Hungarian University of Agriculture and Life Sciences – MATE

Doctoral School of Animal Biotechnology and Animal Science

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

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:	Professor Dr. Miklós Mézes D.Sc., 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

……. ……

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

CONTENTS

LIST OF ABBREVIATIONS

6pgd	6-phosphogluconate dehydrogenase
ALA	α-linolenic acid
ALP	alkaline phosphatase
ALT	alanine transaminase
ARA	arachidonic acid
ASVs	amplicon sequence variants
β-actin	beta actin
BSFLO	black soldier fly larvae oil
cpt1a	carnitine palmitoyltransferase 1A
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DR-1	direct repeat 1
EBP	enhancer binding protein
EFA	essential fatty acid
elovl	elongation of very long-chain fatty acids
EPA	eicosapentaenoic acid
FA	fatty acid
fads	fatty acyl desaturase
fas	fatty acid synthetase
FO	fish oil
g6pd	glucose 6-phosphate dehydrogenase
hadh	hydroxyacyl-CoA dehydrogenase
Hnf4α	hepatocyte nuclear factor 4α
HUFA	Highly unsaturated fatty acid
il-8	interleukin 8
irf-1a	interferon regulatory factor 1a
LA	linoleic acid
LC-PUFA	long-chain (C20-24) polyunsaturated fatty acid
lpl	lipoprotein lipase
MCFA	Medium chain fatty acids
MUFA	monounsaturated fatty acid
PCR	polymerase chain reaction
ppar	peroxisome proliferator activated receptor
PUFA	polyunsaturated fatty acid (≥ 2 double bonds)
qPCR	quantitative PCR
rag-1	recombination activating genes-1
rRNA	ribosomal RNA
sod1	superoxide dismutase 1
srebp-1	sterol-regulatory element binding protein 1
TAG	triacylglycerol
tgf-β1	transforming growth factor beta 1
tlr-5	toll-like receptor 5
VLCFA	very long chain fatty acids (20 carbons or more)
VLC-PUFA	very long-chain (>C24) polyunsaturated fatty acid
VLD L	very low-density lipoprotein.

1. INTRODUCTION

It is widely recognised that an intake of long-chain (LC) 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 and/or n-3 LC-HUFA by humans has been recommended to reduce the risk of cancer, autoimmune and cardiovascular diseases (Leray, 2014; Richter et al., 2016). 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 (VO) with low n-3 LC-HUFA in aquafeeds—a strategy to avoid over-reliance on the scarce fish oil, but it may as well depend on the fish species in question (Boyd, 2015; Richter et al., 2016). 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.

The dietary requirements for LC-HUFA which ensure effective LC-HUFA deposition in fish tissues may differ between species or major fish groups, despite the fact that dietary LC-HUFA is easily deposited in fish tissue (Xu et al., 2020; Rombenso et al., 2021). Therefore, developing strategies to achieve high diet-to-tissue transfer of LC-HUFA in farmed fish is essential. Such strategies should ensure that the desired dietary LC-HUFA are spared from their undesired catabolic fates and that the dietary C18-PUFA are not deposited in tissues at the expense of LC-HUFA (Codabaccus et al., 2012; Turchini et al., 2013; Trushenski and Rombenso, 2020). Noteworthy, it has been reported that feeding the fish with diets containing lipids rich in saturated- and monounsaturated-fatty acids (SFA and MUFA, respectively) can selectively spare LC-HUFA to meet the physiological demands of the species. This is because SFA and MUFA are preferentially catabolized before LC-HUFA (Glencross, 2009). However, in the fish species investigated so far, only minimal to no effect have been demonstrated (Mulligan and Trushenski, 2013; Turchini et al., 2013). In addition, inducing the synthesis of LC-HUFA through fortification of aquafeeds with precursor fatty acid (α-linolenic acid, 18:3n-3 for n-3 LC-HUFA, 18:2n-6 for n-6 LC-HUFA) could be an effective strategy, but this as well depends on the endogenous biosynthetic capacity, the bioenergetics, and the trophic level of the species (Trushenski and Rombenso, 2020; Rombenso et al., 2021). Thus, for some freshwater fish species, it has been possible to completely replace fish oil (FO) with alternative oils without affecting their growth; however, for certain species, it has been discovered that full replacement with lipids limited in LC-HUFA leads to reduced performance (Steffens, 1997; Turchini et al., 2010).

Available scientific data demonstrate that insects are a potential source of aquafeed ingredients, as they form a natural food source for fish and humans (Howe et al., 2014; Davis et al., 2017; Nogales-Mérida et al., 2019; Alfiko et al., 2021). In fish feeding trials, insects have been tested in various forms, and the results have depended on the insect species used, the level of processing, and the level of inclusion in the fish feed (Lewis et al., 2019). One of the insects commonly utilised as a source of aquafeed ingredients is the black soldier fly (*Hermetia illucens*). Lipids extracted from black soldier fly contain relative proportions of SFA, MUFA, and C18-PUFA deemed sufficient to abate LC-HUFA utilisation (Belghit et al., 2018; Nogales-Mérida et al., 2018). However, similar to other insect species, the fatty acid profile of the black soldier fly varies according to the stage of development, type of diet, and rearing conditions (Nogales-Mérida et al., 2018; Alfiko et al., 2021). The high level of SFA in the black soldier fly larvae oil (BSFLO) is mainly attributed to the content of lauric acid, which is a medium-chain saturated fatty acid (Ewald et al., 2020; Moutinho et al., 2023).

The role of lauric acid (C12:0) has been widely investigated in both livestock and human nutrition due to its rapid absorption and oxidation and also due to its antimicrobial and antiviral properties (St-Onge et al., 2008; Dayrit, 2015; Sado-Kamdem et al., 2009). However, results regarding the use of oils rich in medium-chain saturated fatty acids as sources of dietary fatty acids (FA) in various fish species are ambiguous. For instance, although the potential of dietary medium-chain fatty acids (MCFAs) to enhance absorption of protein, lipid, and starch has been demonstrated in rats, MCFAs correlate negatively with growth, feed intake, and fat deposition in the Atlantic salmon (Nordrum et al., 2000) and polka-dot grouper (*Cromileptes altivelis*) (Williams et al., 2006). Rainbow trout (*Oncorhynchus mykiss*, Walbaum) fed a diet rich in MCFA accumulated C12:0 in the whole body, representing more than 20% of total body FA (Figueiredo-Silva et al., 2012). African catfish fed 100% black soldier fly larval meal were associated with reduced feed intake, growth and protein efficiency ratio, with no negative effect on liver indices or hepatic fat deposition. However, the reduced growth was attributed to the reduced level of methionine (Adeoye et al., 2020). According to Fawole et al. (2020), the nutrient utilisation indices were found to be better in African catfish fed 50% black soldier fly larval meal compared with the control, but in this case, there was no significant effect on the 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.

Research 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.

2. LITERATURE REVIEW

2.1. General characteristics and aquaculture production of African catfish

Clarias gariepinus (Burchell, 1822), also known as the North African catfish, is a freshwater species of catfish in the family Clariidae and order Siluriformes. The native range of *C. gariepinus* (Figure 2.1) covers mostly the African continent, as well as Jordan, Israel, Lebanon, Syria, and southern Turkey. The species naturally inhabits floodplains, slow-flowing rivers, lakes, and dams (Skelton, 2001). The natural food of *C. gariepinus* juveniles includes plankton, insects, molluscs, crustaceans, and detritus, while the adults feed mainly on fish and various other food sources available, including plant materials (Bruton, 1979; Khedkar et al., 2003). Their natural feeding habits reflect their nutritional requirements and ability to consume a diverse range of food items. In its natural habitat, reproduction is associated with the rainy season, during which peak egg production by the female and hatching of the fertilised eggs occur. Average production of about 28,000 eggs with a hatching rate of 86.4% has been recorded during the rainy season, and individuals can reach up to 1.5 m in total length (Yalçın et al., 2001; Khedkar et al., 2003).

The species is of great commercial importance, with favourable characteristics such as fast growth rate (e.g., reaching over 1 kg in a year), resistance to diseases, and the ability to tolerate conditions of low dissolved oxygen and high turbidity (Van der Waal, 1998; Khedkar et al., 2003; Lal et al., 2003), making it an excellent species for intensive aquaculture. *C. gariepinus* was adopted as the preferable catfish species for aquaculture in the mid-1970s, and currently, cultivation occurs in many parts of the world (Figure 2.1). Cultivation is practised in ponds, cages, and pens (Khedkar et al., 2003), and spawning is normally induced with 11-desoxycorticosterone-acetate (DOCA) or carp pituitary suspension (CPS) (Khedkar et al., 2003). In *C. gariepinus* aquaculture, feeding husbandry techniques include pond fertilisation (extensive systems), supplementing meals using farm and industrial by-products (semi-intensive systems), and providing nutritionally full, complete feeds such as sinking or extruded fish pellets (intensive systems) (Hecht, 2013). Utilised agricultural and industrial by-products include rice bran, wheat middling, brewer's waste, cottonseed meal, corn meal, and peanut meal. These by-products typically contain 28–35% protein and can be fed directly or processed into pelleted meals. Additionally, non-traditional feed materials such as chicken intestines, abattoir waste, fish market waste, earthworms, and insects are employed (Hecht, 2013).

2.2. General characteristics and aquaculture production of European catfish

The European catfish, also known as wels and sheatfish, *Silurus glanis*, is one of the 20 largest freshwater fish species worldwide (Alp et al., 2004; Cucherousset et al., 2018). The natural geographic range of *S. glanis* covers eastern Europe and western Asia (Figure 2.2). Characterised by its rapid growth and large body weight, individual fish can grow more than 2.7 m in length and achieve a mass of 130 kg (Alp et al., 2004; Cucherousset et al., 2018). The distinct phylogeny and large size indicate that the species can consume large-sized prey, has fast growth rates, a long lifespan, high fecundity, shows nest guarding behaviour, and females produce large eggs (Cucherousset et al., 2018). Its natural diet consists of invertebrates

(especially in fish <30 cm) as well as fish and birds (especially in large fish >120 cm), as indicated by results from stomach content analysis (Carol et al., 2009). The males can mature at a minimum size of 78.8 cm in total length and at age 3, earlier than the females that are reported to mature at a minimum size of about 87.0 cm in total length and at age 4 (Alp et al., 2004). Fecundity estimates (range: 9033 to 340,461 eggs/fish) of mature females were found to be correlated positively with total length and ovary weight of fish (Alp et al., 2004). Similar to other fish species, its growth and survival are affected by water temperature and salinity. For instance, in cold waters with temperatures below 10°C, this species experiences a slow growth rate and may not feed for up to 6 months (David, 2006). The most favourable temperature range for the growth of *S. glanis* was reported to be 25 to 28°C (Hilge, 1985), a temperature range which was also found to be appropriate for the growth and wellbeing of *Clarias gariepinus* (Ogunji and Awoke, 2017). The European catfish (*S. glanis*) has a white flesh that is boneless, easy to cook, and low in fat (6–8%). It has been cultivated in extensive ponds in central and eastern Europe in association with carps for over 100 years (Linhart et al., 2004).

