}) + \text{sum n-3/n-6}),$$

$$\text{h/H} = (C18{:}1n{-}9 + C18{:}2n{-}6 + C20{:}4n{-}6 + C18{:}3n{-}3 + C20{:}5n{-}3 + C22{:}5n{-}3 + C22{:}6n{-}3)/(C14{:}0 + C16{:}0).$$

Where h/H = hypocholesterolaemic/hypercholesterolemic fatty acids.

2.9 | Data Analyses

All statistical analyses were performed using IBM SPSS 29.0 for Windows software. Normality of distribution was tested using Kolmogorov–Smirnov test. Homogeneity of variances between experimental groups was checked using Levene's test. Since the assumption of homogeneity of variance between the groups was not violated ($p > 0.05$), the data were subjected to a one-way analysis of variance (ANOVA). Tukey's multiple comparison test was used to determine significant differences between treatments ($p < 0.05$ was considered significant). Polynomial contrast was applied to check the trend response.

The experimental model: $Yij = \mu + Ti + \varepsilon ij$, where Yij is the dependent variable, μ is the overall mean, Ti is the treatment effect (i = BSF 0, BSF 25, BSF 50 or BSF 75), and εij is the random residual error.

TABLE 1 | Growth performance and nutrient utilization of hybrid African catfish fed varying levels of BSF larval meal-based diets for 8 weeks.

	Test diets				*p*-Values			
Parameters	**Control**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
IW (g)	199.91	200.23	200.33	199.83	0.132	0.533	0.925	0.171
FW (g)	490.84^{b}	475.41^{b}	491.60^{b}	408.24^{a}	12.524	0.022	0.014	0.075
WG (g)	290.94^{b}	275.18^{b}	291.27^{b}	208.41^{a}	12.483	0.022	0.014	0.077
WG (%)	145.52^{b}	$137.45^{a,b}$	145.38^{b}	104.30^{a}	6.211	0.022	0.014	0.080
ADG (g)	5.20^{b}	4.91^{b}	5.20^{b}	3.72^{a}	0.223	0.022	0.014	0.077
RGR	2.46^{b}	$2.37^{a,b}$	2.45^{b}	2.33^{a}	0.062	0.022	0.014	0.080
SGR	0.70^{b}	$0.67^{a,b}$	0.70^{b}	0.55^{a}	0.021	0.018	0.011	0.061
TGC	1.43^{b}	$1.37^{a,b}$	1.43^{b}	1.10^{a}	0.049	0.019	0.012	0.070
FI	8.87	8.55	9.26	8.17	0.200	0.278	0.429	0.337
FCR	1.72^{a}	1.75^{a}	1.78^{a}	2.22^{b}	0.036	0.002	0.001	0.014
PER	$1.41^{a,b}$	$1.41^{a,b}$	1.44^{b}	1.21^{a}	0.034	0.035	0.034	0.044
CF(g/cm^3)	0.85	0.86	0.84	0.80	0.010	0.329	0.138	0.288
SR (%)	100.00	100.00	100.00	100.00	0.000	0.000	0.000	0.000

Note: L, Q = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 3$), pooled standard error of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.
Abbreviations: ADG, average daily growth; CF, condition factor; FCR, feed conversion ratio, FI, feed intake ($gfish^{-1}d^{-1}$); FW, final weight; IW, initial weight; PER, protein efficiency ratio; RGR, relative growth rate; SEM, standard error of the mean; SGR, specific growth rate (% day^{-1}); SR, survival rate; TGC, thermal growth coefficient; WG, weight gain.

3 | Results

3.1 | Growth Performance, Nutrient Utilization, and Organosomatic Indices

Table 1 presents a summary of the growth performance and nutrient utilization of African catfish reared on varying levels of BSF larval meal.

Fish readily accepted all diets, and survival rate was 100% in all dietary treatments. Physical observation of the whole body of fish from each dietary treatment showed no body deformities or alteration in skin, fins and eyes. Fish fed BSF 50 diet had the highest final mean body weight (491.60 g) and weight gain (291.27 g), which are similar to fish fed BSF 25 and the Control groups, but significantly higher ($p = 0.022$) than fish fed BSF 75 diet. A similar linear effect was observed in specific growth rate, thermal growth coefficient, average daily growth rate, and relative growth rate. The FCR increases linearly ($p = 0.001$) with increasing levels of BSF larval meal inclusion. Fish fed BSF 75 had the highest ratio (2.22), which was significantly higher ($p = 0.022$) than those in the other groups. The protein efficiency ratio (PER) indicates how well dietary protein is utilized for growth. A higher ratio signifies more efficient utilization. The highest PER value was observed in fish fed BSF 50 (1.44), which was similar to the Control and BSF 25, but significantly higher ($p = 0.035$) than fish fed the BSF 75 diet. Condition factor was similar among the groups ($p = 0.329$).

TABLE 2 | Organosomatic Indices (%) of hybrid African catfish fed varying levels of BSF larval meal-based diets for 8 weeks.

	Test diets				*p*-Values			
Parameters	**Control**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
HSI	1.42	1.42	1.20	1.20	0.052	0.210	0.062	0.973
SSI	0.05	0.04	0.04	0.04	0.002	0.155	0.069	0.156
VSI	8.45	8.00	7.75	7.66	0.680	0.551	0.194	0.637
IPF	4.00	3.21	3.36	3.57	0.179	0.492	0.507	0.208
RGL	101.23	99.76	103.00	85.00	4.016	0.405	0.233	0.327
F Y	41.55	40.02	41.87	38.00	0.660	0.100	0.090	0.281
CY	85.33	81.81	85.50	81.45	0.940	0.276	0.340	0.885

Note: L, Q = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 3$), pooled standard error of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.
Abbreviations: CY, carcass yield; FY, fillet yield; HSI, hepatosomatic index; IPF, intraperitoneal fat; RGL, relative gut length; SEM, standard error of the mean; SSI, spleen somatic index, VSI, viscerosomatic index.

TABLE 3 | Proximate composition (%, dry matter) of hybrid African catfish fillet fed varying levels of BSF larval meal-based diets for 8 weeks.

	Test diets				*p*-Value			
Parameters	**Ctrl**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
Dry matter	25.10	24.69	24.82	25.11	0.090	0.274	0.813	0.070
Protein	18.17	18.14	18.09	18.20	0.059	0.938	0.953	0.609
Crude fat	5.41	5.03	5.19	5.39	0.072	0.188	0.883	0.050
Ash	1.22	1.23	1.24	1.23	0.007	0.839	0.559	0.557

Note: L, Q = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 3$), pooled standard deviations of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.
Abbreviation: SEM, standard error of the mean.

TABLE 4 | Amino acid profile (% dry matter) of hybrid African catfish fillet fed varying levels of BSF larval meal-based diets for 8 weeks.

	Test die				*p*-Values			
Parameters	**Ctrl**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
Essential AA								
Arginine	0.78	0.76	0.79	0.80	0.009	0.580	0.429	0.458
Histidine	0.49	0.47	0.48	0.50	0.009	0.822	0.811	0.455
Isoleucine	0.76	0.79	0.80	0.77	0.024	0.696	0.790	0.270
Leucine	1.52	1.50	1.54	1.52	0.042	0.937	0.892	0.983
Lysine	2.46	2.30	2.20	2.31	0.134	0.917	0.667	0.626
Methionine	0.41	0.42	0.44	0.41	0.011	0.819	0.866	0.498
Phenylalanine	0.50	0.50	0.48	0.42	0.039	0.916	0.567	0.715
Threonine	0.66	0.68	0.69	0.65	0.029	0.963	0.905	0.636
Valine	0.73	0.73	0.78	0.73	0.025	0.931	0.844	0.702
Non-essential AA								
Alanine	1.09	1.07	1.08	1.13	0.012	0.395	0.179	0.304
Asparagine	1.76	1.76	1.76	1.76	0.056	0.860	0.475	0.720
Cysteine	0.10	0.11	0.08	0.10	0.006	0.399	0.423	0.961
Glutamine	2.51	2.56	2.39	2.43	0.107	0.959	0.723	0.976
Glycine	1.08	0.94	0.94	1.07	0.036	0.347	0.871	0.087
Proline	1.31	1.30	1.52	1.44	0.125	0.923	0.630	0.906
Serine	0.63	0.63	0.64	0.63	0.014	0.996	0.959	0.857
Tyrosine	0.37	0.38	0.36	0.31	0.031	0.877	0.513	0.663

Note: NB: Tryptophan not detected. L and Q = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 3$), pooled standard error of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.
Abbreviations: AA, amino acid; SEM, standard error of the mean.

