Hungarian University of Agriculture and Life Sciences

Doctoral School of Animal Biotechnology and Animal Science

Exploring the Potential Benefits of Insect Oil as Alternative Lipid Source in Aquafeeds for Catfish

Thesis of the PhD Dissertation

Robert Egessa

Gödöllő, Hungary
2025

Hungarian University of Agriculture and Life Sciences, Hungary

Name of Doctoral School: Doctoral School of Animal Biotechnology and Animal Science, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Discipline: Animal Science

Head of Doctoral School: Prof. Dr. Miklós Mézes
Member of the Hungarian Academy of Sciences
Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Feed Safety, Gödöllő, Hungary

Supervisor: Zsuzsanna J. Sandor, PhD
Senior scientist
Head of the Fish Nutrition Research Group
Research Centre for Aquaculture and Fisheries (HAKI), Hungarian University of Agriculture and Life Sciences, Szarvas, Hungary

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Approval of Head of Doctoral School **Approval of Supervisor**

INTRODUCTION AND OBJECTIVES

Introduction

It is widely recognised that an intake of long-chain n-3 highly unsaturated fatty acids (n-3 LC-HUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is associated with health benefits in humans. Fish is regarded as the primary dietary source of n-3 LC-HUFAs. As a result, dietary intake of fish by humans has been recommended to reduce the risk of cancer, autoimmune and cardiovascular diseases. However, it can be argued that many commonly consumed farmed fish have low levels of n-3 LC-HUFA, mainly attributed to the increasing usage of vegetable oils with low levels of n-3 LC-HUFA in aquafeeds, but it may as well depend on the fish species in question. Therefore, maintaining the proper lipid balance to promote fish growth and maximise the accumulation of EPA and DHA in edible tissues is a crucial concern in aquaculture.

Available scientific data demonstrates that insects are a potential source of aquafeed ingredients, as they form a natural food source for fish and humans. One of the insects commonly utilised as a source of aquafeed ingredients is the black soldier fly (*Hermetia illucens*). Lipids extracted from black soldier flies are rich in medium-chain fatty acids (MCFAs) and exhibit relative proportions of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and C18-poly unsaturated fatty acids (C18-PUFAs) deemed sufficient to abate LC-HUFAs utilisation (Belghit et al., 2018). The role of MCFA, such as lauric acid (C12:0), has been widely investigated in livestock and human nutrition due to its rapid absorption and oxidation and also due to its antimicrobial and antiviral properties (Dayrit, 2015). In addition, the potential of MCFA to enhance absorption of protein, lipid, and starch has been demonstrated in rats.

According to Fawole et al. (2020), the nutrient utilisation indices were found to be better in African catfish fed 50% black soldier fly larvae meal compared with the control, with no significant effect on feed intake. Considering these findings, it was hypothesised that feeding catfish with diets rich in MCFA, such as lauric acid, would increase the amount of fat that can be efficiently oxidised, enabling increased retention of n-3 LC-HUFA such as DHA, a poor substrate for mitochondrial β-oxidation (Tocher, 2003). In addition, the growth of fish fed insect oil-based diets would not be affected since proteins would also be spared.

Objectives

The goal of this study was to increase the production of high-quality catfish using sustainable diets with low fish oil content. Thus, the study's overall objective was to examine the potential benefits associated with the use of insect oil (black soldier fly larvae oil, BSFLO) as a feed ingredient in catfish diets. To achieve this overall objective, the following specific research objectives were set:

- To determine the effects of dietary inclusion of BSFLO on the production, digestion, and nutrient utilisation parameters in both the European catfish (*Silurus glanis*) and African catfish hybrid (*Clarias gariepinus* ×*Heterobranchus longifilis*).
- To investigate *in vivo* the effects of BSFLO on the immune system, fatty acid metabolism, and antioxidant defence system of European catfish and African catfish hybrid.
- To characterise the gut microbiota of European catfish and African catfish hybrid fed in *in vivo* trials with diets containing BSFLO relative to a control diet containing fish oil.

MATERIALS AND METHODS

Feed development and trials

Three diets with inclusion of BSFLO were formulated for the two fish species, the main difference being the oil source and physical properties of the pellets (sinking feed for European catfish, floating feed for African catfish). The feeds were formulated to be isoproteic (430 g/kg) and with the same fat content (110 g/kg). The control diet contained a mixture of fish oil (FO) and rapeseed oil RO (50:50); IO50 contained 50% of the FO/RO blend replaced with BSFLO, and IO100 contained 100% BSFLO. The first trial (Trial 1) involved a total of 630 European catfish juveniles (average initial body weight of 28.1 ± 0.17 g) randomly distributed in nine (9) 1 m^3 fibreglass tanks (70 fish per tank). A second trial (Trial 2) involving 900 juvenile hybrid African catfish (average initial weight: 29.1 ± 1.69 g), had a similar setup as the first trial. For each trial, one control group (CTR) and two experimental groups (IO50 and IO100) were set up, and fish were fed for 8 weeks (Trial 1) and 7 weeks (Trial 2), at a 3% feeding rate daily until apparent satiation using automatic feeders.