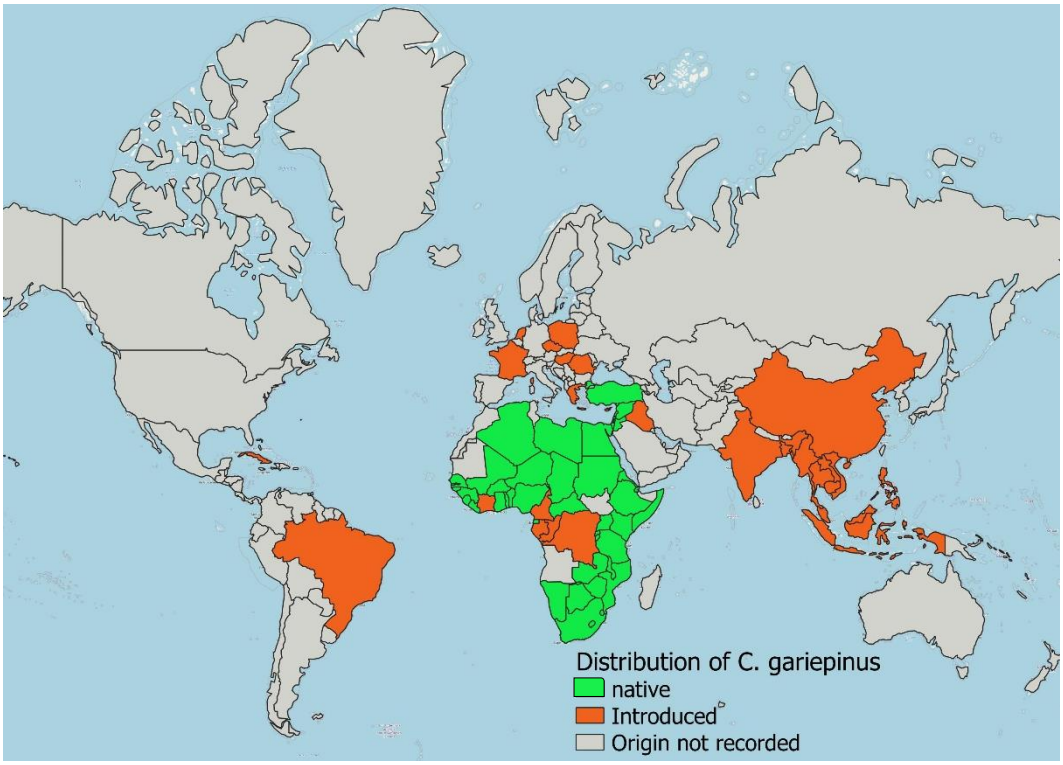

Figure 2.1. Global ranges of native (green) and introduced (red) *C. gariepinus* (Source: CABI, 2022)

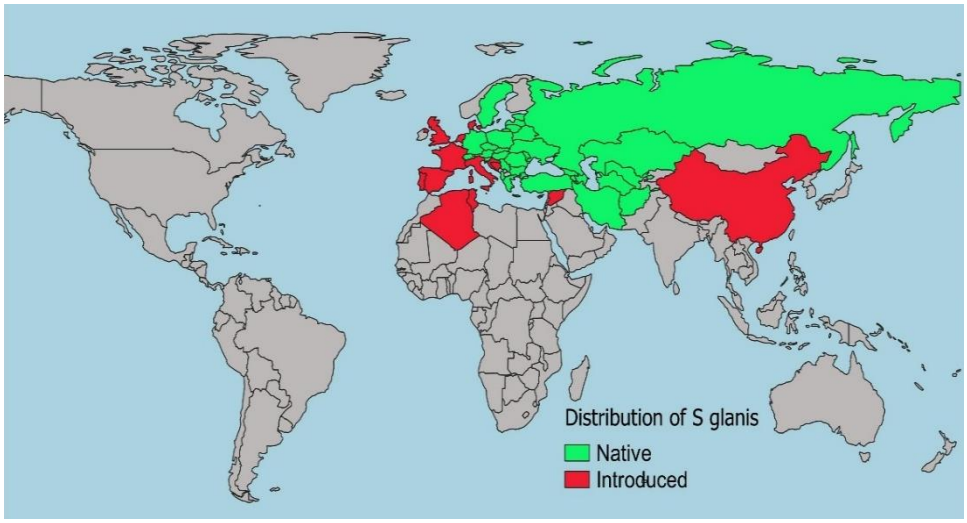

Figure 2.2. Global ranges of native (green) and introduced (red) *S. glanis* (Source: CABI, 2022)

2.3. Fish meal (FM) and alternatives in aquaculture

High-quality FM provides a balanced amount of essential amino acids, vitamins, minerals, and fatty acids (such as DHA, docosahexaenoic acid, and EPA, eicosapentaenoic acid), and therefore, supplementation of fish diets by FM is a good strategy to achieve proper growth and health of farmed fish (Cho and Kim, 2011). The essential amino acids (EAAs) for fish are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Table 2.1). These are not synthesised by fish and must therefore be supplied through diet. Cysteine and tyrosine are synthesised in the body from methionine and phenylalanine, respectively (Lall and Dumas, 2015; Cabano, 2017; Nogales-Mérida et al., 2019). Carnivorous finfish species of commercial importance, such as salmonids, have a high protein requirement (40–55%) in the diet (Figure 2.3), and their compound feeds often contain 40% FM. On the other hand, omnivorous and herbivorous fish have relatively low protein requirements (25–35%), and their diets often contain FM as a secondary source of nutrients and energy or to improve feed palatability (Oliva-Teles et al. 2015; Daniel, 2018). These differences in nutritional requirements of herbivorous, omnivorous, and carnivorous fishes reflect their feeding behaviour (Oliva-Teles et al., 2015).

Freshwater and marine fish also differ in their requirements for essential fatty acids (EFA). For example, freshwater fish's EFA requirements can be met by supplying α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) in their diets, whereas marine fish's EFA requirements can only be met by supplying highly unsaturated fatty acids (HUFA) such as EPA (20:5n-3) and DHA (22:6n-3) in their diets. This is because freshwater fish can successfully elongate and desaturate 18:3n-3 (α-linolenic acid) to 22:6n-3 (DHA) or 18:2n-6 (linoleic acid) to 20:4n-6 (ARA, arachidonic acid), whereas marine fish, which lack or have very low activity of Δ5-desaturase, require dietary LC-PUFA (Lall and Dumas, 2015; Oliva-Teles et al., 2015). Although fish such as salmonids may synthesise EPA and DHA from alpha-linolenic acid (18:3n-3) supplied in the diet, dietary sources of EPA and DHA are more efficient in

depositing them in fish meat (Alfiko et al., 2021). Fish lipids are highly digestible and excellent sources of EFA in both the n-3 and n-6 families. In the FM, the content of fatty acids in the n-3 family is more than that in the n-6 family, with linolenic acid, DHA, and EPA as the predominant n-3 fatty acids (Miles and Chapman, 2006), thereby satisfying the EFA requirements of several fish species. In addition, several fish species can efficiently use diets with carbohydrate levels up to 40–60%, while others do not tolerate more than 10–20% dietary carbohydrates (Oliva-Teles et al., 2015). As a result, an appropriate alternative to FM should preserve the nutritional and functional qualities offered by FM. Plant-based lipid sources contain all the EFAs required in the diets of freshwater fish and can be used as lipid sources for freshwater fish. However, they contain higher concentrations of n-6 than n-3 fatty acids. For instance, oil extracted from soybeans, corn, or cottonseed is rich in linoleic acid, an n-6 fatty acid (Miles and Chapman, 2006).

Table 2.1. Essential amino acid profiles of fish meal and selected alternative protein sources (Derived from Oliva-Teles et al., 2015).

Feedstuff	**Prot.**	**Amino acids (% protein)**											
		Arg	Cys*	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Tyr*	Val
Fishmeal [a]	75.4	5.8	0.8	2.2	4.3	7.0	7.5	2.8	3.8	4.1	1.1	2.9	4.9
Fishmeal [b]	70.6	6.2	0.8	2.4	4.2	7.2	7.5	2.7	3.9	4.1	1.0	3.1	4.9
Meat and bone meal, high fat	54.9	6.9	1.1	2.1	2.9	6.0	5.0	1.3	3.4	3.3	0.6	2.2	4.4
Meat and bone meal, low fat	62.0	6.7	1.2	2.7	2.8	6.2	5.0	1.4	3.5	3.4	0.8	2.3	4.5
Poultry offal meal	60.2	6.6	2.5	1.8	3.9	7.0	4.4	1.4	3.9	3.9	0.7	2.6	5.4
Blood meal	94.1	4.2	1.1	6.2	1.1	12.1	8.7	1.2	6.9	4.7	1.4	3.0	8.5
Feather meal	85.7	6.7	4.3	0.8	4.9	8.0	2.1	0.7	4.7	4.6	0.6	2.5	7.2
Maize distillers [1]	44.0	3.4	2.0	2.4	3.5	12.0	2.6	1.9	4.6	3.2	0.5	4.1	4.4
Maize distillers [2]	29.5	4.3	2.0	2.7	3.8	11.6	3.0	2.0	4.8	3.7	0.8	3.9	5.1
Brewer's yeast	48.6	4.4	0.9	2.0	4.6	6.2	6.3	1.5	3.6	4.4	1.1	2.7	4.9
Maize gluten	67.3	3.1	1.7	2.1	4.1	16.1	1.7	2.4	6.2	3.4	0.5	5.1	4.6
Wheat grain	12.6	4.7	2.2	2.3	3.4	6.5	2.9	1.6	4.5	2.9	1.2	2.7	4.3
Faba bean	29.0	9.0	1.2	2.6	4.1	7.1	6.3	0.8	4.0	3.5	0.8	2.7	4.6
Pea seeds	23.9	8.4	1.4	2.5	4.2	7.1	7.2	1.0	4.7	3.8	0.9	3.1	4.8
Linseed meal [3]	34.2	9.6	1.8	2.6	4.4	5.9	3.9	1.9	4.8	3.8	1.6	2.4	5.2
Canola meal [4]	39.0	5.9	2.5	2.6	4.0	6.8	5.6	2.0	3.9	4.2	1.2	2.9	4.9
Sunflower meal [5]	37.7	8.5	1.7	2.5	4.1	6.2	3.5	2.3	4.4	3.6	1.2	2.4	4.9
Rapeseed meal [6]	38.3	6.1	2.3	2.6	4.0	6.7	5.5	2.1	3.9	4.4	1.3	3.1	5.1
Soybean meal [7]	49.3	7.5	1.6	2.7	4.6	7.7	6.3	1.4	5.1	3.7	1.4	3.5	4.5
Soybean meal [8]	53.5	7.3	1.6	2.7	4.6	7.7	6.3	1.4	5.1	3.8	1.4	3.5	4.8
Cottonseed meal [9]	45.0	11.1	1.6	2.9	3.2	5.9	4.2	1.4	5.1	3.3	1.1	2.9	4.2