The results of organosomatic indices (Table 2) showed no significant differences ($p > 0.05$) among the different dietary treatments.

3.2 | Proximate, Amino Acids, and Fatty Acids Composition of African Catfish Fillets

The proximate composition, amino acids and fatty acids profiles of African catfish fillets are presented in Tables 3–5, respectively. No significant differences ($p > 0.05$) were observed in the different dietary groups for dry matter, crude protein, crude fat, saturated fat, and ash content (Table 3).

The amino acid profile of the catfish fillets (Table 4) indicates no significant difference ($p > 0.05$) among the various dietary treatments. Leucine and lysine are the most abundant essential amino acids (EAA), while alanine, asparagine, glutamine, glycine, and proline are the most abundant nonessential amino acids observed.

TABLE 5 | Fatty acid profile (% total fatty acid) of hybrid African catfish fillet fed varying levels of BSF larval meal-based diet for 8 weeks.

	Test diets				*p*-Values			
Parameters	**Ctrl**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
C12:0 (lauric acid)	1.29[a]	4.66[b]	4.74[b]	6.41[c]	0.564	<0.001	0.001	0.001
C14:0 (mystic acid)	3.13[b,c]	3.00[a,b]	2.75[a]	3.35[c]	0.073	0.003	0.279	0.001
C16:0 (palmitic acid)	25.85	23.93	24.94	23.01	0.479	0.168	0.079	1.000
C17:0 (heptadecanoic acid)	1.30	1.14	1.26	1.25	0.046	0.732	0.948	0.458
C18:0 (stearic acid)	9.85	9.26	9.25	9.01	0.157	0.289	0.089	0.579
Total SFAs	42.06	42.53	41.55	43.75	0.441	0.368	0.317	0.343
C16:1n7 (palmitoleic acid)	3.93[b]	2.66[a]	2.24[a]	2.74[a]	0.198	<0.001	0.001	0.001
C18:1n9c (oleic acid)	38.53[b]	32.61[a]	31.30[a]	32.69[a]	0.928	0.002	0.002	0.004
Total MUFAs	42.82[b]	35.52[a]	33.86[a]	34.32[a]	1.204	0.002	0.001	0.011
C18:2n6c (linoleic acid)	7.63[a]	13.77[b]	17.06[c]	15.12[b]	1.076	<0.001	0.001	0.001
C18:3n3 (α-Linolenic acid)	4.32[b]	3.16[a,b]	2.82[a]	2.16[a]	0.270	0.007	0.001	0.444
C20:4n6 (ARA)	0.26	0.30	0.41	0.26	0.045	0.773	0.520	0.586
C20:5n3 (EPA)	0.71	0.94	0.79	0.61	0.094	0.719	0.632	0.347
C22:6n3 (DHA)	1.47	2.09	2.48	1.97	0.326	0.801	0.573	0.455
Total PUFAs	15.12[a]	21.95[b]	24.60[b]	21.93[b]	1.233	0.011	0.008	0.013
Sum EPA + DHA	2.07	2.61	2.93	2.38	0.390	0.914	0.757	0.556
DHA/EPA	3.29[a]	4.04[a]	5.62[b]	4.24[a,b]	0.290	0.008	0.019	0.013
EPA/ARA	3.48	3.11	1.54	2.50	0.330	0.170	0.118	0.283
Sum n-3	6.38	5.77	5.75	5.21	0.396	0.830	0.398	0.966
Sum n-6	8.74[a]	16.18[b]	18.85[b]	16.71[b]	1.185	<0.001	0.001	0.001
n-3/n-6	0.73[b]	0.35[a]	0.30[a]	0.31[a]	0.058	<0.001	0.001	0.004
n-6/n-3	1.39[a]	2.85[a,b]	3.53[b]	3.32[b]	0.293	0.011	0.004	0.047
PUFA/SFA	0.36[a]	0.52[b]	0.60[b]	0.50[b]	0.029	0.004	0.006	0.003
Atherogenic index	0.69[a]	0.71[a,b]	0.70[a,b]	0.76[b]	0.011	0.034	0.014	0.188
Thrombogenic index	0.85	0.84	0.85	0.86	0.018	0.990	0.886	0.813
h/H ratio	1.83	1.97	1.99	2.01	0.033	0.195	0.063	0.327

Note: FA include: C4:0, C6:0, C8:0, C10:0, C11:0, C13:0, C15:0 C17:0, C20:0, C21:0, C22:0, C23:0, C24:0, C14:1, C15:1, C17:1, C16:1n7, C18:1n9t, C20:1n9, C22:1n9, C24:1n9, C18:2n6t, C20:3n3, C20:2n6, C20:3n6. L, Q, and C = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 3$), pooled standard error of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.

Abbreviations: AI, atherogenicity index; h/H, hypocholesterolemic/hypercholesterolemic fatty acids; SEM, standard error of the mean; TI, thrombogenicity index.

Table 5 shows the fatty acid profile of the hybrid African catfish fillets. In contrast to the observed marked differences in the fatty acid content of the Control diet relative to the BSF larval diets, the fatty acid values of the fillets were similar across treatments. The most abundant saturated fatty acids (SFAs) in fillets were lauric acid (C12:0), mystic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). Lauric acid increased significantly ($p = 0.001$) with increasing levels of BSF larval meal. In all, no significant differences ($p = 0.368$) were observed in the total SFA content of catfish fillets among the dietary treatments and no trend response was observed.

Palmitoleic acid (C16:1n7) and oleic acid (C18:1n9) are the main constituents of monounsaturated fatty acids (MUFAs) in fillets of the experimental catfish. The concentration of palmitoleic acid and oleic acid in fish fillets was significantly higher ($p = 0.001$ and 0.002, respectively) in the Control group compared to the BSF groups with linear and quadratic effects. Consequently, fillets of fish fed the Control diet retained significantly higher concentrations ($p = 0.002$) of total MUFA (42.82%) compared to the BSF groups.

PUFAs consist of short-chain PUFAs (mainly LA C18:2n6 and α-linolenic acid [ALA] C18:3n3) and long-chain PUFAs (mainly arachidonic acid C20:4n6, eicosapentaenoic acid C20:4n3 and docosahexaenoic acid C22:6n3). The levels of LA increased linearly and quadratically across the dietary groups, with the peak mean value observed in fish fed BSF 50%, which was significantly different ($p = 0.001$) from the other groups. The ALA in fillets

decreased linearly with increasing levels of BSF larval meal. Fish fed on the Control diet had the highest concentration of ALA (4.32%), which was similar to fish fed BSF 25 but significantly higher ($p = 0.007$) than those fed BSF 50 and 75 diets.

In terms of LC-PUFA, there were no significant differences or trends in ARA, EPA, and DHA among all the dietary treatments. However, the values are generally higher in fish fed BSF diets compared to those that received the Control diet, resulting in a significantly higher total PUFA ($p = 0.011$) in fish fed BSF larval diets than in the Control group, with observed increased linear and quadratic effects.