At the end of feeding experiments, fish were individually measured and dissected for tissue samplings. 10 fish were randomly sampled, and the total length measured for each fish, followed by dissection to obtain the liver weight for calculation of Fulton index and hepatosomatic index, respectively. For whole-body analysis of proximate composition and fatty acid profiles, three fish per tank (9 fish per dietary group) were sampled and freeze-dried until analysis. Three additional fish per tank were sampled, and blood collected and centrifuged to obtain plasma for analysis of biochemical and immunological parameters. The same samples of fish were dissected to obtain the liver for analysis of antioxidative status. Whole intestines were sampled and stored in

8% formalin solution until histological processing. Four fish samples per tank (12 fish per group) were dissected to obtain the spleen, head kidney, and liver, for gene expression analysis. The remaining portion of the liver was used for total lipids and fatty acid analysis. Fish remaining in the tanks were fed the diets for an additional week, before collecting intestinal faecal content for microbiota analysis. An additional three fish per tank were sampled, collecting mid-intestine for digestive enzyme activity assessment, and an additional five to eight fish/tank were sacrificed to collect faeces for digestibility studies.

Analysis and calculations

The feeds and whole fish body were analysed for dry matter, crude protein, crude lipid, ash content, and gross energy. Yttrium, dry matter, and crude protein content were also determined in the lyophilised faeces and respective feeds. Fatty acid composition of the insect oil, diets, whole fish, and fish livers were determined. Plasma biochemical and immunological parameters, digestive and antioxidant enzyme activities, gene expression, intestinal histology, and gut microbiota structure were analysed. The next production and nutrient utilisation parameters were determined: specific growth rate, feed conversion ratio, protein and fat retention, protein efficiency ratio, Fulton and hepatosomatic index, apparent digestibility coefficients and lipid quality indices of fish. One-way ANOVA was used to assess differences in the measured parameters between dietary treatments. To determine if the data followed a linear or quadratic model in response to insect oil, orthogonal polynomial contrast analysis was performed. The differences in alpha diversity indices of microbiota between dietary groups were evaluated using Kruskal–Wallis test, followed by Wilcox pair-wise comparison test. The differences in beta-diversity were evaluated using PERMANOVA. All the statistical testing were performed in R software and results considered significant at 95%.

RESULTS AND DISCUSSION

Growth performance, nutrient utilisation and body composition

The growth performance, nutrient utilisation, and body indices of European catfish and African catfish hybrid indicated no statistical differences ($p<0.05$) between the dietary groups (Tables 1 and 2). These results demonstrate that BSFLO can be used to replace up to 100% of fish oil and/or vegetable oil in the diets of the two species without negatively impacting growth and nutrient utilisation. Similarities in trypsin, lipase, and amylase activities between the dietary groups (Figure 1) indicated that the digestive processes were not affected when BSFLO was used as a source of lipids in the diets of the two species.

Table 1. Growth performance, nutrient utilisation, and body indices of European catfish (*Silurus glanis*) fed the CTR, IO50 and IO100 diets for eight weeks. Data expressed as mean ± standard deviation (SD)

Parameters	Dietary groups			ANOVA
	CTR	IO50	IO100	*p*-value
IBW (g)	27.94±0.24	27.89±0.09	28.13±0.34	0.505
FBW (g)	197.58±2.76	202.18±0.54	198.82±5.88	0.364
WG (%)	607.15±8.93	624.98±3.97	606.79±12.64	0.085
SGR (%/day)	3.31±0.03	3.35±0.01	3.32±0.03	0.167
FCR	0.60±0.01	0.60±0.01	0.60±0.01	0.948
PER	3.83±0.04	3.87±0.06	3.86±0.07	0.639
CF (%g cm^{-3})	0.55±0.02	0.55±0.05	0.54±0.02	0.583
HSI (%)	1.99± 0.19	2.07± 0.24	2.10± 0.25	0.446
FR (%)	78.32 ± 6.42	78.98 ± 11.78	72.63 ± 12.24	0.729
PR (%)	52.88±1.89	53.56±0.98	53.53±1.35	0.819
SR (%)	100±0.00	100±0.00	100±0.00	-

IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PR, protein retention; FR, fat retention; PER, protein efficiency ratio; CF, condition factor (Fulton index); HSI, hepatosomatic index; SR, survival rate

Table 2. Growth performance, nutrient utilisation, and body indices of African catfish hybrid fed the CTR, IO50 and IO100 diets for seven weeks. Data expressed as mean ± SD.

Parameters	Dietary groups			ANOVA
	CTR	IO50	IO100	*p*-value
IBW (g)	28.81±1.26	30.31±2.27	28.27±1.78	0.574
FBW (g)	155.27±4.45	162.47±0.19	160.87±3.78	0.229
WG (%)	439.11±8.11	437.61±39.63	469.77±22.53	0.488
SGR (%/day)	3.56±0.04	3.66±0.14	3.64±0.03	0.493
FCR	0.75±0.02	0.74±0.03	0.73±0.01	0.836
PER	3.10±0.10	3.15±0.14	3.17±0.07	0.786
CF (%g cm^{-3})	0.81±0.00	0.83±0.01	0.81±0.01	0.266
HSI (%)	1.04±0.05	1.04±0.14	1.13±0.14	0.702
FR (%)	122.03±8.04	119.11±13.89	111.72±8.43	0.503
PR (%)	46.28±1.73	46.18±1.60	46.62±0.95	0.924
SR (%)	91.5±3.50	89.5±1.50	89.0±0.00	0.960

IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PR, protein retention; FR, fat retention; PER, protein efficiency ratio; CF, condition factor (Fulton index); HSI, hepatosomatic index; SR, survival rate