[a] high protein; [b] 60-68% protein in feed; [1] Wet grain and solubles; [2] Dried grains and solubles; [3] Expeller extracted; [4] Solvent extracted; [5] Solvent extracted, dehulled and partially dehulled; [6] Solvent extracted, low erucic, low glucosinolates; [7] High oil (expeller); [8] High protein (dehulled); [9] Low fibre, low oil; Prot- refers to Protein; and * indicates conditionally essential amino acid.

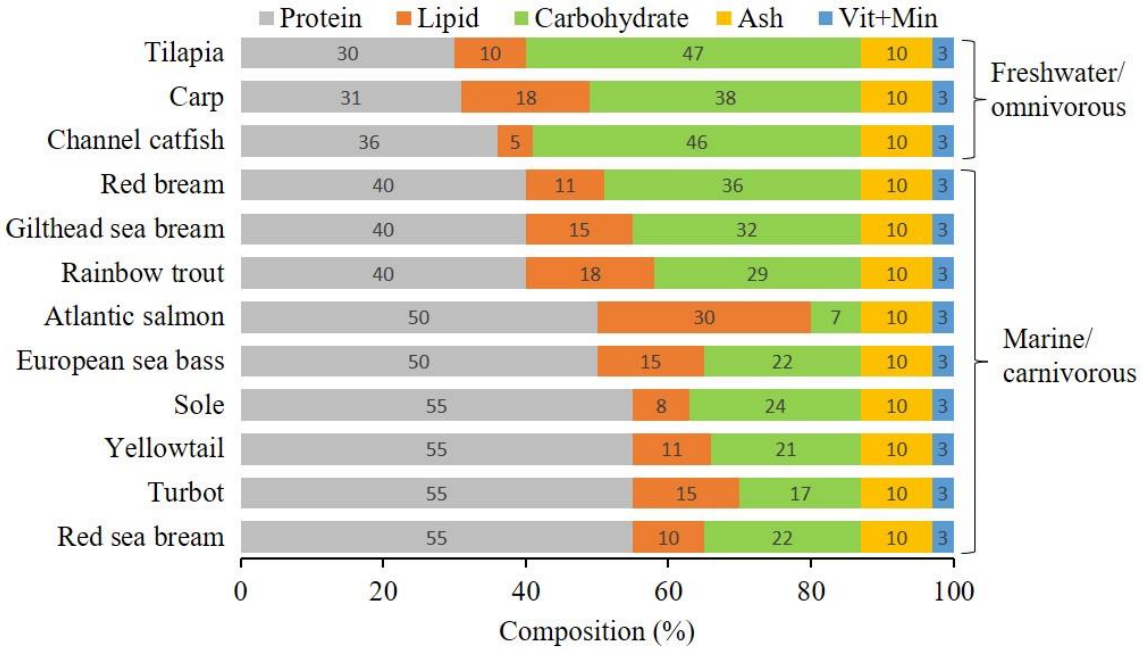

Figure 2.3. Nutritional requirements (%) for various fish species (Source: Oliva-Teles et al., 2015).

2.4. Insects as aquafeed ingredients

Insects form a natural food source for fish and humans and supply large amounts of protein (9.3–76%), fat (7.9–40%), energy (890–4,900 kcal/kg, fresh weight), essential minerals, and vitamins (Howe et al., 2014; Davis et al., 2017; Nogales-Mérida et al., 2019; Alfiko et al., 2021). Therefore, insects are good sources of EAA and EFA. The amounts and types of fatty acids vary according to the species, stage of development (larva, pupa, prepupa, and imago), type of diet, and rearing conditions (Tables 2.2 and 2.3), resulting in variable fatty acid contents (Nogales-Mérida et al., 2019; Alfiko et al., 2021). Insects also contain antimicrobial peptides, which could be beneficial to fish health through their activity against pathogens (Nogales-Mérida et al., 2019). Moreover, many insects are herbivores or omnivores and can be reared on a variety of feed materials, including plant materials, agricultural wastes, and food wastes. Thus, many insects have the capability of recycling organic waste and feeding on waste foodstuffs, although the kind of rearing substrate used influences their fatty acid profile (Newton et al., 2005; Barroso et al., 2014; Davis et al., 2017; Nogales-Mérida et al., 2019; Alfiko et al., 2021). In addition, insects including silkworms (*Bombyx mori*), black soldier flies (*Hermetia illucens*), houseflies (*Musca domestica*), yellow mealworms (*Tenebrio molitor*), lesser mealworms (*Alphitobius diaperinus*), house crickets (*Acheta domesticus*), banded crickets (*Gryllodes sigillatus*), and field crickets (*Gryllus assimilis*) were approved for the production of feed in aquaculture under EU legislation (Alfiko et al., 2021; Terova et al., 2021). Other insects that have been tested in aquaculture include blowflies (*Chrysomya megacephala*), grasshoppers (*Zonocerus variegatus*), common flies (*Lucilia sericata*), termites (*Macrotermes subhyalinus*), and the superworm (*Zophobas morio*) (Cabano, 2017; Nogales-Mérida et al., 2019).

A comparison of the nutritional compositions of insects, FM, and soybean meal reveals a crude protein content of 40–65% (on a dry matter basis) in insects, similar to that in soybean meal but less than that in FM (Table 2.2). However, their lipid level is higher than FM's content, although the amounts of omega-3 fatty acids in fish oil (FO) are much higher than in insect oils, which have large quantities of saturated fatty acids (Tables 2.2 and 2.3). Insect meals

such as house cricket, mealworm, and housefly maggot meals have higher unsaturated fatty acid concentrations (about 60–70%) than that in black soldier fly larvae, with an unsaturated fatty acid content <37% (Table 2.3). The low content of EPA and DHA in the insect species limits their application in aquaculture.

Table 2.2. Proximate, amino acid and fatty acid compositions of insect meals compared to fish meal (FM) and soybean meal values from literature (Derived from Alfiko et al., 2021).

	SPM	BSFL	HFM	YMW	LMW	HC*	BC*	FM	SM
Proximate composition and mineral elements (%)									
Crude protein	60.7 (81.7)	42.1 (56.9)	50.4 (62.1)	52.8 (82.6)	57.3 (62.6)	63.3 (76.5)	59.8 (69.0)	70.6	51.8
Lipids	25.7	26.0	18.9	36.1	8.5	17.3	13.3	9.9	2.0
Ca	0.38	7.56	0.47	0.27	0.13	1.01	0.20	4.34	0.39
P	0.60	0.90	1.6	0.78	0.11	0.79	1.04	2.79	0.69
Essential amino acid composition (g/16 g nitrogen)									
Arginine	5.6	5.6	4.6	4.8	5.6	6.1	5.3	6.2	7.64
Histidine	2.6	3	2.4	3.4	3	2.3	3	2.4	3.06
Isoleucine	5.1	5.1	3.2	4.6	4	4.4	4.8	4.2	4.16
Leucine	7.5	7.9	5.4	8.6	5.8	9.8	8	7.2	7.58
Lysine	7	6.6	6.1	5.4	4.7	5.4	5.9	7.5	6.18
Methionine	3.5	2.1	2.2	1.5	2.3	1.4	1.4	2.7	1.32
Phenylalanine	5.2	5.2	4.6	4	3.4	3	2.5	3.9	5.16
Threonine	5.1	3.7	3.5	4	3.5	3.6	4.2	4.1	3.78
Tryptophan	0.9	0.5	1.5	0.6	0.8	0.6	0.6	1	1.36
Valine	5.5	8.2	4	6	4	5.1	6	4.9	4.5
Fatty Acid composition (%)									
Saturated fatty acids									
12:0 (Lauric)		21.4	–	0.5	0.1	–	0.1	–	–
14:0 (Myristic)		2.9	5.5	4	1.4	0.7	1.65	3.4	
16:0 (Palmitic)	24.9	16.1	31.1	21.1	26.4	23.4	23.5	16.1	10.6
18:0 (Stearic)	5.4	5.7	3.4	2.7	10.9	9.8	7.35	4.6	3.8
Monosaturated fatty acids									
16:1n-7 (Palmitoleic)	0.8	–	13.4	4	1.1	1.3	3.78	0.8	–
18:1n-9 (Oleic)	24.3	32.1	24.8	37.7	35.9	23.8	29.14	12.7	21.8
Polyunsaturated fatty acids (%)									
18:2n-6 (Linoleic)	6.3	4.5	19.8	27.4		38	29.78	1.4	53.7
18:3n-3 (Linolenic)	36.0	0.19	2	1.2	0.4	1.2	2.13	0.6	5.8
20:5n-3 (EPA)	0.2	0.03	–	–	–	–	–	11.1	–
22:6n-3 (DHA)	–	0.006	–	–	–	–	0.07	29.1	–

SPM, Silkworm pupae meal; BSFL, Black soldier fly larvae; HFM, housefly maggot meal; YMW, yellow meal worm; LMW, lesser meal worm; HC, house cricket; BC, banded cricket; FM, fish meal; SM, Soymeal; * Data from whole body meal; For crude protein, data in () are from defatted meals. For fatty acid composition values correspond to cow manure as substrate.