The DHA/EPA ratio showed a linear and quadratic relationship. The ratios ranged between 3.29 and 5.62. The highest mean value was observed in fillets of fish fed BSF 50 (5.62), which is comparable to BSF 75 (4.24) but significantly higher ($p = 0.008$) than BSF 25 (4.04) and the Control group (3.29). No significant differences were seen in the summation of n-3 fatty acids ($p = 0.830$). However, fillets of catfish fed BSF larval diets contain lower n-3 fatty acids compared to the Control group. On the other hand, the sum n-6 was significantly higher ($p = 0.001$) in fillets of fish fed on BSF diets than in the Control group, with linear and quadratic effects. The study observed linear and quadrilinear responses in n-3/n-6 and n-6/n-3 ratios. The n-3/n-6 ratio was found to be significantly lower ($p = 0.001$) in the fillets of catfish fed BSF larval diets compared to those fed the Control diet.

Conversely, the n-6/n-3 ratio was significantly higher ($p = 0.011$) in fish fed BSF larval diets compared to the Control group. The PUFA/SFA ratio ranges from 0.36 to 0.60 and was significantly higher ($p = 0.004$) in the BSF diets than in the Control group. The highest atherogenic index was observed in BSF 75 (0.76), which was similar to BSF 25 and BSF 50 but significantly higher ($p = 0.034$) than in the fish fed the Control diet (0.69). The thrombogenic index and hypocholesterolemic/hypercholesterolemic ratio (h/H) were not statistically significant across the dietary groups, with p-values of 0.990 and 0.195, respectively.

3.3 | Blood Biochemistry

3.3.1 | Hematological Parameters

Data obtained from the full blood count (Table 6) showed no significant differences ($p > 0.05$) in all parameters across the dietary treatments. However, some patterns are observed: the BSF groups exhibited higher levels of RBC, HGB, and HCT compared to the Control group. Additionally, the BSF groups showed reduced levels of MCH and MCHC across all the treatments relative to the Control. Conversely, PLT count decreased with increasing dietary BSF levels. WBC and GRAN levels were higher in the BSF groups than in the Control. In contrast, lower levels of LYM were observed in fish fed BSF larval meal diets relative to the Control diet.

3.3.2 | Plasma Biochemical Indices

The plasma biochemical indices of hybrid African catfish fed on graded levels of BSF larval meal diets is shown in Table 7. There were no significant differences ($p > 0.05$) in most plasma indices, except for globulin, albumin–globulin ratio, and cholesterol. Globulin levels were lower in the BSF groups compared to the Control group, which had the highest value (30.20 g/L), similar to BSF 75 but significantly ($p = 0.015$) higher than BSF 25 and BSF 50. The albumin-globulin ratio was highest in BSF 50 group, which was comparable to BSF 25 but significantly different ($p = 0.003$) from BSF 75 and the Control groups. Cholesterol levels in the BSF groups were lower compared to the Control group, which had the highest level (8.53 mmol/L), similar to BSF 50 but significantly different ($p = 0.0017$) from BSF 25 and BSF 75

TABLE 6 | Hematological indices of hybrid African catfish fed varying levels of BSF larval meal-based diets for 8 weeks.

	Test diets				*p*-Values			
Parameters	**Control**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
RBC (10^{12}/L)	2.89	2.90	3.35	3.14	0.107	0.377	0.222	0.606
HGB (g/dL)	11.79	11.80	13.54	12.42	0.331	0.204	0.219	0.384
HCT (%)	35.23	36.20	38.50	37.55	0.749	0.439	0.018	0.530
MCV (fL)	146.04	149.79	145.53	145.41	0.847	0.212	0.413	0.251
MCH (pg)	40.54	40.49	40.35	39.52	0.207	0.270	0.088	0.348
MCHC (g/dL)	27.79	27.09	27.76	27.20	0.256	0.565	0.452	0.932
PLT (10^9/L)	8.96	6.63	5.85	4.11	0.708	0.103	0.016	0.827
WBC (10^9/L)	44.53	46.48	54.76	50.48	1.669	0.130	0.076	0.346
GRAN (%)	19.49	20.39	23.60	21.79	0.750	0.236	0.134	0.363
LYM%	66.99	66.34	63.02	64.24	1.208	0.602	0.285	0.773

Note: L, Q = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 9$), pooled standard error of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.
Abbreviations: GRAN, granulocyte; HCT, hematocrit, HGB, hemoglobin; LYM, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration, MCV, mean corpuscular volume of red blood cell; PLT, platelet; RBC, red blood cells; SEM, standard error of the mean; WBC, white blood cells.

TABLE 7 | Plasma biochemical indices of hybrid African catfish fed varying levels of BSF larval meal-based diets for 8 weeks.

	Test diets				*p*-Values			
Parameters	**Ctrl**	**BSF 25**	**BSF 50**	**BSF 75**	**SEM**	**ANOVA**	**L**	**Q**
Total protein (g/l)	45.30	37.74	37.87	40.04	1204	0.080	0.125	0.038
Albumin (g/l)	15.10	13.37	15.18	11.88	0.658	0.236	0.182	0.542
Globulin (g/l)	30.20^{b}	$24.37^{a,b}$	22.68^{a}	$28.16^{a,b}$	0.981	0.015	0.297	0.002
A/G ratio	0.50^{a}	$0.55^{a,b}$	0.67^{b}	0.42^{a}	0.027	0.003	0.495	0.002
Glucose (mmol/L)	9.41	7.83	8.39	10.19	0.400	0.152	0.397	0.036
Cholesterol (mmol/L)	8.53^{b}	6.80^{a}	$7.48^{a,b}$	6.87^{a}	0.231	0.017	0.021	0.162
Triglyceride (mmol/L)	1.63	1.33	1.52	1.45	0.093	0.726	0.685	0.552
Creatinine (mmol/L)	23.15	19.28	21.78	22.24	0.873	0.465	0.977	0.233
Lactate (mmol/L)	5.91	5.05	5.33	5.57	0.248	0.682	0.747	0.298
Urea (mmol/L)	2.52	2.13	2.45	1.89	0.116	0.197	0.131	0.712
Phosphorus (mmol/L)	5.48	6.08	5.01	5.00	0.169	0.065	0.080	0.326
ALT (U/l)	29.86	31.15	29.30	28.00	0.814	0.605	0.324	0.440
AST (U/l)	50.18	53.11	53.59	54.68	1.024	0.474	0.145	0.661

Note: L, Q = Linear and quadratic polynomial contrasts, respectively. Values are means ($n = 6$), pooled standard error of the mean. Values of different superscripts in a row are significantly different ($p < 0.05$). The absence of a letter indicates no significant difference between treatments.
Abbreviations: A/G, Albumin Globulin, ALT, alanine aminotransferase, AST, aspartate aminotransferase; SEM, standard error of the mean.