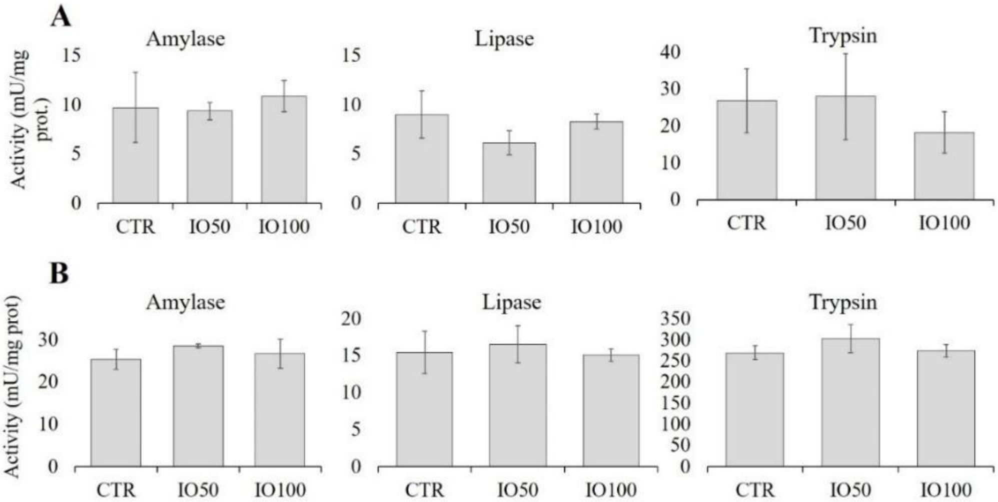

Figure 1. Digestive enzyme activity (mU/mg prot.) of European catfish (A) and African catfish hybrid (B) juveniles fed different oil-based diets.

There were no major differences in whole-body crude protein, crude lipid, and ash between the dietary groups of both species. Significant differences were observed in the fatty acid profile of the whole fish body. The whole-body w% of MUFA, EPA, DHA, and n-3 PUFA significantly decreased with an increase in dietary insect oil fraction. In addition, a significant difference was observed in the w% of whole-body n-3 PUFA in both species. While the whole-body total PUFA as well as n-6 PUFA indicated no statistical difference between the dietary groups of European catfish, there was a statistical difference in the whole-body w% of total PUFA as well as the w% of n-6 PUFA in African catfish hybrid. The n-6 PUFAs, including γ-linolenic acid (C18:3n-6), dihomo-γ-linolenic acid (C20:3n-6), and arachidonic acid (ARA, C20:4n-6), were different between the dietary groups of each species.

The main findings on fatty acid metabolism

The substitution of a mixture of FO and RO with BSFLO did not result in liver lipid deposition, but the fatty acid (FA) profiles were significantly influenced by the diets (Tables 3 and 4). Such results were also reported for juvenile *Onychostoma macrolepis* fed diets containing up to 50% BSFLO replacing FO (Gou et al., 2023) and Atlantic salmon fed a diet containing BSFL (Belghit et al., 2019). The reduced lipid deposition in the liver can be attributed to the high content of MCFAs, such as lauric acid, in the insect oil-based diets. A comparison of the lauric acid content of the liver with that in the corresponding diets revealed a much lower content in the livers than in the diets, indicating that lauric acid was oxidised to provide energy rather than stored. In both trials, the liver DHA/EPA ratio generally increased with an increase in BSFLO inclusion in the diets, similar to the trends in DHA/EPA in the diets, but with the DHA/EPA ratio more pronounced in the liver than in the diets. The increased DHA/EPA ratios were due to the decrease in EPA and increase in DHA content with BSFLO inclusion, which could be a consequence of desaturation of EPA and selective retention of DHA or retention of DHA and selective catabolism of EPA.

Table 3. Liver fatty acid composition (w% of total FA) and total lipid content (wet weight basis) of European catfish fed experimental diets for 8 weeks (means ±SD).

Fatty acid	Dietary groups			ANOVA
	CTR (n=6)	IO50 (n=8)	IO100 (n=6)	*p*-value
Total lipid (g/100g)	1.36±0.49	1.56±0.371	1.19±0.41	0.205
C12:0	0.34±0.24^{a}	0.44±0.12^{ab}	0.97±0.62^{b}	**0.004**
C14:0	1.20±0.32^{a}	1.54±0.18^{ab}	1.78±0.41^{b}	**0.016**
C16:0	18.07±3.09	18.00±1.70	17.24±0.56	0.728
C18:0	10.76±2.57	9.75±2.73	10.91±1.60	0.618
C18:1n-9	32.27±2.80	30.10±2.79	28.13±4.11	0.116
C18:2n-6	12.09±5.53	9.52±3.05	7.63±1.91	0.103
C18:3n-6	0.11±0.13^{a}	0.30±0.17^{ab}	0.44±0.08^{b}	**0.003**
C18:3n-3	0.48±0.16	0.38±0.19	0.26±0.17	0.124
C20:2n-6	0.86±0.35	1.07±0.30	0.88±0.22	0.361
C20:3n-6	2.05±0.68^{a}	2.97±0.78^{ab}	3.00±0.40^{b}	**0.032**
C20:4n-6	2.23±0.74^{a}	3.72±1.22^{b}	4.75±0.54^{b}	**<0.001**
C20:5n-3	0.58±0.29	0.53±0.14	0.40±0.01	0.369
C22:2n-6	0.71±1.14	0.26±0.14	0.32±0.11	0.795
C22:6n-3	5.08±1.81	5.68±2.01	5.96±1.79	0.712
SFA	33.52±6.98	33.70±3.47	35.32±2.19	0.749
MUFA	41.72±1.94	41.39±2.97	40.04±5.03	0.678
PUFA	24.76±6.17	24.91±2.35	24.64±3.69	0.993
PUFA n-6	18.62±5.09	18.31±1.46	18.01±2.74	0.949
PUFA n-3	6.14±2.12	6.59±2.28	6.63±1.89	0.902
PUFA/SFA	0.80±0.35	0.75±0.13	0.70±0.10	0.717

Statistical *p*-values refer to differences between dietary groups (CTR, IO50 and IO100). Values in same line with different superscript letters are significant ($p < 0.05$). Total SFA includes C4:0, C6:0, C8:0, C10:0, C11:0, C13:0, C15:0, C17:0, C19:0, C20:0, C22:0, C23:0, and C24:0 detected but not included in the table. Total MUFA includes C14:1n-5, C15:1n-5, C16:1n-7, C17:1n-7, C20:1n-9, C22:1n-9, and C24:1n-9 detected but not included in the table.