In addition, defatting of the insect meal can be used to improve the quality of insect meals by removing unnecessary lipids and FA that do not match the fish's dietary requirements (Henry et al. 2018). Additionally, chitin, the main component of insect cuticles, and the indigestible polysaccharide may be removed, producing defatted and dechitinised insect meals with improved protein quality and feed digestibility (Rumpold and Schlüter, 2013; Alfiko et al., 2021). Apart from black soldier fly meal, which contains a relatively high calcium content, the rest of the insect species have very low calcium levels (Table 2.2), requiring the addition of

calcium to insect-based aquafeeds. Alternatively, the insect substrates can be fortified with calcium to increase the calcium content in insect meals for fish (Alfiko et al., 2021). Phosphorus is an important mineral with an essential role in bone mineralisation. Although fish can absorb minerals directly from the environment, phosphorus is usually a limiting mineral because of its relatively low quantities in water (Cabano, 2017).

Table 2.3. Fatty acid composition (%) of insect species at different stages of development (Source: Nogales-Mérida et al. 2019)

Fatty acid profile	**BSF**		**HF**			**MW**		**ZM**	**HC**	**FO**	**SBO**
	Larva	Prepupa	Larva	Pupa	Adult	Larva	Adult	Larva	Adult		
Saturated Fatty acids											
12:00	43.19	42.65				0.24		0.57	0.20	3.80	
14:00	7.76	6.31	3.78	2.70	6.80	2.42	1.84	0.89	1.12	5.73	0.10
16:00	13.47	14.82	25.50	34.85	22.80	17.64	18.65	17.44	24.32	13.05	10.48
18:00	2.13	3.35	3.25	2.75	4.50	3.60	6.17	4.84	7.32	2.98	3.88
20:00			3.80	0.14	1.30	0.52		0.11	0.00	0.17	0.40
Monounsaturated Fatty Acids											
16:1n-7	2.00	4.14	15.91	5.59	24.00	1.63	2.20	0.56	1.56	5.51	0.09
18:1n-9	10.36	14.75	30.71	22.83	29.30	38.84	36.74	12.50	29.14	14.47	20.86
Poly Unsaturated Fatty Acids											
18:2n-6	16.28	9.25	13.29	36.27	3.30	25.32	32.46	13.79	37.87	2.35	53.48
18:3n-3	0.85	0.50	3.35	2.73	1.30	1.14	0.74	0.79	1.35	0.62	7.71
20:4n-6	0.27	0.10	0.08	0.05		0.07		0.05		1.07	
20:5n-3	0.00	0.27	0.03	0.15		0.04		0.04	0.64	9.87	
22:6n-3		0.10	0.03	0.03		0.04		0.03		10.24	
n-3	0.85	0.83	2.02	2.91	1.30	1.15	0.74	0.82	1.67	20.73	7.71
n-6	16.46	9.28	10.66	36.32	3.30	25.34	32.46	13.82	37.87	3.42	53.48
n-3/n-6	0.06	0.08	0.33	0.08	0.39	0.05	0.02	0.08	0.05	6.06	0.14

BSF, Black soldier fly; HF, House fly; MW, Mealworm; HC, House cricket; ZM, *Zophobas morio*; FO, fish oil; SBO, soybean oil.

2.5. Application of insect products in catfish diets

In feeding experiments conducted on catfish, insect meals including silkworm pupa (SWP) meal, housefly maggot (HFM) meal, mealworm (MW) meal, and black soldier fly larvae meal (BSFL) meal have been incorporated into experimental diets. Feeding defatted or non-defatted SWP meal is reported to result in high digestibility in catfish. The dietary inclusion of non-defatted SWP meal at a level of 50% in aquafeeds did not negatively affect growth performance, FCR, and organoleptic quality of catfish (Alfiko et al., 2021). Feeding experiments examining the effect of HFM meal on growth performance have been performed for *Clarias gariepinus* (African catfish), *Heterobranchus longifilis* and their hybrids, with results suggesting a HFM meal inclusion level not exceeding 30% (Alfiko et al., 2021). Studies involving the use of live maggots as feed for *C. gariepinus* resulted in poor growth, suggesting that live maggots could only be used as a supplementary diet. In a related study, it was demonstrated that HFM meal could be used as a partial replacement for FM in African catfish fingerlings at a 60–70% inclusion level for good growth and nutritional utilisation (Alfiko et al., 2021). Total (100%) and partial (50%) replacement of FM with BSFL meal, MW meal or a 1:1 combination of both ingredients in the diet of African catfish had no significant effect on the growth performance

and feed utilisation during the 6 weeks of feeding. However, significant differences were observed in fish fed with a diet where FM was totally replaced with MW meal (Gebremichael et al., 2023), which could be due to BSFL meal being more digestible than MW meal in this species (Sándor et al., 2022).

In addition, both fresh and dried MW meal are acceptable as an alternative protein source for aquaculture and appear to be a viable alternative protein source for catfish. African catfish fed diets with up to 40% MW meal replacement exhibited no major difference in growth and feed utilisation efficiency when compared to the control group. In relation to BSFL meal, the FM may be replaced with BSFL meal up to 50% without impacting the growth, nutrient utilisation, survival rate, and welfare of the African catfish fingerlings. In other catfish species, yellow catfish juveniles (*Pelteobagrus fulvidraco*) fed with a diet containing 75% of FM replaced with MW meal did not affect the growth performance and immunological response (Alfiko et al., 2021). Furthermore, incorporating 18% of MW meal into the diet improved the immune response and bacterial resistance of yellow catfish without affecting their growth. Moreover, 25% replacement of FM with BSFL meal resulted in varying results, ranging from no effect on the growth or immunity index to the best growth performance in yellow catfish (Nogales-Mérida et al., 2019; Alfiko et al., 2021). In addition, a 30% FM substitution considerably enhanced cholesterol and nitric oxide concentrations in plasma, along with the prevention of superoxide radical anion production. However, channel catfish (*Ictalurus punctatus*) fed on chopped black soldier fly larvae were comparable to the control group in terms of body weight and total length and indicated that BSFL meal could be beneficial as an FM replacement up to a 7.5% inclusion (Alfiko et al. 2021).

2.6. Fatty acids in fish diet, biological roles, and fatty acid metabolism

2.6.1. Fatty acids and their biological role in organisms

Fatty acids (FA) are organic molecules with a carboxylic acid group at the end of the aliphatic chain containing four or more carbons, usually an even number up to 24 (Castro et al., 2016). They occur either as free molecules or as complex lipids, including membrane phospholipids and triglycerides, which are basically two and three FA bonded to a glycerol molecule, respectively (Tocher, 2003). Their properties and functions are governed by characteristics such as their length, degree of unsaturation, and position of their double bonds (Calder, 2005). Fatty acids can be designated as saturated, monounsaturated or polyunsaturated fatty acids based on the number of carbon-carbon double bonds present in the chain. Saturated fatty acids (SFA) have only single carbon-carbon bonds in their chains, while monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) have one carbon-carbon double bond and two or more carbon-carbon double bonds, respectively (Table 2.4). The C16:0 and C18:0 forms are predominant naturally occurring SFAs in animal fats, including fish lipids, although a range of chain lengths from C12 to C24 can be found. In the MUFA, the predominant ones are the 18:1n-9 and 16:1n-7 (Tocher, 2003).

Table 2.4. Fatty acid nomenclature

Nomenclature		Trivial name	Systematic name
n	Δ		
8:0		Caprylic	Octanoic
10:0		Capric	Decanoic
12:0		Lauric	Dodecanoic
13:0		Tridecylic	Tridecanoic
14:0		Myristic	Tetradecanoic
14:1n-5	$14:1\Delta^{9}$	Myristoleic	9-tetradecenoic
15:0		Pentadecylic	Pentadecanoic
15:1		Ginkgolic	10-Pentadecanoic
16:0		Palmitic	Hexadecanoic
16:1n-7	$16:1\Delta^{9}$	Palmitoleic	9-hexadecenoic
17:0		Margaric	Heptadecanoic
17:1		-	10-Heptadecanoic
18:0		Stearic	Octadecanoic
18:1n-9	$18:1\Delta^{9}$	Oleic	9-Octadecenoic
18:2n-6	$18:2\Delta^{9,12}$	Linoleic	9,12-Octadecadienoic
18:2 n-9		Linolelaidic	9, trans-12-Octadecadienoic
18:3n-3	$18:3\Delta^{9,12,15}$	α-linolenic	9,12,15-Octadecatrienoic
18:3n-6		γ-Linolenic	6,9,12-Octadecatrienoic
18:4n-3	$18:4\Delta^{6,9,12,15}$	Stearidonic	6,9,12,15-Octadecatetraenoic
20:0		Arachidic	Eicosanoic
20:1n-9	$20:1\Delta^{11}$	Gondoic	11-Eicosenoic
20:2n-6		Eicosadienoic	
20:3n-3	$20:3\Delta^{11,14,17}$	Eicosatrienoic	11,14,17-eicosatrienoic
20:3n-6		Homo-γ-linolenic	8,11,14-Eicosatrienoic
20:4n-3	$20:4\Delta^{8,11,14,17}$	Eicosatetraenoic	8,11,14,17-eicosatetraenoic
20:4n-6	$20:4\Delta^{5,8,11,14}$	Arachidonic	5,8,11,14-Eicosatetraenoic
20:5n-3	$20:5\Delta^{5,8,11,14,17}$	Eicosapentaenoic	5,8,11,14,17-Eicosapentaenoic
21:0		-	Heneicosanoic
22:0		Behenic	Docosanoic
22:1n-9	$22:1\Delta^{13}$	Erucic	13-Docosenoic
22:2n-6	$22:2\Delta^{13,16}$	-	13,16-Docosadienoic
22:4 n-6	$22:4\Delta^{7,10,13,16}$	Adrenic	7,10,13,16-Docosatetraenoic
22:5n-3	$22:5\Delta^{7,10,13,16,19}$	Docosapentaenoic	7,10,13,16,19-Docosapentaenoic
22:6n-3	$22:6\Delta^{4,7,10,13,16,19}$	Docosahexaenoic	4,7,10,13,16,19-Docosahexaenoic
23:0		-	Tricosanoic
24:0		Lignoceric	Tetracosanoic
24:1n-9	$22:1\Delta^{15}$	Nervonic	15-tetracosenoic
24:5n-3	$24:5\Delta^{9,12,15,18,21}$	Tetracosapentaenoic	9,12,15,18,21-tetracosapentaenoic
24:6n-3	$24:6\Delta^{6,9,12,15,18,21}$	Tetracosahexaenoic	6,9,12,15,18,21-tetracosahexaenoic