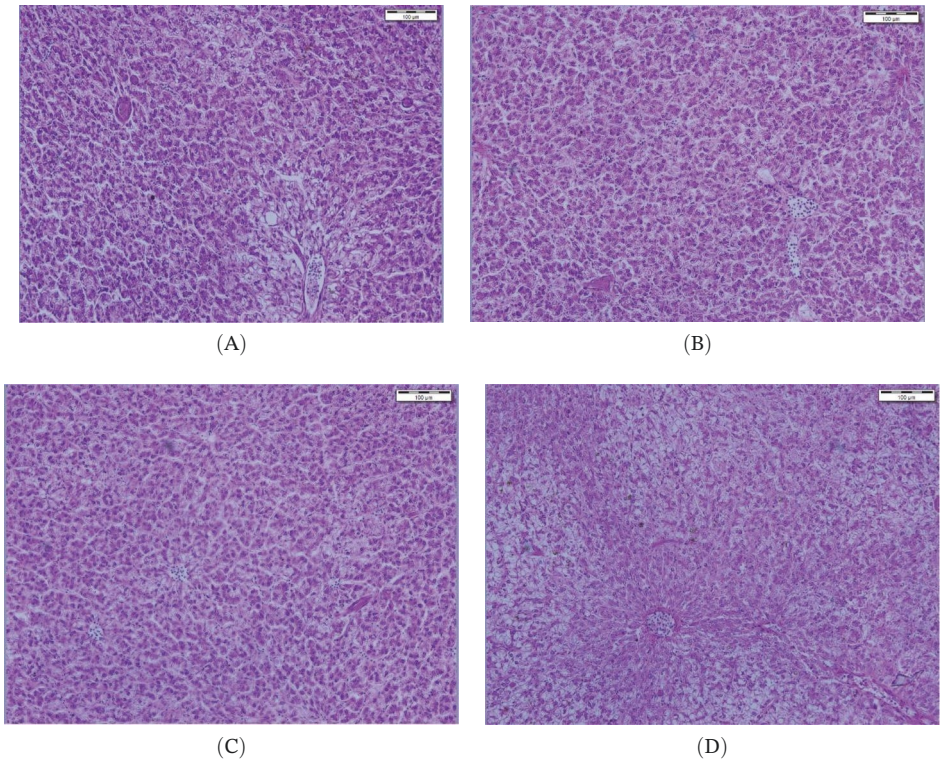

FIGURE 1 | Liver histology of hybrid African catfish fed varying levels of BSF larval meal-based diets for 8 weeks (HE; ×200, Scale bar = 100 μm). A = Control diet, B = BSF 25, C = BSF 50, D = BSF.

groups. Although not statistically significant, a downward trend was observed in total protein, triglycerides, creatinine, lactate, and urea, whilst an upward trend was noticed in AST. Glucose, albumin, and ALT levels remained stable.

3.4 | Liver Histology

The liver histological appraisal of hybrid African catfish (Figure 1) across all dietary treatments shows normal sinusoidal organization, with no vacuolization or evidence of inflammation. The

hepatocyte has a normal structure, uniform appearance, and distribution; the nuclei are regular and not eccentric.

4 | Discussion

4.1 | Diet Composition

Focus has shifted to include not only formulating a diet that satisfies the nutritional requirements of target fish species but also producing fish that meet human dietary requirements. This study demonstrates the feasibility of combined dietary replacement of fishmeal and fish oil with BSF larval meal and corn oil, assessing nutritional safety, physiological parameters, and fillet quality, and offering new insight into the nutritional plasticity of hybrid African catfish, *C. gariepinus* × *H. longifilis*. This approach reflects a more realistic, sustainable, and economically viable strategy for feed formulation.

The experimental diets were formulated to be isonitrogenous, using a conversion factor of N × 6.25. However, because the BSF larval meal contains an appreciable amount of chitin (a non-protein N) [45, 46], the crude protein content of the diets may be overestimated. Future studies on insect meal should consider using the recommended correctional factor of N × 5.33 [47] or the summation of amino acids.

The amino acid content of BSF larval meal diets is similar to the Control diet (Table 8) and appears to meet the EAA requirements of African catfish [42, 48, 49]. Leucine and lysine were the most abundant EAA observed in all the experimental diets, reflecting their richness in the insect larval meal [4, 50]. The comparable levels of amino acids in all experimental diets suggest that fish were fed a similar quality of protein, and the substitution of fishmeal with BSF larval meal did not negatively affect the amino acid composition of the test diets. This supports previous findings suggesting that the amino acid profile of BSF larvae is well-balanced and similar to that of fishmeal [16, 51, 52].

The fatty acid composition of corn oil (Table 9) shows that linoleic (C18:2n6) is the most prevalent (55.46%), followed by oleic acid C18:1n9 (30.11%) and palmitic acid C16:0 (10.91%). These results closely align with the previous report by NRC [53].

Palmitic acid is the most abundant SFA in the diets, closely followed by lauric acid. BSF larval meal has been reported to have a high concentration of lauric acid, which contributed to the increased levels of SFA in BSF larval diets compared to the Control diet [16, 54, 55]. Oleic acid (C18:1n9) was the major component of MUFA detected in experimental diets. The concentration of oleic acid decreased in the test diets when fishmeal and fish oil were substituted with BSF larval meal and corn oil, respectively. Consequently, total MUFA decreases across treatments. The present finding aligns with Guerreiro et al. [56] and

TABLE 8 | Amino acid composition (%, dry matter) of BSF larval meal-based diets fed to hybrid African catfish for 8 weeks.

	Test diets			
Parameters	**Ctrl**	**BSF 25**	**BSF 50**	**BSF 75**
Essential AA				
Arginine	1.79	1.5	1.51	1.41
Histidine	1.26	1.18	1.29	1.16
Isoleucine	1.51	1.51	1.62	1.83
Leucine	2.86	2.79	2.77	2.31
Lysine	3.27	4.41	3.91	3.83
Methionine	1.23	0.89	0.95	1.09
Phenylalanine	1.51	0.91	0.78	0.65
Threonine	2.6	2.02	1.85	1.62
Valine	1.93	1.7	1.71	1.51
Non-essential AA				
Alanine	2.5	2.63	2.47	2.19
Asparagine	3.98	3.44	3.21	2.39
Cysteine	0.25	2.63	2.47	2.19
Glutamine	6.61	5.49	5.28	4.98
Glycine	2.55	3.05	2.61	2.40
Proline	4.12	4.43	4.94	4.98
Serine	1.79	1.56	1.49	1.30
Tyrosine	0.81	0.64	0.55	0.56

Note: NB: Tryptophan not detected.
Abbreviation: AA, amino acid.

TABLE 9 | Ingredient composition (g/kg, DM) and inclusion levels of BSF larval meal.

	Test diets			
Ingredients	**Control**	**BSF 25**	**BSF 50**	**BSF 75**
Fishmeal	400	300	200	100
BSF meal	0	160	260	360
JPC 56 soy conc.	200	150	170	160
Blood meal	20	20	20	20
Min./vit. premix	20	20	20	20
Glucose	10	10	10	10
Vitamin C	0.1	0.1	0.1	0.1
Fish oil	91	0	0	0
corn oil	0	85	83	82
Novilpel	10	10	10	10
threonine	8	8	7	8
Lysine	10	11	10	10
Methionine + cystin	5	5	5	5
Tryptophan	1	1	1	1
Wheat	224.9	219.9	203.9	213.9
Total	1000	1000	1000	1000
Proximate composition (%, DM)				
Dry matter	89.96	89.69	89.64	90.22
Crude protein	40.65	40.38	39.81	39.53
Crude fat	13.63	14.02	14.47	15.34
Fiber	1.38	2.29	2.76	3.81
Ash	13.87	11.08	10.86	8.18
Calcium	2.54	2.16	1.97	1.45
Phosphorus	2.18	1.8	1.64	1.14
NFE	20.43	21.92	21.74	23.36
GE (MJ/kg)	13.15	13.74	14.43	15.33
ADF	1.78	2.70	3.28	4.43
Chitin	—	2.46	2.84	3.75

Note: NFE = (nitrogen-free extract) = [100 – (% moisture + protein% + lipid% + ash% + fiber%)].Fishmeal (herring, CP = 67%, fat 9%). BSF larval meal[2] (defatted) (GRINSECT, Hódmezővásárhely, Hungary), moisture, 4%, crude protein, 55.7%, crude fat, 10%. JPC 56 soy conc. (CP = 56%, crude fat = 3.3%). Blood meal (CP = 95%, crude fat = 1.7%). Corn oil (VFI GmbH, Wels, Australia) Energy, 3404 kJ/100 g; fat 92 g/100 g (SFA, 12.8; MUFA, 29; PUFA, 51 g). Wheat (CP = 12.3%, crude fat 1.3%). Mineral and vitamin premix: Lysine 0.780 %, Methionine 4.257 %, Meth + cistine 4.257%, Calcium, 18.800%; Phosphorus, 9.122%; Sodium, 5.322%; Magnesium, 0.136%; Zinc 4000.000 mg/kg; Copper, 600.000 mg/kg; Iron, 4000.000 mg/kg Manganese, 2668.000 mg/kg; Iodine, 56.000 mg/kg; Selenium, 11.700 mg/kg; Cobalt, 12.000 mg/kg; A vitamin, 440,000.000 IU/kg; D-3 vitamin, 116,000.000 IU/kg; E vitamin, 2000.000 mg/kg; K-3 vitamin, 66.000 mg/kg; B-1 (tiamin) 66.000 mg/kg; B-2 (riboflavin), 198.000 mg/kg; Pantoth. acid 478.280 mg/kg; B-6 (piridoxin) 110.000 mg/kg; B-12(kobalamin) 1.100 mg/kg; Biotine 4.400 mg/kg; 080 B-3 (nikotinic) 935.770 mg/kg; Folic acid 22.000 mg/kg; Choline-chloride, 18,000.000 mg/kg; Antioxidants, 55.000 mg/kg; Canthaxanthin 180.000 mg/kg; Phytase, 10,000.000 Ftu/k; Xylanase, 27,000.000 U/kg; Apo-esther, 92.000 mg/kg; Protease 450.000 U/kg; Sepiolite (E562), 1770.000 mg (Source: KJK-Agroteam Kft, Dombóvár, Hungary).
Abbreviations: ADF, acid detergent fiber; GE, gross energy.