Table 4. Liver FA composition (w% of total FA), and total lipid content (wet weight basis) of African catfish hybrid fed diets for 7 weeks (means ±SD, n=3)

	Dietary groups			ANOVA
FA	CTR	IO50	H100	*p*-value
Total lipid (g/100g)	8.18 ±0.66	9.11±2.59	8.00 ±0.19	0.967
C12:0	0.09±0.03^{a}	0.63±0.21^{b}	1.42±0.28^{c}	**<0.001**
C14:0	1.15±0.06^{a}	1.61±0.08^{b}	2.18±0.03^{c}	**<0.001**
C16:0	24.55±1.79	23.93±1.91	23.41±0.47	0.682
C18:0	11.60±0.76	11.69±0.35	12.16±0.43	0.456
C18:1n-9t	0.23±0.02^{a}	0.27±0.01^{b}	0.29±0.01^{b}	**0.007**
C18:1n-9c	42.42±2.16	41.50±0.90	39.21±0.22	0.066
C18:2n-6t	0.24±0.04	0.26±0.02	0.40±0.22	0.477
C18:2n-6c	6.01±0.40	6.87±1.21	6.38±0.60	0.482
C18:3n-6	0.26±0.04^{a}	0.34±0.02^{b}	0.42±0.01^{c}	**0.002**
C18:3n-3	0.46±0.01	0.41±0.22	0.31±0.05	0.423
C20:2n-6	0.56±0.05	0.63±0.10	0.56±0.03	0.422
C20:3n-6	1.49±0.16^{a}	1.90±0.15^{b}	2.31±0.02^{c}	**<0.001**
C20:4n-6	1.17±0.14^{a}	1.56±0.18^{b}	2.04±0.09^{c}	**<0.001**
C20:5n-3	0.53±0.01^{b}	0.46±0.07^{ab}	0.29±0.01^{a}	**0.001**
C22:2n-6	0.01±0.02	0.02±0.01	0.03±0.01	0.236
C22:6n-3	1.65±1.30	0.82±1.18	1.82±0.05	0.377
SFA	38.51±2.37	38.65±1.98	40.01±0.58	0.567
MUFA	49.11±2.48	48.08±1.10	45.44±0.09	0.067
PUFA	12.38±1.71	13.27±1.99	14.55±0.67	0.304
n-3 PUFA	2.64±1.32	1.68±1.19	2.42±0.02	0.526
n-6 PUFA	9.74±0.77	11.58±1.64	12.13±0.66	0.087
PUFA/SFA	0.32±0.06	0.35±0.07	0.36±0.02	0.645

Statistical *p*-values refer to differences between groups (CTR, IO50 and IO100). Values in same line with different superscript letters are significant ($p < 0.05$). Total SFA includes C4:0, C6:0, C8:0, C10:0, C11:0, C15:0, C17:0, C19:0, C20:0, C22:0, C23:0, and C24:0 detected but not included in the table. Total MUFA includes C14:1n-5, C15:1n-5, C16:1n-7, and C17:1n-7, C22:1n-9, C20:1n-9 and C24:1n-9 detected but not included in the table.

To understand the effect of dietary BSFLO on lipid metabolism, a number of genes involved in PUFA elongation (*elovl2* and *elovl5*) and desaturation (*fads2*), de novo fatty acid synthesis (*fas, g6pd* and *6gpd*), fatty acid oxidation (*cpt1a* and *hadh*) and triacylglycerol metabolism (*lpl* gene), and transcription factors (*pparα, pparγ, srebp-1* and *hnf4α*) were evaluated. There were no statistical differences in the relative expression of *fas, g6pd, 6pgd, fads2, elovl2,* and *elovl5,* between the dietary groups in both species. In the European catfish, the relative expression of *cpt1a* was significant, being highest in the group fed IO100 diet (Figure 2A). Although *cpt1a* expression was also highest in African catfish hybrid fed IO100, the results were similar between the dietary groups (Figure 2B). The *cpt1a* gene encodes a protein that enables the delivery of long-chain fatty acids into the mitochondria for β-oxidation. Thus, increased expression of *cpt1a* indicated increased oxidation of long chain fatty acids such as EPA (Tocher, 2003).

In both species, the dietary groups fed IO100 diet presented a significant upregulation of *lpl* gene encoding lipoprotein lipase. Lipoprotein lipase hydrolyses triacylglycerols present in plasma lipoproteins, thereby providing free fatty acids for either storage or oxidation in tissues (Lu et al., 2013). Among the transcription factors, only *hnf4α* was significant in European catfish, being highest in the group fed IO100 diet while in African catfish hybrid, both *hnf4α* and *pparα* were significantly lowest in fish fed IO50 when compared to the group fed IO100 diet (Figure 2).