The unsaturated fatty acids can also be named based on the omega (ω or n-) nomenclature and the delta (Δ) nomenclature. The omega (ω or n-) nomenclature is based on the number of carbon atoms in the fatty acid chain, the number of double bonds (unsaturation) within the chain, and the position of the first double bond relative to the methyl ($-CH_3$) terminus of the FA chain (Tocher, 2003; Glencross, 2009). For example, FA with the formula 22:6n-3 means a FA with 22 carbon atoms and six double bonds, with the first double bond situated 3 carbon atoms from

the methyl end. The Δ-nomenclature on the other hand, specifies the positions of all double bonds from the carboxyl group carbon. With reference to the formula 22:6n-3 (n-nomenclature), the Δ-nomenclature therefore becomes 22:6Δ4,7,10,13,16,19. The geometric configuration of most unsaturated FAs in fish is the *cis* configuration, containing double bonds at three carbon intervals separated by a methylene group (Tocher, 2003; Glencross, 2009). Although the Δ-nomenclature is more precise as it specifies the double bond positions along the FA chain, the n- nomenclature is the most frequently used in fish nutrition (Tocher, 2003). Fatty acids are also known by their English names, often reflecting their origin, such as α-linolenic acid from linseed oil, and Greek-Latin names that reflect their number of carbon atoms and double bonds. Thus, "docosahexaenoic acid" reflects 22 carbon atoms and six double bonds (Table 2.4).

Lipids, along with proteins, form the major organic constituents of fish, with carbohydrates being quantitatively much less prominent in fish (Tocher, 2003). Lipids such as triglycerides are an efficient form of high-energy storage molecules in fish. Fatty acid catabolism takes place in the mitochondria and peroxisomes, as a β-oxidation process involving sequential cleavage of two-carbon units (Henderson, 1996). The mitochondrial β-oxidation is quantitatively more important and can utilise a wide range of fatty acid substrates (Henderson, 1996). The unique capability of fish to readily metabolise these compounds makes fish such as salmon capable of surviving long periods of food deprivation. With the exception of docosahexaenoic acid (DHA), oxidation of FA is determined by FA concentrations and enzyme specificities. In addition, there is an order of preference with saturated and monounsaturated FA preferentially oxidised before PUFA and PUFA before HUFA. DHA is a poor substrate for mitochondrial β-oxidation, as removal of the Δ4 double bond requires peroxisomal oxidation. It can thus efficiently be retained in tissues in spite of dietary concentration (Tocher, 2003). Phospholipids, on the other hand, are the major lipid component of membranes of cells and organelles. They maintain the structure and function of cellular biomembranes (Tocher, 2003; Tocher and Glencross, 2015). PUFA determines the physical properties (melting temperature) of phospholipids, hence impacting the fluidity of cell membranes. In so doing, PUFA acts as an active antifreeze for membrane lipids, which is important for fish and other poikilotherms that remain active at low temperatures (Das, 2008; Nakamura and Nara, 2004). Moreover, membrane-associated processes such as permeability, cell division, and inflammation are also regulated by LC-PUFA, indicating their role in controlling and regulating cellular metabolism and physiology (Schmitz and Ecker, 2008; Vagner and Santigosa, 2011).

LC-PUFA also control fatty acid synthesis by activating transcription factors and regulating expression of genes, including those coding for fatty acid synthase (Qiu, 2003). They also play important roles in the maturation of teleosts, sperm, and embryonic and larval development (Butts et al., 2015; Tocher and Glencross, 2015). Moreover, LC-PUFAs are reported to be associated with oocyte maturation, higher egg fertilisation success, hatching and larval survival rates (Sorbera et al., 2001; Yanes-Roca et al., 2009). The role of DHA in the structure and functioning of neural membranes is pivotal for the proper development of neural tissues. In addition, ARA plays a role in cell signalling, immune response, and in the regulation of ionic balance (Glencross, 2009; Tocher and Glencross, 2015).

The role of lipids and their constitutive FA in fish health has been clearly demonstrated in a number of investigations. Fish use both innate/nonspecific mechanisms such as humoral (chemical secretions, e.g., cytokines and eicosanoids) and cellular agents (granulocytes, monocytes/macrophages, thrombocytes, nonspecific cytotoxic cells, and lymphocytes), and adaptive/specific mechanisms. However, the innate strategy appears dominant due to the challenges associated with the aquatic environment (Arts and Kohler, 2009). Upon penetration

of the physical barriers (scales, skin or mucus) by pathogens, an inflammatory response is launched through the release of cytokines and eicosanoids. Cytokines such as interleukins, chemokines, and interferons are known to bind to specific cell-surface receptors, causing a cascade of intracellular signalling events that alter cell functions. Eicosanoids such as prostaglandins and leukotrienes are hormone-like compounds which are important modulators of inflammation and specific immunity responses (Arts and Kohler, 2009). ARA, EPA and dihomo-gamma-linolenic acid (DG-LIN, 20:3n-6) are precursors for the synthesis of eicosanoids such as prostaglandins, prostacyclins, leukotrienes, and thromboxanes, which regulate important signalling pathways (Arts and Kohler, 2009; Guillou et al., 2010; Tian et al., 2022). The generation of eicosanoids from precursor FA molecules occurs via two enzymes, cyclooxygenase (COX) and lipoxygenase (LOX) (Figure 2.4). Stress, cytokines, and other stimuli trigger the release of phospholipase, which migrates to the cell membrane, catalysing ester hydrolysis of phospholipid or diacylglycerol to free EPA, ARA, and/or DG-LIN, which are subsequently oxygenated via a number of pathways, producing eicosanoids (Figure 2.4). ARA-derived eicosanoids (2-series prostaglandins and 4-series leukotrienes and lipoxins) promote inflammation, while EPA and DG-LIN-derived eicosanoids (3-series prostaglandins and 5-series leukotrienes and lipoxins) are described as less potent or anti-inflammatory (Arts and Kohler, 2009).

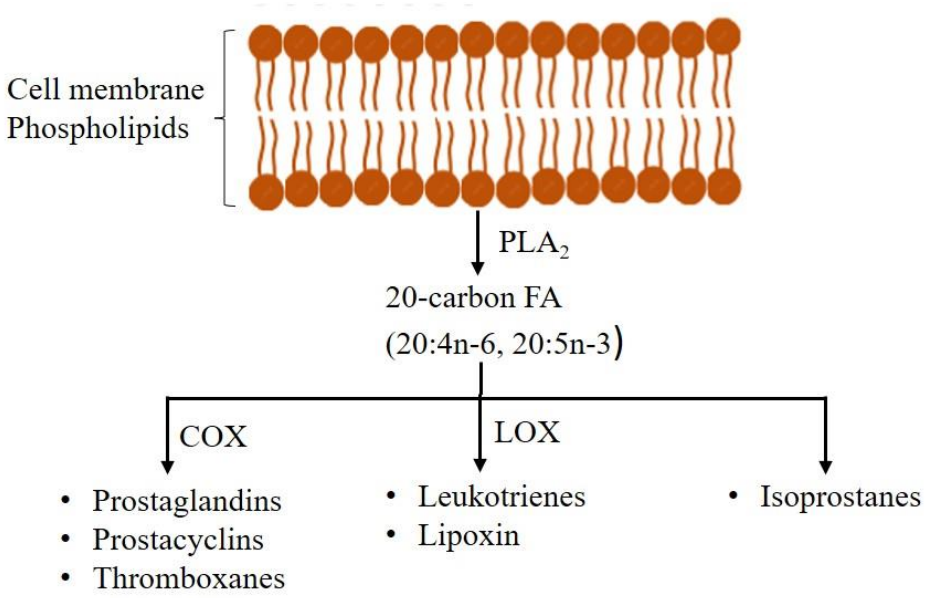

Figure 2.4. Eicosanoid synthesis pathways (PLA_2, phospholipase 2; COX, cyclooxygenase; LOX, lipoxygenase. Derived from Rowley et al., 1995 and Arts and Kohler, 2009.

2.6.2. Essential fatty acid requirements in fish

Essential fatty acids (EFA) must be obtained from the diet since they cannot be synthesised by fish (Oliva-Teles et al., 2015). The EFA requirements can be divided into three gradually increasing levels (Tocher, 2015). The first level is the physiological EFA, the absolute dietary PUFA requirement of species of fish that prevents the manifestation of EFA deficiency conditions. The second level is the amount required for maintaining optimal health and growth performance of the fish, while the third level, the highest of all three, is the amount required to guarantee the nutritional value of the fish for consumers in terms of LC-PUFA deposition in fish muscle. These requirements are species specific and dependent on the capacity of a species to synthesise fatty acid. The LC-PUFA play physiologically important roles in fish, and their requirements in the diet of fish are primarily determined by the ability of a species to

endogenously synthesise them from linoleic acid (LA) and alpha-linolenic acid (ALA) precursors supplied in the diet (Tocher et al., 2003; Monroig and Kabeya, 2018; Xie et al., 2021).