Abdel-Tawwab et al. [57] in diets of meagre *Argyrosomus regius* juveniles and European sea bass *Dicentrarchus labrax*, respectively. In contrast, Caimi et al. [58] observed an increasing value of oleic acid in a diet for Siberian sturgeon (*Acipenser baerii*) juveniles when fishmeal was substituted with defatted BSF larvae, resulting in increased MUFA across the groups.

LA and ALA are short-chain PUFAs that are essential for their role in various physiological processes and for promoting healthy growth and development in fish [59]. In the present experiment, the concentration of LA in the diets was much greater than that of ALA, primarily due to the presence of BSF larval meal and corn oil. LC-PUFAs, such as ARA, EPA, and DHA, play vital

roles in the physiological welfare, growth, and survival of fish [60]. DHA and EPA are regarded as the most important fatty acids for their crucial role in preservation of structural membranes of cells, development of the brain, neural, and visual systems, stress and disease resistance, promote growth and survival of fish, especially during the early stages of development [61–64]. ARA is significant for its role as a precursor to eicosanoid production, which regulates osmoregulation, cardiovascular function, neural control, and reproduction. [65–68]. Most fish are inefficient at synthesizing these LC-PUFAs; therefore, they obtain these nutrients from their diet [69–71]. While marine ingredients such as fishmeal and fish oil are rich in LC-PUFA, several researchers have reported that insect meals are either deficient [46, 56, 72, 73] or contain negligible amounts [4, 16, 46] of these important LC-PUFAs; thus limiting their use in aquafeed and therefore recommend enrichment of insect larvae meant as ingredient for fish feed utilization [39].

The sharp decrease in ARA, EPA, and DHA across the experimental diets could be attributed to a deficiency of these essential fatty acids in BSF larval meal [38, 40, 74] and corn oil (NRC, 1993; [75]). The observed moderate increase in total PUFA across the diets was mainly due to LA, which was by far the most abundant PUFA. Consequently, total n-3 decreased, and n-6 increased across the group. Nevertheless, the DHA/EPA ratio (2.3–2.51) meets the nutritional requirements of fish [62]. Similar findings were previously reported by Lock et al. [54], Borgogno et al. [74], Mancini et al. [38], and Hu et al. [76]).

4.2 | Growth Performance

Replacing fishmeal with up to 75% BSF meal and completely substituting fish oil with corn oil resulted in no mortality, and feed intake was similar ($p > 0.05$) across all dietary treatments, which positively reflects the fish's adaptability and acceptability of the new diet. This is consistent with other studies on fishmeal replacement with BSF larval meal [55, 77–79].

Replacing 50% of fishmeal with BSF meal resulted in the highest growth performance (final weight, weight gain, SGR). However, at 75% level, growth performance and nutrient utilization (FCR, PER) significantly decline ($p < 0.05$). The lower growth in fish fed BSF 75 (360 g kg^{-1} dietary BSF meal), relative to other treatments, was likely not due to palatability or toxicity/pathogenic issues, but rather a deficiency in nutrient uptake and utilization, plausibly due to its high chitin content (3.75%) (Table 9).

Chitin is a polymer of N-N-acetylglucosamine, a primary structural component of invertebrate exoskeleton. Although chitinolytic activity was reported in several fish species, including the African catfish [80, 81], most fish lack sufficient chitinolytic enzymes (chitinase and chitobiase) in the gastrointestinal tracts for efficient digestion [72, 82, 83].

Chitin in BSF meal may bind with nutrients, such as proteins and lipids, thereby reducing digestibility and decreasing the bioavailability of EAAs and fatty acids necessary for growth and development ([19, 84, 85]. It has been reported that dietary chitin as low as 1.6% inhibits nutrient uptake in the intestinal tract, resulting in reduced growth in turbot *Scophthalmus maximus* [37]. For example, the apparent digestibility coefficient (ADC) of protein and lipid for BSF larval meal was 63% and 78%, respectively, whereas that of fishmeal was 88%–98% and 98.7% [37]. Villanueva-Gutiérrez et al. [86] mentioned a chitin content of 2.73% in BSF larval diet as a possible reason for depressed growth in totoaba (*T. macdonaldi*). On the contrary, Piccolo et al. [87] found no significant reduction in protein and lipid digestibility in sea bream *Sparus aurata* fed dietary 500 g kg^{-1} defatted *Tenebrio molitor*, which contains 4.6% chitin.

The bioactive compounds in insect meal (chitin, antimicrobial peptides, lauric acid) may positively impact fish's immune system and gut health due to their immunostimulant properties [88–90]. However, high inclusion of these compounds may divert energy towards innate immune responses and increase cell turnover, thereby reducing the proportion of dietary energy and protein available for fish growth [91], potentially raising the FCR.

Replacing fishmeal and fish oil with BSF meal and corn oil increases the dietary n-6/n-3 ratio, significantly reduces the availability of EPA and DHA, which are essential for maintaining membrane function, promoting growth, and enhancing metabolic efficiency [62, 67]. A deficiency in n-3 LC-PUFAs may result in reduced dietary energy available for growth and increased maintenance energy requirements [16, 42, 92].

The observed linear increase in FCR ($p = 0.001$) in a dose-dependent manner aligns with previous findings by Li et al. [93], Caimi et al. [58], and Mastoraki et al. [94], for Jian carp, Siberian sturgeon and European sea bass, respectively. Similarly, Nayak et al. [95] observed an increasing FCR with increasing substitution of fish oil with linseed oil in diets for silver barb (*Puntius gonionotus*) fingerlings. In contrast, Lock et al. [54] observed a decline in FCR with higher levels of BSF larval meal in diets for Atlantic salmon, possibly due to increased fat deposition in the organs.

The present study supports previous findings by Adeoye et al. [21] and Xiao et al. [96] in which replacement of fishmeal with BSF meal up to 50% (75 g kg^{-1} dietary inclusion) and 48% (223 g kg^{-1}) in African catfish (*C. gariepinus*) and yellow catfish (*Petteobagrus fulvidraco*), respectively, did not have adverse effects on fish growth and body composition. Replacing 50% of fishmeal (150 g kg^{-1}) with BSF larval meal in the diet for Chinese largemouth catfish (*Silurus merdionalis*) for 56 days resulted in significantly improved growth performance compared to the fishmeal-based control diet [97].

Additionally, a high dietary inclusion of BSF up to 400 g kg^{-1} (50% FM replacement) did not affect the growth and survival rate of rainbow trout *Oncorhynchus mykiss* [55]. Similar observations were made in the Jian carp *Cyprinus carpio var. Jian* [93] and the zebra fish *Danio rerio* [98]. Additionally, in various insect species, fishmeal could be replaced with the yellow mealworm (*Tenebrio molitor*) larval up to 50% (corresponding to 120 and 400 g kg^{-1}) meal in diets for the common catfish, *Ameiurus melas* and the blackspot sea bream, *Pagellus bogaraveo*, respectively [99, 100] without impairing growth and nutrient utilization. Substituting fishmeal with housefly maggot *Musca domestica* meal up to 50% (180 g kg^{-1}) in diets for Nile tilapia *Oreochromis niloticus* has no significant impact on growth, flesh quality and innate immunity [101].