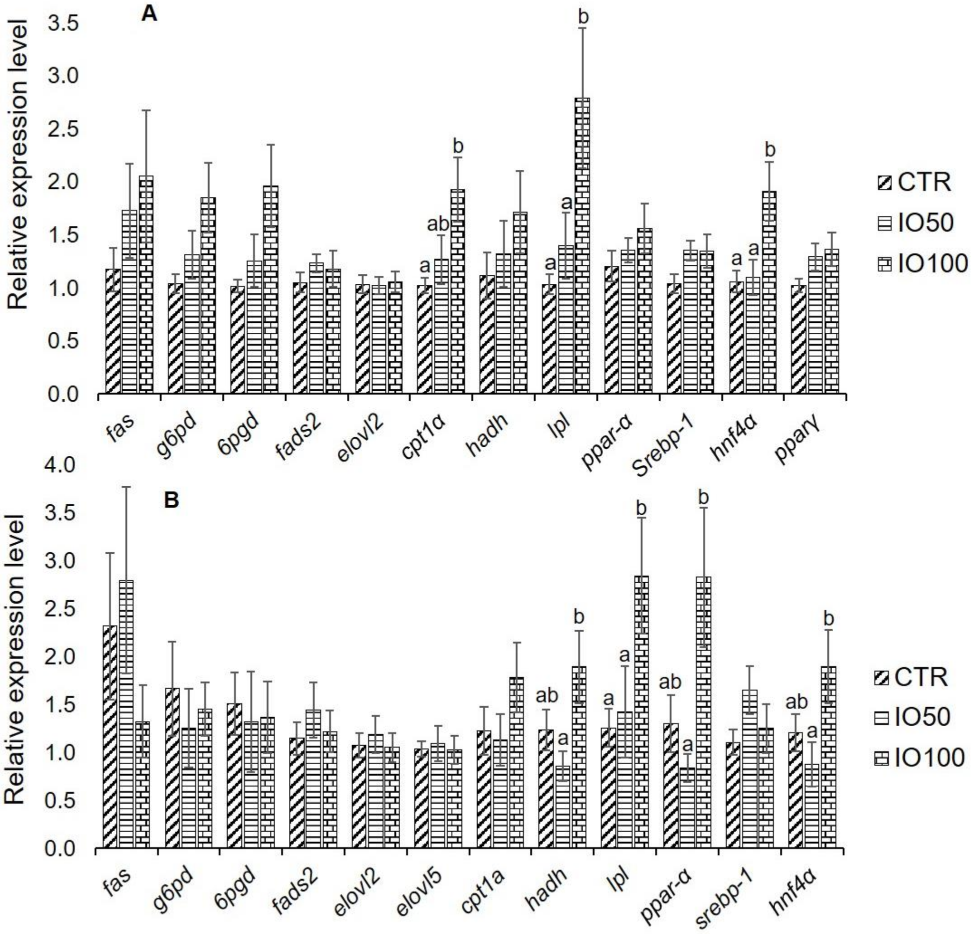

Figure 2. The relative expression levels of genes involved in *de novo* fatty acid synthesis (*fas*, *g6pd* and *6gpd*), elongation and desaturation (*elovl2*, *elovl5* and *fads2*), β-oxidation and triacylglycerol metabolism (*cpt1a*, *hadh* and *lpl*), and transcriptional regulation (*pparα*, *pparγ*, *srebp-1* and *hnf4α*), in the livers of European catfish (**A**) and African catfish hybrid (**B**). Letters (a, b) denote significant differences between the dietary groups for any given gene.

Main finding on antioxidative capacity and immune system

Plasma biochemical parameters are considered an important indicator for evaluating the effects of diet on the health and nutritional status of fish. In the present study, plasma cholesterol, creatinine, total bilirubin, total protein, and immunoglobulin contents, as well as myeloperoxidase and lysozyme activities, were not significantly affected. However, differences in the content of albumin, globulin, and the activities of alkaline phosphatase and lipase were observed between the dietary groups of European catfish and not in African catfish hybrid (Figure 3). In addition, glucose content significantly differed between dietary groups of African catfish hybrid but not between groups of European catfish. Sudha et al. (2022) reported major differences in the contents of total protein, albumin, cholesterol, glucose, as well as the albumin-to-glucose ratio between groups of stripped catfish fed FO and those fed BSFLO-based diets.

Similarly, no major differences in serum biochemical parameters were observed in Jian carp fed BSFLO and vegetable oils (Li et al., 2016). In addition, serum biochemical parameters and lysozyme activities of barramundi fed on partially defatted BSFL protein and oil were similar between the dietary groups (Hender et al., 2021). Rainbow trout fed on diets in which FO was replaced with BSFLO had significantly different glucose and phosphorus content between the dietary groups (Dumas et al., 2018). Changes in albumin level reflect changes in liver function, and the higher albumin level in European catfish fed IO100 diet may indicate upregulated metabolic activities since albumins are involved in the transport functions (Manna et al., 2021).

In African catfish hybrid, a linear increase in myeloperoxidase activity with increasing BSFLO fraction in the diets was observed, highlighting its influence on the non-specific immune function. Unlike ALT activity, which was similar between dietary groups, suggesting that BSFLO inclusion in the diets did not negatively affect liver health, the ALP activity was significantly higher in European catfish fed the IO100 diet. Thus, higher ALP activity in the IO100 group of European catfish was most likely associated with higher metabolic activity (Manna et al., 2021).