In fish species with high conversion capacity (mainly freshwater fish), the C_{18}-PUFA (LA and ALA) present at about 1% of the diet dry weight can meet their EFA requirement. It was suggested that the EFA requirements of freshwater fish can be classified into three types: (1) a rainbow trout type, where n-3 PUFA like 18:3n-3 is required; (2) a tilapia type requiring n-6 PUFA (e.g., l8:2n-6); and (3) a carp type requiring both n-3 and n-6 PUFA (e.g., both l8:3n-3 and l8:2n-6) (Kanazawa, 1985). Although several studies support the knowledge that n-6 PUFA alone would meet the requirements for essential fatty acids in tilapia, the addition of n-3 PUFA in the diet would improve fish performance under certain conditions (Corrêa et al., 2023). However, in some fish species, an excessive supply of dietary 18:3n-3 can block or depress the Δ6-desaturation of 18:2n-6 required to produce ARA, which can be detrimental in species that require more ARA than EPA (Izquierdo et al., 2001). However, for species with limited or no ability to biosynthesise LC-PUFA from their C_{18}-PUFA (LA and ALA) precursors, especially marine species, HUFA must be supplied through diet (Glencross, 2009; Lall and Dumas, 2015; Oliva-Teles et al., 2015). Moreover, a wide range of EFA requirements exists even in fish capable of endogenous LC-PUFA synthesis. For instance, in fish species such as rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) with the ability to convert ALA to EPA and DHA, direct provision of LC-PUFA in the diet resulted in better growth of the species (Wirth et al., 1997; Satoh et al., 1989a; Wilson and Moreau, 1996). However, in *Clarias gariepinus*, dietary provision of n-3 LC-PUFA did not increase their growth performance beyond those of fish fed the C_{18}-PUFA diets (Legendre et al., 1995; Hoffman and Prinsloo, 1995). Therefore, for some species, LC-PUFA can be regarded as semi-essential, as the rate of conversion from C_{18}-PUFA to LC-PUFA (C20 or C22) may be insufficient to support optimal growth, especially at the larval stage, when fish are undergoing fast somatic growths and neural tissues accumulating LC-PUFA are rapidly developing (Glencross, 2009).

2.6.3. Lipid biosynthesis enzymes

Many enzymes, genes, and transcriptional factors are involved in the metabolism of lipids in fish. The origin of lipids can be endogenous synthesis from nonlipid sources through lipogenesis or provided in the diet. The liver is known as the main site for lipogenesis in fish because the activity of lipogenic enzymes is substantially higher in hepatocytes than in adipocytes. Lipogenesis necessitates the sequential action of two enzyme systems: acetyl-CoA carboxylase (Acc), which generates malonyl-CoA from acetyl-CoA, and fatty acid synthase (Fas), which uses malonyl-CoA to synthesise saturated long-chain fatty acids via a series of condensation and reduction reactions involving the use of nicotinamide adenine dinucleotide (NADPH). Enzymes such as glucose 6-phosphate dehydrogenase (g6pd) and 6-phosphogluconate dehydrogenase (6pgd) provide the NADPH for fatty acid synthesis (Henderson, 1996). Other enzymes important in lipogenesis include ATP citrate lyase, involved in the conversion of citrate to acetyl-CoA. Desaturation of the synthesised saturated fatty acids, mainly palmitic acid (16:0) and stearic acid (18:0), by the microsomal stearoyl-CoA desaturases (Δ9 desaturases), produces palmitoleic acid (16:1n–7) and oleic acid (18:1n–9), respectively (Castro et al., 2016).

Dietary C_{18} PUFA, such as ALA and LA, can be desaturated by fatty acyl desaturases (Fads) and elongated by elongase of very long-chain fatty acids (Elovl), producing LC-PUFA (Table 2.5). Desaturating enzymes perform dehydrogenation reactions resulting in the introduction of

a double bond at specific positions in fatty acid chains (Nakamura and Nara, 2004). As previously mentioned, FA unsaturation is a major determinant of the melting temperature of a triglyceride as well as the fluidity of biological membranes. Because of the fundamental function of unsaturated long-chain fatty acids in cellular membranes, fatty acyl desaturases are conserved across kingdoms. Desaturases can be classified into membrane-bound and soluble forms on the basis of subcellular localisation, as well as into methyl-end and front-end desaturases based on the end of the fatty acyl chain from which the desaturase derives its specificity (Castro et al., 2016; Nakamura and Nara, 2004).

In the vertebrates, well-known Fads enzymes constitute three members, which in mammals constitute Fads1, Fads2 and Fads3 (Castro et al., 2016). In the cartilaginous fish (as well as in mammals), the genes *fads1* (for Fads1 protein) and *fads2* (for Fads2 protein) encode Δ5 and Δ6 desaturases, respectively (Guillou et al., 2010; Xie et al., 2021). With the exception of the Elopomorpha species, such as the Japanese eel *Anguilla japonica*, which possesses both Fads1 and Fads2 desaturases, teleost fish possess only Fads2 with diverse activities (Castro et al., 2016; Xie et al., 2021). Fads2 are named by the fixed position of the double bond they create, counting from the carboxyl (front) end of the FA, and are often termed as "front-end" desaturases (Nakamura and Nara, 2004; Xie et al., 2021). For instance, Δ6, Δ5 and Δ4 Fads2 create double bonds at positions 6, 5 and 4 of the fatty acyl chain, respectively. The Δ6 Fads2 catalyse the desaturation of 18- and 24-carbon PUFA in the biosynthesis pathways of both n-3 and n-6 LC-PUFA (Figure 2.8). In addition, many Δ6 Fads2 also catalyse the desaturation of 20:3n-3 and 20:2n-6, thereby exhibiting Δ8 activity and presenting an alternative pathway to the Δ6Δ5 pathway of EPA and ARA synthesis (Figure 2.5). Most teleost Fads2 are bifunctional Δ6Δ5 Fads2. In addition, Fads2 with Δ4 activity has been characterised in a number of teleost species (Table 2.5).

Table 2.5. Teleost species with a complete LC-PUFA biosynthetic enzymes characterized (source: Xie et al., 2021)

Teleosts	**Fads and Elovl present**
Freshwater species	
African catfish (*Clarias gariepinus*)	Δ6Δ5 Fads2, Elovl5, Elovl4, Elovl2, Elovl8
Mexican silverside (*Chirostoma estor*)	Δ6Δ5 Fads2, Δ4 Fads2, Elovl5
Rainbow trout (*Oncorhynchus mykiss*)	Δ6 Fads2, Δ5 Fads2, Elovl5, Elovl4, Elovl2
Silver barb (*Barbonymus gonionotus*)	Δ6Δ5 Fads2, Elovl5
Striped snakehead (*Channa striata*)	Δ6Δ5 Fads2, Δ4 Fads2, Elovl5
Tambaqui (*Colossoma macropomum*)	Δ6Δ5 Fads2, Elovl5
Tench (*Tinca tinca*)	Δ6Δ5 Fads2, Elovl5, Elovl2
Tilapia (*Oreochromis niloticus*)	Δ6Δ5 Fads2, Δ4 Fads2, Elovl5
Zebrafish (*Danio rerio*)	Δ6Δ5 Fads2, Elovl5, Elovl4, Elovl2
Diadromous species	
Atlantic salmon (*Salmo salar*)	Δ6 Fads2, Δ6Δ5 Fads2, Elovl5, Elovl4, Elovl2
Japanese eel (*Anguilla japonica*)	Δ6 Fads2, Δ5 Fads1, Elovl5, Elovl2
Marine species	
Rabbitfish (*Siganus canaliculatus*)	Δ6Δ5 Fads2, Δ4 Fads2, Elovl5, Elovl4, Elovl8
Salema (*Sarpa salpa*)	Δ6 Fads2, Δ5 Fads2, Elovl5
Sand sole (*Pegusa lascaris*)	Δ6Δ5 Fads2, Δ4 Fads2, Elovl5
Thicklip grey mullets (*Chelon labrosus*)	Δ6 Fads2, Δ5 Fads2, Elovl5

The elongase protein family includes seven proteins (Elovl 1–7). These enzymes catalyse the addition of two-carbon units to the carboxyl end of a fatty acyl CoA, with malonyl CoA as the two-carbon donor and NADPH as the reducing agent. The elongation process consists of condensation, reduction, dehydration and reduction reactions, catalysed by four enzymes, similarly to the *de novo* synthesis of palmitic acid by the Fas enzyme. Elongation primarily occurs on the cytoplasmic side of the endoplasmic reticulum, as well as in the mitochondria (Cook and McMaster, 2002). Elongase proteins differ in their elongation capacity. For instance, Elovl-1, 3, 6 and 7 elongate saturated and monounsaturated fatty acids; Elovl2 and Elovl5 elongate PUFA, while Elovl4 elongates both very long chain (VLC)-SFA and VLC-PUFA (Jakobsson et al., 2006; Guillou et al., 2010). The functional characterisation of Elovl2, Elovl4, and Elovl5 indicated that Elovl5 mainly elongates PUFA of chain lengths C18 and C20, whereas Elovl2 and Elovl4 act on PUFA substrates of chain lengths C20 and C22, although elovl2 is capable of elongating C_{18} PUFA as well but at a comparatively lower efficiency (Castro et al., 2016; Monroig et al., 2018). The Elovl4 has been shown to elongate PUFA of chain length > C24 and SFA of chain length > C24 and up to C36 (Agbaga et al., 2008; Li et al., 2017).

2.6.4. Biosynthesis of long-chain polyunsaturated fatty acids ($LC_{(20-24)}$-PUFA) in fish

The ability of fish to synthesise fatty acids *de novo* is limited to palmitic acid (16:0) and stearic acid (18:0) biosynthesis in a process known as lipogenesis (Polakof et al., 2011). The fatty acid synthase enzyme system catalyses the synthesis of saturated long-chain fatty acids from acetyl CoA, malonyl CoA, and nicotinamide adenine dinucleotide phosphate (NADPH), resulting in palmitic acid (16:0) as the main product and stearic acid (18:0) as a minor product of elongation of palmitic acid (Castro et al., 2016). In fish, palmitic acid and stearic acid can be desaturated by the microsomal stearoyl-CoA desaturases (Δ9 desaturases), producing monounsaturated fatty acids (MUFA), namely palmitoleic acid (16:1n–7) and oleic acid (18:1n–9), respectively. Modification of MUFA by enzymes known as methyl-end desaturases introduces a new double bond between the existing double bond and the methyl end ($–CH_3$) of the fatty acyl chain, resulting in the synthesis of PUFA (Sperling et al., 2003; Monroig and Kabeya, 2018). For instance, oleic acid is converted to linoleic acid (LA) by the methyl-end desaturase Δ12 desaturase. Linoleic acid can then be converted to α-linolenic acid (ALA) by Δ15 desaturase (Monroig and Kabeya, 2018). However, as observed for other vertebrates, fish cannot biosynthesise LA and ALA *de novo* because they lack Δ12 and Δ15 desaturases, which were discovered in plants, marine microbes (including microalgae), heterotrophic protists and bacteria, and invertebrates (Tocher et al., 2003; Monroig and Kabeya, 2018; Xie et al., 2021). Therefore, LA and ALA must be provided in fish diets. Moreover, the longer-chain derivatives of LA and ALA, namely arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and DHA (22:6n-3), are essential for some fish.