The present finding contradicts other studies, which indicate that substituting fishmeal with BSF larval meal at levels above 50% does not negatively impact the growth performance of African catfish.

Replacing 75% of fishmeal with BSF meal (171.8 g kg^{-1}) did not compromise the growth or health of African catfish [20]. Additionally, Olaniyi and Salau [102] reported that incorporating 436.5 g kg^{-1} (75% FM replacement) of housefly maggot meal into the diet did not adversely affect the growth of African catfish. Similarly, including lesser mealworm (*Alphitobius diaperinus*) meal at 157.5 g kg^{-1} (75% FM replacement) in diet for European perch (*Perca fluviatilis*) did not compromise production performance [103].

Complete replacement of fishmeal with BSF larval meal has been reported for the Atlantic salmon [46, 54, 104] and for European sea bass (*Dicentrarchus labrax*; [57]). Kishawy et al. [105] reported that substituting fishmeal with BSF meal up to 100% (100 g kg^{-1}) in Nile tilapia diet did not impair growth or feed efficiency. Similarly, Taufek et al. [106] found that complete replacement of fishmeal with cricket (*Gryllus bimaculatus*) meal (300 g kg^{-1}) supports healthy growth and nutrient utilization in African catfish. Furthermore, fishmeal was entirely replaced with defatted housefly maggot meal in the diets for African catfish *C. gariepinus* [107, 108] and Nile tilapia *Oreochromis niloticus* [107] without negatively affecting fish growth or health. The differences may be due to factors such as fish species and size, the BSF larval matrix, the processing method of the larval ingredient or the actual dietary dosage of larval meal used in the feed formulation [109, 110].

Previous studies have demonstrated successful partial or total dietary fish oil substitution with vegetable oils and/or animal fat such as BSF larval oil, as was reported in African catfish *C. gariepinus* [111–113], *H. longifilis* [114], Nile tilapia [115, 116], Atlantic salmon [117], and Jian carp [118]. In a 10-week study on the striped catfish *P. hypophthalmus*, [119] examined BSF larval oil and a series of vegetable oils (moringa, black cumin seed, and flax seed) as a complete replacement for fish oil (23 g kg^{-1}). Each diet was formulated to contain 80 g kg^{-1} fishmeal and 174 g kg^{-1} BSF larval meal. The authors concluded that the growth and feed efficiency metrics (final body weight, weight gain, SGR, TGC, and FCR) of fish fed BSF larval oil and moringa oil diets were comparable to those of fish fed the fish oil-based control diet. Similarly, Babalola et al. [120] reported significant growth and feed efficiency in *H. longifilis* when fed a diet containing 398 g kg^{-1} fishmeal and a complete replacement of 60 g kg^{-1} fish oil with palm kernel oil. A complete replacement of a 1:1 mixture (25 g kg^{-1}) of fish oil and rapeseed oil with BSF larval oil in the diet for hybrid African catfish (*C. gariepinus* × *H. longifilis*) did not affect growth and feed utilization [121].

In contrast to the present study, Lin and Shiau [28] found that a complete substitution of fish oil (40 g kg^{-1}) with corn oil in a diet for grouper (*Epinephelus malabaricus*) resulted in depressed growth, which was attributed to the low DHA/EPA ratio as a result of the dietary change. However, fish that were fed diets containing a blend of fish oil and corn oil (3:1) and (1:1) exhibited similar high growth rates and nutrient efficiency as those fed the fish oil control diet.

No significant differences were seen in organosomatic indices (HSI, SSI, VSI, IPF, RGL, carcass yield, and fillet yield) in fish fed BSF larval diets compared to the Control group. This suggests that the alternative ingredients did not adversely affect protein and lipid deposition at the organs and carcass levels. These findings align with similar studies in African catfish *C. gariepinus* [20, 22], striped catfish [122], Jian carp *Cyprinus carpio* var. Jian [123], Japanese sea bass *Lateolabrax japanicus* [124] and European sea bass *Dicentrarchus labrax* [125], where substitution of fishmeal with BSF larval meal had no significant influence on HSI, SSI, VSI, and IPF. Similarly, Tran et al. [103] reported that feeding lesser mealworm (*Alphitobius diaperinus*) meal to European perch does not influence the organosomatic indices (HSI, SSI, VSI, MFI, fillet yield).

4.3 | Proximate Composition, Amino and Fatty Acid Profile, Fillet Nutritional Quality

In this study, the incorporation of BSF larval meal and corn oil in the diets of hybrid African catfish had no significant influence on the proximate composition of the fillets. These findings align with Gebremichael et al. [22], Abdel-Tawwab et al. [57], and Busti et al. [126], who found no significant differences in dry matter, crude protein, crude fat, and ash content in African catfish *C. gariepinus*, European seabass *Dicentrarchus labrax*, and sea bream *Sparus aurata*, respectively. Similarly, Wang et al. [101] found no significant difference in the proximate composition of Nile tilapia fillets fed diets containing housefly (*Musca domestica*) maggot in dry matter, crude protein, crude lipid, ash, and gross energy. However, some authors observed differences in dry matter, lipid, and ash content in fillets of rainbow trout and European sea bass fed on BSF larval-based diets [72, 127]. The inclusion of BSF larval meal and corn oil in the test diets did not affect the amino acid profile of the fillets, as the values are similar across all treatments. This contrasts with report by Iaconisi et al. [128], which found significant differences in some amino acids, notably histidine and leucine, in the muscle of gilthead sea bream and rainbow trout fed yellow mealworm (*Tenebrio molitor*) larval-based diets.

The fatty acid profile of fish fillet, primarily triacyl glycerides (Table 5), generally reflects the fatty acid composition of the diets. However, some fatty acids are either retained in the flesh in the same proportion or selectively utilized [129, 130].

Lauric acid and palmitic acid are the most abundant SFA in BSF larvae meal [16, 39, 57]. The same pattern was seen in the test diets. The notable increase in lauric acid, along with stable levels of palmitic acid and stearic acid, resulted in unchanged total SFA across the groups. Palmitic and stearic acids are long-chain fatty acids (LCFAs) that are less affected by short-term dietary changes due to their metabolic stability and slower oxidation rates; therefore, they are readily retained in tissues [131]. In contrast, lauric acid, a medium-chain fatty acid (MCFA), is rapidly metabolized for energy, resulting in low tissue retention [54, 56]. This was evidenced by the attenuation of lauric acid in fillets relative to diets. Similar observations were made in Jian carp [118] and Atlantic salmon [46, 54]. The significant decrease in sum MUFA in fish that received BSF diets may be largely due to the low oleic acid content found in BSF meal, which was reflected in the fatty acid composition of the diets (Table 10).

Conversely, the notable increase in sum PUFA in the BSF groups may be attributed to LA, which is abundant in corn oil, (accounting for ~58% of total fatty acids) [23], resulting in the substantial accumulation of linolic acid in the muscle, raising the level of n-6 fatty acids and contributing to the observed decrease in the n-3/n-6 ratio. A decline in n-3/n-6 ratio in fillet is a typical outcome when fishmeal and fish oil are replaced with alternative

TABLE 10 | Fatty acid composition (% total fatty acids) of corn oil and experimental diets fed to hybrid African catfish for 8 weeks.