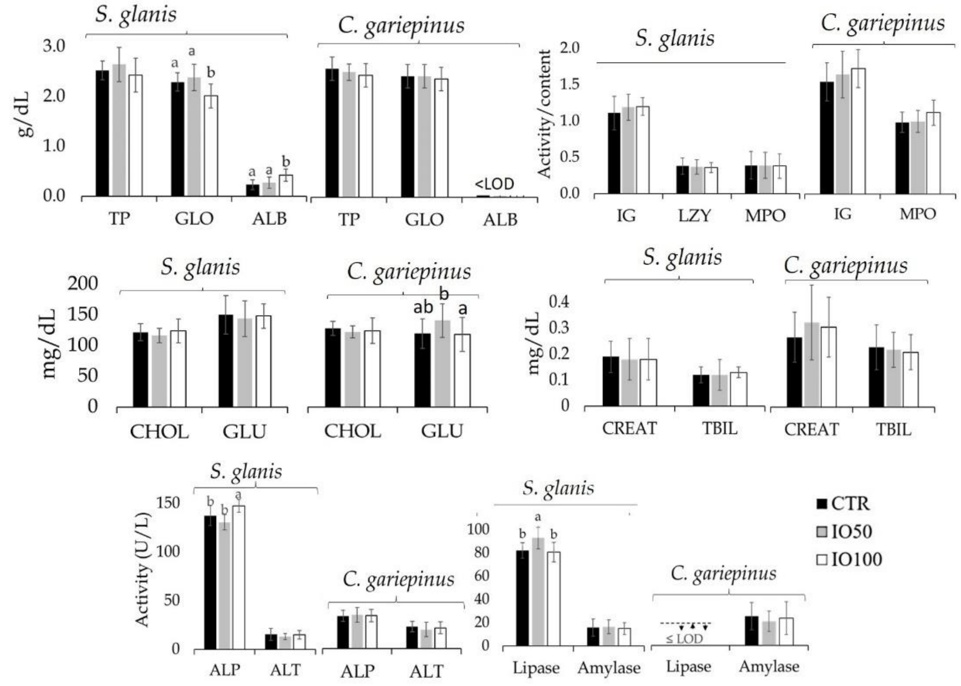

Figure 3. Plasma biochemical and immunological parameters (mean±SD, n=9) of European catfish (*S. glanis*) and African catfish (*C. gariepinus*) hybrid fed with CTR, IO50 and IO100 diets. Alanine transaminase, ALT; alkaline phosphatase, ALP; cholesterol, CHOL; glucose, GLU; creatinine, CREAT; total bilirubin, TBIL; total protein, TP; albumin, ALB; globulin, GLO; immunoglobulin, IG (g/dL); lysozyme, LZY (µg/mg prot); Myeloperoxidase, MPO (OD_{450nm}).

The liver antioxidant indices of the two species are shown in Figure 4. There was no significant induction of SOD, as well as differences in the contents of MDA, GSH, and TAOC between the dietary groups of European catfish. In addition, the hepatic expression of the *sod1* gene was also similar between the dietary fish groups. Similar results on SOD, GSH, and TAOC were obtained for African catfish hybrid, but MDA differed significantly between the dietary groups. The MDA is an end product of lipid peroxidation which is capable of damaging cell structure and function (Cherian et al., 2019). The lower content of MDA in insect oil-based dietary groups relative to the CTR group suggested relatively lower levels of oxidative stress in the insect oil-based groups.

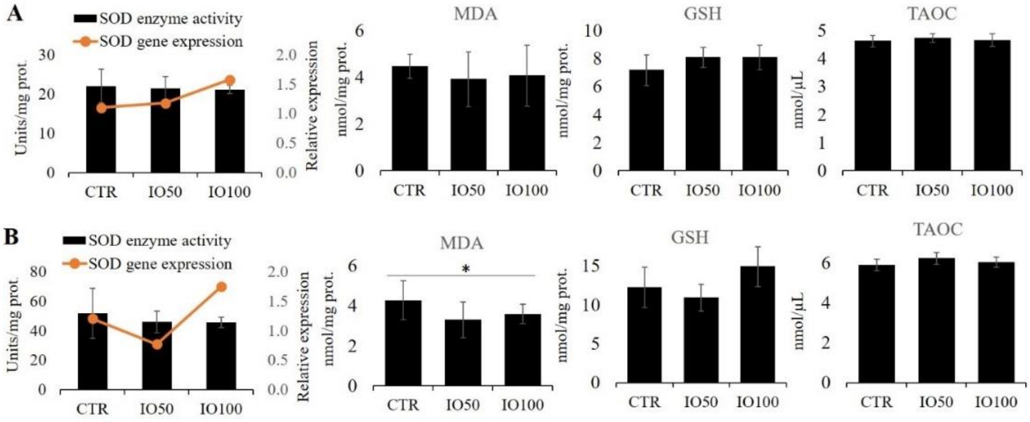

Figure 4. The effects of dietary black soldier fly larvae oil on the expression and activity of SOD enzyme, as well as on the contents of MDA, GSH and TAOC in the liver of European catfish (A*)* and African catfish hybrid (B). Data presented as mean ± SD. * indicates significant results.

The relative expression of immune related genes including toll-like receptor 5 (*tlr-5*), interleukin 8 (*il-8*), interferon regulatory factor 1 (*irf-1*), recombination activating gene 1 (*rag-1*), and transforming growth factor β1 (*tgf-β1*) were evaluated in the spleen and head kidneys (Figures 5). With the exception of *irf-1*, whose expression was significantly higher in the spleen of fish fed IO100 diet than in fish fed the CTR diet, the expression of *tlr-5*, *il-8*, *rag-1* and *tgf-β1* were similar between the dietary groups of European catfish. Similar results were obtained in the expression of *tlr-5* and *tgf-β1* in the spleen of African catfish hybrid. In the head kidney of European catfish, all the genes indicated no major difference between fish fed the different diets.