Freshwater fish have a higher capacity to convert dietary C_{18} PUFA (LA and ALA) to C_{20-24} LC-PUFA (ARA, EPA, and DHA) than marine fishes (Sargent et al., 2002; Castro et al., 2012). This capacity for endogenous biosynthesis of LC-PUFA from C_{18} PUFA depends on the presence, expression and activity of key enzymes, diet, trophic level, and environmental conditions including salinity, temperature, and photoperiod (Zheng et al., 2005; Fonseca-Madrigal et al., 2014; Xie et al., 2021). The bioconversion from ALA to EPA and LA to ARA requires a series of desaturation and elongation reactions catalysed by Fads2 and Elovl proteins and may involve the Δ6/Δ5 or Δ8/Δ5 desaturation pathways utilising the same enzymes (Sargent et al., 2002; Xie et al., 2021). The Δ6/Δ5 pathway involves a Δ6 desaturation of LA (18:2n-6) and ALA (18:3n-3) to 18:3n-6 and 18:4n-3, respectively, a two-carbon chain elongation step to 20:3n-6 and 20:4n-3, and a subsequent Δ5 desaturation leading to the

production of ARA (20:4n-6) and EPA (20:5n-3), respectively (Figure 2.5). The elongation step can be catalysed by Elovl5 and Elovl2. In addition, the ability of fish Elovl4 to catalyse this elongation step has been reported (Gregory and James, 2014).

The alternative Δ8 desaturation pathway for the production of ARA from 18:2n-6 and EPA from 18:3n-3 starts with an elongation step to 20:2n-6 and 20:3n-3, respectively, followed by a Δ8 desaturation step yielding 20:3n-6 and 20:4n-3 and finally a Δ5 desaturation step (figure 2.5). The biosynthesis of DHA from EPA may either occur directly via the "Δ4 pathway", involving a Δ4 desaturation of docosapentaenoic acid (DPA, 22:5n-3) following an elongation step from EPA, or via the longer "Sprecher pathway". The Sprecher pathway involves two consecutive elongations of EPA to produce 24:5n-3, followed by a Δ6 desaturation to form 24:6n-3, which then undergoes partial β-oxidation in the peroxisomes to produce DHA (Figure 2.5). In most fish species, the Sprecher pathway is the most common pathway for DHA synthesis. The elongation steps are catalysed by Elovl2, 4 and 5, with Elovl2 identified as the most efficient (Castro et al., 2016).

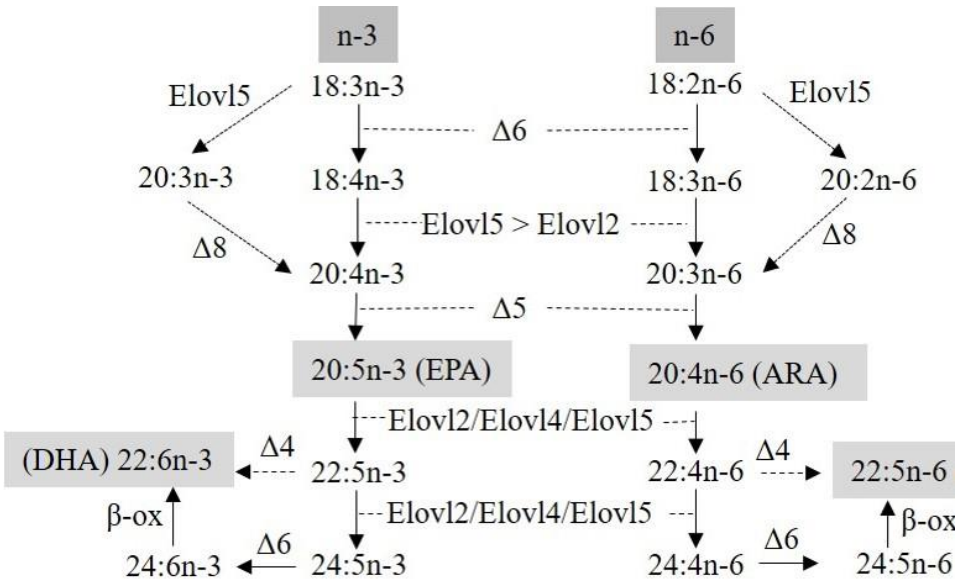

Figure 2.5. Schematic illustration of the biosynthesis pathway of long-chain (C20-24) polyunsaturated fatty acids (LC-PUFA) from α-linolenic (18:3n-3) and linoleic (18:2n-6) acids in fish. Redrawn from Oboh et al., 2016; Ayisi et al., 2018 and Monroig et al., 2018.

2.6.5. Lipid transport and lipolysis enzymes

The fatty acids derived from the diet or formed endogenously are subjected to various processes within the fish. For instance, they can be esterified into phospholipids (structural lipids of biomembranes) or incorporated into reserve lipids, the triacylglycerols (TAGs). Triacylglycerols are the main form of storage for a wide spectrum of fatty acids, ranging in chain length from C12 to C24 with the number of carbon-carbon bonds ranging from zero to six double bonds depending on the fatty acid composition. In mammals and fish, fatty acids from the pancreatic lipase-mediated digestion of dietary lipids are absorbed by intestinal enterocytes along with another digestion product, 2-monoacylglycerols. Within the intestinal cell, the fatty acids are re-esterified to the monoacylglycerol to form TAGs, which are transported as chylomicrons through blood to adipose and muscle tissues (Henderson, 1996). Re-esterification of fatty acids into TAGs and packaging into smaller lipoprotein particles similar to chylomicrons has been observed in fish. Apolipoproteins are the major proteins associated with chylomicron assembly and have been genetically characterised in fish (Kamala

et al., 2013). Before the TAGs of chylomicrons can be assimilated by muscle and adipose tissues, they are first hydrolysed by lipoprotein lipase, and the resulting fatty acids are absorbed by the adipose or muscle cells and re-esterified within the cell into triacylglycerols (Henderson, 1996). Although not well characterised in fish, it is thought that fatty acids are first activated to their CoA derivatives before being incorporated into triacylglycerols and phospholipids via the microsomal glycerol-3-phosphate pathway as seen in mammals (Henderson, 1996).

To utilise TAGs, they must be mobilised from the storage tissue (e.g., adipose tissue) through lipolysis. During lipolysis, TAGs stored in cellular lipid droplets are sequentially hydrolysed, generating fatty acids, a critical process in the metabolism of stored lipids. The process involves the activities of three specific lipases, namely, hormone-sensitive lipase (HSL), monoacylglycerol lipase, and adipose triacylglycerol lipase (ATGL). The ATGL initiates lipolysis by cleaving the first FA from TAG, producing diacylglycerol (DAG). Following this reaction, HSL then acts on DAG, releasing additional FA and the monoacylglycerol (MAG) molecule, which is then hydrolysed by monoacylglycerol lipase (MAGL). During lipolysis, lipoproteins carry TAGs to tissues such as the muscle and adipose tissue via blood, consequently leading to their entry into the mitochondria where β–oxidation occurs to yield energy (Henderson, 1996; Eichmann et al., 2012; Ayisi et al., 2018).

2.6.6. Transcriptional regulation of lipid biosynthesis genes

Transcription factors (TFs) and regulatory loops interact intricately to control several genes involved in lipid metabolism and transport. The characterisation of the promoter regions of several genes involved in lipid biosynthesis revealed the presence of multiple binding elements (*cis*-elements) for transcription factors (Carmona-Antoñanzas et al., 2014; Xie et al., 2021). These include the stimulatory protein 1 (Sp1) binding element in Atlantic salmon Δ6 *fads2*, rabbitfish Δ6Δ5 *fads2* and *elovl5*, and Channa striata *elovl5* promoters; CCAAT/enhancer binding protein (C/EBP) element in the Atlantic cod Δ6 *fads2* gene promoter; nuclear factor 1 (NF-1) element in rabbitfish Δ4 *fads2* and Δ6Δ5 *fads2*; and the peroxisome proliferator response element (PPRE) in rabbitfish Δ6Δ5 *fads2* and golden pompano Δ4 *fads2*, *elovl5* and *elovl4a* promoters (Goh et al., 2020; Xie et al., 2021). Other binding elements include direct repeat 1 (DR-1), a binding site for the hepatocyte nuclear factor 4α (Hnf4α) reported in the promoter of rabbitfish Δ4 *fads2*, Δ6Δ5 *fads2*, and *elovl5*; and sterol response elements (SRE), a binding site for Srebp. The SRE is considered to be the most conserved element, being present in the promoters of lipid biosynthesis genes, including several *fads2* and *elovl* genes, acetyl-CoA carboxylase (*acc*), steroyl-CoA desaturase (*scd*) and fatty acid synthase (*fas*) genes in fish (Carmona-Antoñanzas et al., 2014; Xu et al., 2019; Goh et al., 2020; Xie et al., 2021). Therefore, these binding elements play a role in the control of these genes and serve as essential components in teleost fish genetic regulatory networks.

Investigations on promoter activity and gene expression analysis reveal Hnf4α, Srebp-1, Lxr, Ppar, and Sp1 are the main TFs involved in the transcriptional regulation of FA biosynthesis in teleosts. In mammals, Hnf4α is a ligand-activated protein of the nuclear receptor family with hepatic expression, comprising a DNA binding domain (DBD) and a ligand binding domain (LBD). Hnf4α was reported to upregulate the expression of Δ6Δ5 *fads2*, Δ4 *fads2*, and *elovl*5 in rabbitfish, thereby promoting LC-PUFA biosynthesis. Predictions also indicated the occurrence of the Hnf4α binding site in the promoter of the *cptIα1b* (Carnitine palmitoyltransferase I alpha 1b) and *cptIα2a* (Carnitine palmitoyltransferase I alpha 2b) genes in grass carp (*Ctenopharyngodon idella*), suggesting that Hnf4α could be involved in modulating fatty acid β-oxidation as well (Xie et al., 2021). In mammals, SREBP-1c, one of the three isoforms of SREBP (SREBP-1a, -1c, and -2), a member of the basic helix–loop–helix

leucine zipper family, binds SRE and regulates target gene expression. The expression of SREBP-1c is upregulated by LXR (LXRα and LXRβ), a member of the nuclear hormone receptor superfamily. Cholesterol metabolites known as oxysterols activate LXRs as natural ligands (Nakamura et al., 2004). LXR can heterodimerise with RXR (retinoid X receptor) and bind to the LXR response element (LRE) of the SREBP-1c promoter, promoting SREBP-1c expression, which in turn then upregulates genes involved in FA biosynthesis. In teleosts, Srebp-1 and Lxrα have been reported in Atlantic salmon, rainbow trout, Japanese seabass, and rabbitfish. Similar to observations in mammals, the teleost Lxrα-Srebp-1 pathway was reported to up-regulate the transcription of *fads2* and *elovl* genes and appears conserved between teleosts and mammals (Xie et al., 2021). However, unlike mammals, where two subtypes of LXR (LXRα and LXRβ) have been identified, a single Lxr subtype has been identified in fish, showing a highly conserved structure across teleost fish, amphibians, birds, and mammals. The Lxr protein regulates multiple genes of the lipid biosynthetic pathway, mediating cross-regulation between cholesterol and fatty acid biosynthesis (Carmona-Antoñanzas et al., 2014; Xie et al., 2021). In addition, Srebp also associates with other factors such as nuclear transcription factor Y (NF-Y) and Sp1 in regulating cholesterol and fatty acid biosynthesis (Goh et al., 2020).