		Test diets			
Parameters	**Corn oil**	**Ctrl**	**BSF 25**	**BSF 50**	**BSF 75**
C12:0 (lauric acid)	—	0.37	11.11	13.85	20.59
C14:0 (mystic acid)	—	3.72	3.83	4.01	4.91
C15:0 (pentadecanoic acid)	—	0.58	0.23	0.14	0.08
C16:0 (palmitic acid)	10.91	24.01	18.96	17.11	15.45
C17:0 (heptadecanoic acid)	—	1.48	0.37	0.48	0.25
C18:0 (stearic acid)	1.67	7.68	4.97	4.1	3.06
C20:0	0.35	0.28	0.21	0.20	0.16
C22:0	0.08	0.13	0.10	0.06	0.05
Total SFA	13.02	38.33	40.45	40.37	45.12
C16:1n7 (palmitoleic acid)	0.12	4.23	2.62	2.31	2.32
C17:1 (heptadecanoic acid	—	0.14	0.07	0.05	0.03
C18:1n9 (oleic acid)	30.11	29.77	25.51	24.27	21.99
Total MUFA	30.41	34.14	28.2	26.63	24.34
C18:2n6c (linoleic acid)	55.46	11.76	24.63	28.51	28.15
C18:3n3 (α-Linolenic acid)	1.11	4.61	2.25	1.61	1.03
C20:4n6 (ARA)	—	0.47	0.19	0.12	0.06
C20:5n3 (EPA)	—	3.25	1.3	0.85	0.37
C22:6n3 (DHA)	—	7.41	2.98	1.91	0.93
Total PUFA	56.57	27.53	31.35	33.00	30.54
Sum EPA + DHA	—	10.66	4.28	2.28	1.30
EPA/ARA	—	6.91	6.84	7.08	6.17
DHA/EPA	—	2.28	2.29	2.25	2.51
Sum n-3	1.11	15.27	6.53	4.37	2.33
Sum n-6	55.46	12.26	24.82	28.63	28.21
n-3/n-6	0.02	1.25	0.26	0.15	0.08
n-6/n-3	49.96	0.80	3.80	6.55	12.11

Note: Fatty acids include: C4:0, C6:0, C8:0, C10:0, C11:0, C13:0, C15:0 C17:0, C21:0, C23:0, C24:0, C14:1, C15:1, C17:1, C16:1n7, C18:1n9t, C20:1n9, C22:1n9, C24:1n9, C18:2n6t, C20:3n3, C20:2n6, C20:3n6.

ingredients such as insect meal and vegetable oils [75, 95, 112, 115, 132, 133].

Despite the marked reduction in dietary LC-PUFA resulting from the inclusion of BSF meal and corn oil, the levels of ARA, EPA, DHA, and the sum n-3 fatty acids in the fillet remained relatively stable. This contrasts with increased LA and decreased ALA in fillet tissue, parallel with dietary supply. This divergent response does not suggest enhanced de novo biosynthesis from C18 precursors, as there was no dominant compensatory mechanism, leading to either conservation of ALA or increased EPA/DHA deposition in fillet tissue [42, 134–136]. Instead, it likely reflects preferential retention or selective conservation of LC-PUFA in muscle tissue, a phenomenon previously documented in various freshwater and euryhaline teleosts such as Atlantic salmon [117, 137], gilthead seabream, European seabass [138], rainbow trout [139, 140], largemouth bass *Micropterus salmoides* [141], and striped catfish *Pangasianodon hypophthalmus* [142], African catfish, *C. gariepinus* [143] when fish oil is partially or totally replaced with an alternative lipid source such as vegetable oils or animal fat.

This retention highlights the physiological importance of these fatty acids (in maintaining cell membrane integrity and synthesizing eicosanoids), resulting in reduced catabolism compared with MUFAs and SFA [10, 11, 65, 131, 144, 145]. Besides, regulation of lipid metabolism in the muscle tissue is species-specific [10, 11, 146]. It appears that hybrid African catfish specifically modulate LC-PUFA by selectively oxidizing excess n-6 and MUFA for β-oxidation, while sparing EPA and DHA, thus prioritizing their presence in phospholipids, which are essential for structural functions [75, 147, 148].

African catfish are considered relatively lean-fleshed (< 2%) or low-fat (2%–4% ww fat) teleosts [149], typically with low levels of intramuscular lipid deposition [150]. The lipids found in their muscle are predominantly structural and are tightly regulated to maintain membrane integrity [10, 11]. Consequently, a short dietary change may have a limited influence on the muscle compared to its impact on the liver and visceral tissues.

From a fish health perspective, a moderate increase in muscle LA (n-6) and lauric acid (C12:0) may not be inherently detrimental to fish. LA may serve as an efficient energy substrate, while lauric acid is known for its antimicrobial and immunomodulatory properties, which may improve gut health and enhance immune responses [88, 90].

Producing fish that meet human dietary requirements for n-3/n-6 fatty acids is essential, as these fatty acids offer numerous health benefits, including the prevention of coronary heart diseases and the reduction of inflammatory disorders [150–152]. This study found that the n-3/n-6 ratios in the fillets of fish fed BSF diets (0.30–0.35) were substantially lower than in the Control diet (0.73). Additionally, these ratios were lower than those reported for the muscle of other fish species fed BSF larval-based diets, such as hybrid African catfish (heteroclarias) [121], African catfish (*C. gariepinus*) [22] and Jian carp (*Cyprinus carpio var. Jian* [118]). The low n-3 muscle fatty acid profile may reflect a trade-off between sustainability and optimal lipid nutrition. Nevertheless, the n-3/n-6 ratios observed in this study fall within the recommended range (0.25 to 1) for human dietary intake [153].

From a human nutrition perspective, in addition to the n-3/n-6 ratio, the nutritional quality of fatty acids in fish muscle could be assessed using various indices, including the PUFA/SFA ratio, the atherogenicity index (AI), the thrombogenicity index (TI) and the hypocholesterolemic/hypercholesterolemic index (h/H) [151]. The significantly improved PUFA/SFA ratio reflects a higher intake of dietary PUFA, particularly LA from corn oil. PUFA is recognized for its cardiovascular benefits in humans, as it helps lower low-density lipoprotein (LDL) cholesterol levels [154, 155]. In this study, the PUFA/SFA ratio ranged from 0.50 to 0.60, surpassing the minimum threshold of > 0.45 for a healthy diet [156, 157].

The significantly higher AI in fish fed BSF 75 (0.76) relative to those fed the Control diet (0.69), suggests a theoretical potential towards promoting atherosclerosis due to higher levels of SFA. The absence of significant differences in TI across treatments suggests that hybrid African catfish can preferentially retain anti-thrombotic fatty acids (MUFA and LC-PUFA) in their muscle tissue despite dietary changes. For fish meat to be considered beneficial for human health in reducing the risk of coronary heart disease, both AI and TI levels should be below 1.0 [55]. In the current study, both AI and TI levels were below 1.0 and within the range (0.48–0.83) reported for various fish species [156]. The stable h/H index further confirms that dietary changes did not compromise the cholesterol-lowering potential of catfish fillets.

Overall, the nutritional quality indices suggest that the fillets of hybrid catfish raised on BSF-corn oil-based diets are nutritionally acceptable. Increasing consumers' awareness and acceptance of fish cultured on insect-based diets could enhance human nutrition and contribute to sustainability by reducing reliance on finite marine resources while supporting circular bioeconomy principles.

4.4 | Blood Biochemistry and Liver Histology

4.4.1 | Hematological Parameters

Blood biochemical parameters, such as full blood count and plasma or serum analysis, are utilized to evaluate the physiological and health status of fish. They offer comprehensive insights into oxygen transport capacity, immune potential, nutritional status, stress level, disease, and toxicity in fish [32, 158–160]. Hematological parameters of fish are influenced by internal factors such as species, age, sex, and inflammation, while external factors include nutrition, stocking density, handling, and water quality parameters [158, 161].