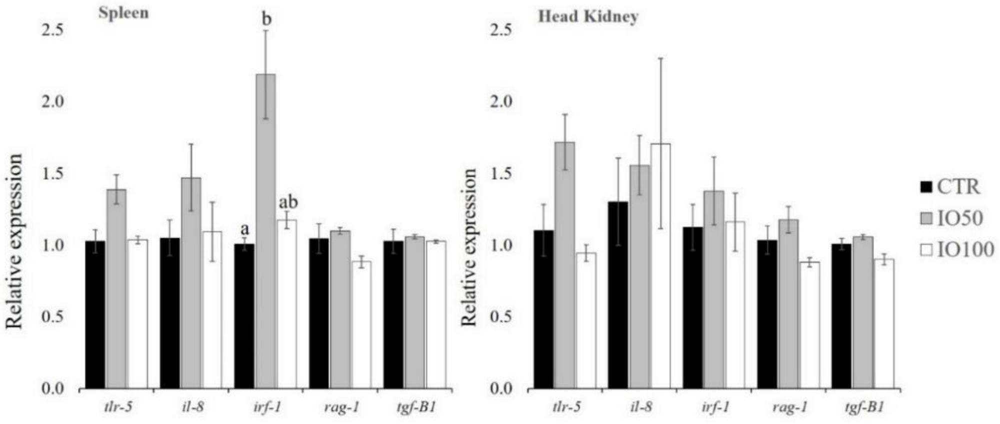

Figure 5. Relative expression (mean ± SE, n = 12) of immune response genes in European catfish fed the CTR, IO50 and IO100 diets. Letters **a** and **b** denote significant differences.

Results on the intestinal microbiota composition and inferred functions

The intestinal bacterial community in European catfish was dominated by either Fusobacteriota or Spirochaetota (Figure 6 A) while that of African catfish hybrid was dominated by Fusobacteriota (Figure 6 B). Other bacteria phyla included Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota, and Verrucomicrobiota, among others. These phyla were also reported in channel catfish and blue catfish— Fusobacteriota and Firmicutes being the dominant (Bledsoe et al., 2018; Xia et al., 2022). In this study, Fusobacteriota was dominated by the genus *Cetobacterium*, consistent with results reported on blue catfish (Bledsoe et al., 2018) and African catfish (Skvortsova et al., 2023). The genus *Brevinema* was the most dominant in the phylum Spirochaetota and was identified in faecal samples of European catfish in varying abundances. In the Atlantic salmon fed dietary insect meal during the seawater phase, *Brevinema* was found to be more associated with the mucosa than the digesta (Li et al., 2021). The dominant genus of Firmicutes in European catfish was *Exiguobacterium* while *Romboutsia* was the dominant Firmicute in African catfish hybrid. The genus *Romboutsia* (Firmicutes) in addition to *Cetobacterium* (Fusobacteriota) and *Plesiomonas* (Proteobacteria) have been characterised as common members in the guts of healthy yellow tail fish (Yang et al., 2023). In our study, there were no major differences in abundance of these genera between fish fed the CTR, IO50, and IO100. The high lipid content of SFAs such as lauric acid has been reported to be associated with increased abundance of lactic acid bacteria (Huyben et al., 2020). In our study such enrichment was not observed similar to the observations reported on Atlantic salmon (Weththasinghe et al., 2022). The relative abundances of bacterial pathways were statistically similar between the bacterial communities of fish fed the different diets. The most abundant pathways were associated with metabolism, environmental information processing, cellular processes, and genetic information processing, similar to observations reported on cobia fish (Reinoso et al., 2023).

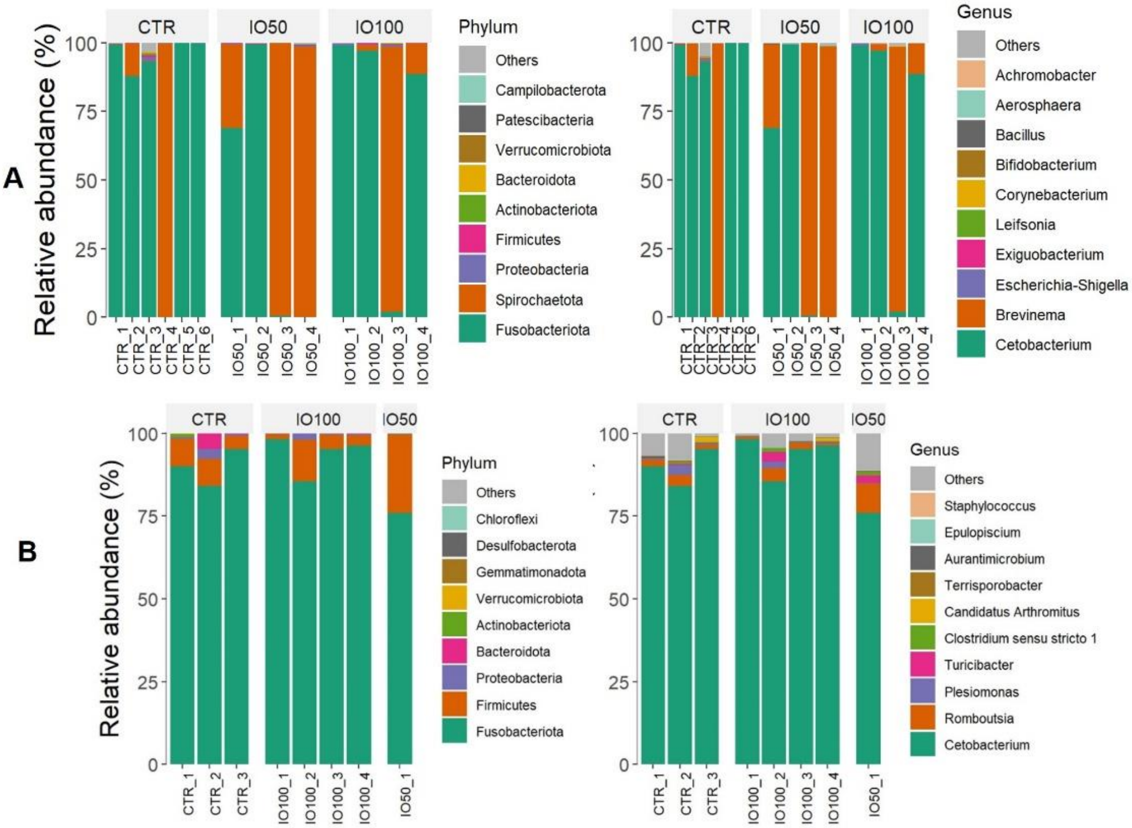

Figure 6. Relative abundance of bacterial phyla and genera in faecal samples of European catfish (A) and African catfish hybrid (B) fed with the CTR, IO50 and IO100 diets.

CONCLUSIONS AND RECOMMENDATIONS

This study investigated the potential benefits associated with the substitution of a mixture of fish oil and vegetable oil, commonly used in commercial diets of catfish, with black soldier fly larvae oil (BSFLO) in the diets of European catfish and African catfish hybrids. The dietary black soldier fly larvae oil promoted the growth performance and feed utilisation in the two species. The whole-body fatty acid profile largely mirrored the fatty acid profile of the diets, but some deviations were observed in the fatty acid profile of the liver. The liver and whole-body lipid quality indices, such as the Atherogenic Index, Thrombogenic Index, and Polyene Index, across the dietary groups of both species were low and favourable for human consumption. However, further studies on the sensory quality of the resulting flesh of catfish fed BSFLO-based diets are recommended.

Differences in the expression of hepatic genes involved in lipid metabolism were observed between the dietary groups and fish species, reflecting differences in the lipid metabolic reactions between the two species. Based on the expression of immune-related genes, the insect oil-based diets could strengthen the immune response in catfish. However, this observation needs further investigation involving challenged animals. Nonetheless, a linear increase in myeloperoxidase activity with increasing insect oil fraction in the diets was recorded, indicating the improvement of innate immune responses. The activity of SOD and contents of MDA, GSH, and TAOC across dietary groups of fish indicated that the use of BSFLO-based diets reduces oxidative stress. This was also reflected in the Firmicutes to Bacteroidota ratio, which was improved in the fish fed insect oil diets and increased with an increase in insect oil fraction in the diets. The histological evaluation showed normal histological structures of the intestine in both species, and no major differences were detected in the diversity, community composition and inferred functional capacity of the bacterial communities between the dietary groups of each species following feeding with the experimental diets. However, notable differences in the bacterial community composition were observed between the two fish species.

NEW SCIENTIFIC RESULTS

1. I have demonstrated that complete substitution of a mixture of fish and vegetable oils commonly used in commercial diets of catfish, with black soldier fly larvae oil in the diets of European catfish (*Silurus glanis* L) and African catfish hybrid (*Clarias gariepinus* × *Heterobranchus longifilis*) promotes adequate growth, nutrient utilisation, and metabolic health in juvenile fish. Based on my investigation, it will be possible recommendation of black soldier fly larvae oil in the diets of catfish with 0.60-0.73 g/g FCR and 3.35-3.66 %/day SGR on 25-26 C° rearing temperature.

2. Also, I have observed following feeding that there is a general tendency for liver antioxidant capacity to increase with increase in insect oil inclusion in the diets of both species, indicating that the dietary use of black soldier fly larvae oil could be associated with potential health benefits for fish.

3. I have demonstrated for the first time that the inclusion of black soldier fly larvae oil rich in medium chain fatty acids in diets, speeds up the β-oxidation process in the liver of European catfish, as well as African catfish hybrid. I have proven that dietary intake of black soldier fly larvae oil at 5% does not affect the total liver lipid content of fish but increases the proportion of DHA (C22:6n-3) and ARA (C20:4n-6), especially in fish fed 100% insect oil. In addition, I have detected the positive effects on the expression of several lipid-related genes in the livers of both catfish species.

4. I have assessed for the first time the microbiota diversity in European catfish as well as in African catfish hybrid following feeding with black

soldier fly larvae oil. I have demonstrated that dietary utilisation of black soldier fly larvae oil does not negatively impact the diversity, community composition and functional profile of gut microbiota, but improves the Firmicutes/Bacteroidota ratio known to be associated with enhanced oxidative response and more resistance to pathogens.

5. I have also demonstrated that European catfish fed diets containing 100% black soldier fly larvae oil had a higher metabolic reaction than African catfish hybrid similarly fed same diets under similar rearing conditions, and this was reflected in the activities and/or content of plasma biochemical indicators including alkaline phosphatase and the level of albumin.

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PUBLICATIONS RELATED TO THE THESIS

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