The hematological parameter values recorded herein are within the recommended range for *C. gariepinus* [162, 163] and closely align with those reported by other researchers [159, 164]. The hematological profile showed no statistically significant differences across the various treatments, indicating that incorporating BSF meal and corn oil into the diets for African catfish did not negatively impact fish blood health or compromise the physiological condition. This finding is consistent with previous studies by Fawole et al. [20] and Adeoye et al. [21], which showed that dietary inclusion of BSF larval meal at 171.8 and 150 g kg^{-1}, respectively, did not significantly alter the hematological profile of the African catfish. Nevertheless, some discernible patterns were identified, which may be viewed as indicative rather than definitive.

The moderately increased levels of RBC, HGB, and HCT alongside stable MCV but mildly reduced MCH and MCHC in the BSF group relative to the Control group may suggest stimulation of erythropoiesis with similar cell size but slightly less HGB-rich content (hypochromia), which may potentially reflect a suboptimal supply of essential hematopoietic micronutrients such as iron, vitamin B12, and folate. Chitin in BSF meal may bind with functional iron, thereby reducing its bioavailability for heme synthesis [158, 165, 166]. Additionally, replacing fish oil with corn oil (high in LA but negligible EPA/DHA) may alter cell membrane composition, potentially increasing lipid peroxidation, thereby impairing heme synthesis, despite an increase in total RBC production [165, 167]. The increased erythropoietic output could be a compensatory response to sustain adequate oxygen transport under the new dietary regime [167, 168].

The moderate rise in leukocyte count (WBC) and granulocytes, alongside a subtle reduction in LYM count, in the BSF diets compared to the fishmeal control diet, may reflect a mild activation of the immune response and a shift in the leukocyte profile towards neutrophils or monocytes [160, 169]. This may suggest a dominance of the innate immune response over adaptive immunity, possibly triggered by bioactive compounds in BSF meal (e.g., chitin, AMPs, and lauric acids) and corn oil lipid milieu (which favors arachidonic acid-derived eicosanoids and granulocyte recruitment) [79, 83, 170]. These factors tend to stimulate innate immunity, leading to increases in neutrophils, monocytes, and total WBC count, resulting in a relative decrease in LYMs [159]. A moderate reduction in PLT count may result from minor modulation of thrombopoiesis influenced by dietary factors such as chitin and fatty acids, which may affect blood clotting parameters and PLT regulation [33, 171, 172]. Overall, the hematological patterns suggest that incorporating BSF meal and corn oil in hybrid African

catfish diets does not cause overt hematological impairments, although there may be mild immune-modulating effects.

4.4.2 | Plasma Biochemical Indices

The results of plasma biochemical indices indicate that replacing fishmeal and fish oil with BSF larval meal and corn oil in the diet did not adversely affect the African catfish, as most parameters remained stable. However, significant decreases in globulin and cholesterol levels were observed. The reduction likely reflects physiological and dietary adaptation rather than impairment of immune or metabolic function. In teleosts, plasma globulin encompasses immunoglobulins and acute-phase proteins [158, 160, 173]. The observed reduction, in the absence of mortality, elevated liver enzymes (AST and ALT) and inflammatory histology, while total protein and hematological indices remained unaffected, suggests a mild shift towards activation of innate immune function rather than immunosuppression [158].

Similarly, the reduction in plasma cholesterol levels, which may be a positive health indicator, may be attributed to the dietary plant-based corn oils and BSF meal components, such as phytosterols and chitin, which are associated with hypocholesterolemic effects [27, 93, 114, 174]. Additionally, the reduction in cholesterol level may be attributed to lauric and other MCFAs, which are rapidly oxidized for energy, reducing the fat available for storage as adipose tissue, and in turn limit liver lipid accumulation [54, 93, 174].

Furthermore, the normal levels of triglyceride and hepatosomatic indices across the treatments suggest lipid digestion and metabolic regulation were not adversely affected [175, 176]. The finding aligns with previous reports on African catfish *H. longifilis* [114], European sea bass [14], and Japanese sea bass [124] fed BSF larval diets. In contrast, Egessa et al. [121], Fawole et al. [20], Sudha et al. [119], and Tran et al. [92] reported that hybrid African catfish, African catfish, striped catfish and European perch (*Perca fluviatilis*) maintained stable serum cholesterol fed BSF-based diets.

Other indices, including albumin, glucose, lactate, creatinine, and urea, showed no significant differences, suggesting that the dietary modifications did not adversely affect energy metabolism, muscle function, renal health, or nitrogen excretion [159, 175, 177, 178]. Overall, the biochemical responses suggest that the hybrid African catfish can maintain metabolic and immune homeostasis despite the dietary changes.

4.4.3 | Liver Histology

The liver is the primary site of manifestation for dietary imbalances due to its central role in metabolism, making it susceptible to cellular injury, lipid accumulation (fatty liver), or inflammatory responses [164]. Histological examination of the liver of the African catfish (Figure 1) indicates that the dietary modification did not adversely affect hepatic structure or function. The normal hepatocyte structure, regular nuclear shape, normal sinusoidal organization, and no obvious vacuolization, inflammation, or necrosis indicate that both nutrient metabolism and detoxification processes were maintained. The finding is further supported by the hematological and plasma biochemistry results, which show no significant differences across most parameters. Contrarily, Jimoh et al. [179] noticed vacuolation of the hepatocytes of the liver in *C. gariepinus* fed cooked sesame meal-based diets.

5 | Conclusion

The study evaluated the effects of replacing fishmeal and fish oil with BSF and corn oil on growth performance, amino acids, fatty acids composition, blood biochemistry, and liver histology of hybrid African catfish. Fish readily accepted all diets, and no mortality was recorded. The amino acid content of fillets of fish fed the BSF diets was relatively similar to that of the fishmeal-based Control treatment. The ARA, EPA DHA, and sum n-3 PUFA contents in fillets of fish fed BSF larval diets were comparable to the Control group. Results of the blood biochemistry and liver histology indicate that the dietary changes did not compromise the general health status of cultured African catfish. Overall, the findings of this study suggest that BSF larval meal may partially replace dietary fishmeal with up to 50% (200 g kg^{-1}) and corn oil may completely replace fish oil, in a practical diet for hybrid African catfish without exerting adverse impacts on growth, feed conversion, fillet nutritional quality, and physiological well-being.

Nomenclature

ADC:	Apparent digestibility coefficient
ALA:	Alpha linolenic acid
AMP:	Anti-microbial peptides
AMP:	Atherogenic index
AI:	Alpha-linolenic acid
ARA:	Arachidonic acid
BSF:	Black soldier fly
DHA:	Docosahexaenoic acid
DM:	Dry matter
EAA:	Essential amino acids
EPA:	Eicosapentaenoic acid
FA:	Fatty acids
FAO:	Food and Agriculture Organization
FCR:	Feed conversion ratio
h/H:	Hypocholesterolemic/hypercholesterolemic index
HSI:	Hepatosomatic index
LA:	Linoleic acid
LC:	Long-chain
MCFA:	Medium-chain fatty acids
MUFA:	Monounsaturated fatty acid
n-3:	Omega-3 fatty acids
n-6:	Omega-6 fatty acids
NEAA:	Non-essential amino acids
NRC:	National Research Council
PUFA:	Polyunsaturated fatty acids

SC: Short chain

SFA: Saturated fatty acids

SGR: Specific growth rate

SSI: Spleen-somatic index

TI: Thrombogenic index

VSI: Viscerosomatic index.

Author Contributions

Christopher Teye-Gaga: conceptualization, data curation, formal analysis, methodology, writing – original draft, writing – review and editing. **Péter István Molnár:** data curation, supervision, writing – review and editing. **Attila Kertész:** data curation, supervision, writing – review andediting. **John Kiguru Maina:** data curation, supervision, writing – review and editing. **Elshafia Ali Hamid Mohammed:** data curation, supervision, writing – review and editing. **Gabriella Gulyás:** data curation, supervision, validation, writing – review and editing. **Levente Czeglédi:** data curation, project administrator, supervision, validation, writing – review and editing. **Milán Fehér:** data curation, project administrator, supervision, validation, writing – review and editing. **Péter Bársony:** conceptualization, data curation, methodology, supervision, validation, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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