The peroxisome proliferator-activated receptor (PPAR), with three subtypes (PPARα, PPARβ and PPARγ) in mammals, is a family of ligand-activated nuclear hormone receptors with DBD and LBD domains. Observations on sea bass (*Dicentrarchus labrax*) indicated that the expression profile of *pparα*, *pparγ*, and *pparβ* is similar to that found in mammals, with the liver and adipose tissue being the main sites of *pparα* and *pparγ* expression, respectively, and Pparβ being expressed in a number of tissues (Boukouvala et al., 2004). Similar to LXR, activated PPAR heterodimerises with RXR and typically binds to PPRE elements in mammals. PPARα is reported to up-regulate mammalian LC-PUFA biosynthesis by promoting the expression of *ElOVl5* and Δ6 *FADS2*, while PPARγ has a potential down-regulation effect (Li et al., 2019; Xie et al., 2021). In teleosts, Pparα is reported to up-regulate *fads*2 expression and enhance LC-PUFA production in rainbow trout and golden pompano, but studies indicate that Pparα has no significant influence on *fads*2 expression in Japanese seabass and large yellow croaker. Additionally, Pparα had no effect on key enzymes involved in LC-PUFA biosynthesis in rabbitfish, whereas Pparγ down-regulated expression of the Δ6Δ5 *fads2* gene and decreased LC-PUFA biosynthesis in rabbitfish. The transcription factor SP1, which is involved in maintaining the expression of housekeeping genes lacking a TATA box, is present in the Δ6 *fads2* promoter in Atlantic salmon but not in Atlantic cod, orange-spotted grouper, European seabass, or Japanese seabass. The absence of sp1 in marine fish species may account for the low promoter activities of Δ6 *fads2* of those species and could partly explain the low LC-PUFA biosynthetic capacity of marine carnivorous fish (Xie et al., 2021).

2.6.7. Dietary regulation of desaturases and other enzymes

Genes encoding lipid metabolism enzymes such as stearoyl CoA desaturase (*scd*), acetyl CoA carboxylase (*acc*), fatty acid synthase (*fas*), elongases (*elovl*), and delta-5 and -6 desaturases (Δ6Δ5 *fads*) can be suppressed by PUFA-rich diets (Clarke and Jump, 1996; Sampath and Ntambi, 2005; Qin et al., 2009; Li et al., 2020). PUFA suppress the activities of these genes by inhibiting the rate of transcription, resulting in a decline in corresponding mRNA concentration and an eventual decline in enzymatic activity (Clarke and Jump, 1996; Sampath and Ntambi, 2005). This regulatory activity by PUFAs occurs through their interaction with several transcription factors that bind cis-elements in the promoters of genes of lipid metabolism. For instance, PUFA inhibits the expression and proteolytic processing of Srebp-1c, a transcription

factor that binds the SRE response element found in the promoters of *fas*, *scd*, *elovl,* and *fads2*, thereby suppressing the induction of lipogenic genes (Nakamura et al., 2004; Xie et al., 2021). In mammals, dietary PUFA at 2–3% of diet weight is sufficient to suppress the induction of lipogenic genes by inhibiting the expression and proteolytic processing of SREBP-1c (Nakamura et al., 2004). The mature Srebp-1c is a product of the proteolytic cleavage of the precursor protein. The precursor Srebp-1c, with two membrane-spanning domains, is found inserted in the membrane of the endoplasmic reticulum (Figure 2.6). The C-terminal end of this precursor protein is bound to the Srebp cleavage-activating protein (SCAP), as illustrated in mammals (Nakamura et al., 2004; Sampath & Ntambi, 2005). A drop in the cellular sterol levels results in the movement of the Srebp-SCAP complex to the Golgi apparatus, where it undergoes proteolytic cleavage, producing the C and N terminal domains. The amino-terminal domain migrates to the nucleus and activates target genes (Figure 2.6). Although the exact mechanism by which PUFA suppresses proteolytic cleavage of SREBP-1c is largely unknown, increased intracellular sterol levels seem to result in a decline in SREBP maturation. Thus, PUFAs may function by increasing cellular cholesterol levels via increased sphingomyelinase activity, which results in increased hydrolysis of sphingomyelin in the plasma membrane and increased cholesterol levels in the endoplasmic reticulum (Sampath and Ntambi, 2005).

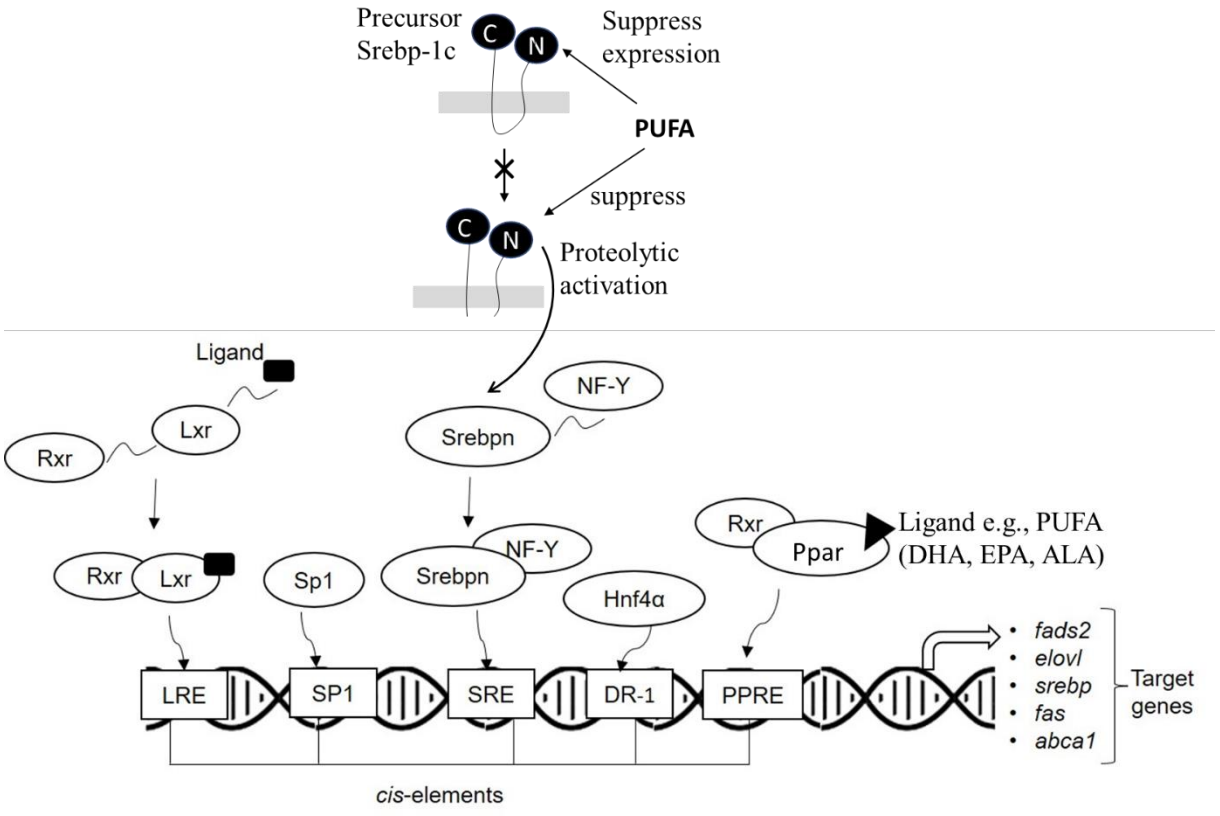

Figure 2.6. Schematic illustration indicating the role of PUFA and selected transcription factors and their binding elements in regulating the transcription of genes involved in lipid biosynthesis in fish.

Pparα and Pparβ are involved in regulating fatty acid β-oxidation while Pparγ is involved in adipocyte function and differentiation, lipid storage by adipocytes, and glucose responsiveness and is mainly expressed in adipocyte tissue, with moderate levels in the liver and lower levels in muscle (Ayisi et al., 2018, Wang et al., 2022). The binding of an agonistic ligand activates Ppars. Activated Ppar forms a heterodimer with the retinoid X receptor (Rxr), enabling it to

bind PPRE in the promoters of target genes (Figure 2.6). PUFA and MUFA are the natural ligands that activate Ppar in teleosts, and their binding affinities to Ppar differ. For example, PUFA such as EPA can bind to Pparα with higher affinity than SFA, MUFA and n-6 PUFA (Ayisi et al., 2018). Thus, dietary PUFA, such as DHA and EPA, are associated with increases in the activities and mRNA of FA oxidation enzymes in both mitochondria and peroxisomes, mediated by Pparα. Other PUFA such as α-linolenic acid and γ-linolenic acid have similar effects, whereas linoleic acid does not have a significant effect over saturated fats (Nakamura et al., 2004). High expression of Pparα mRNA is observed in the liver, that utilize FA for energy and is required for the induction of genes involved in mitochondrial and peroxisomal β-oxidation. Pparα plays a central role by inducing the genes for mitochondrial and peroxisomal FA oxidation as well as those for ketogenesis in mitochondria. Fatty acids released from adipose tissue during fasting are considered endogenous ligands with strong binding affinity for Pparα. It thus appears that Pparα together with srebp-1c, acts as a sensor of HUFA status and stimulate the induction of Δ6-desaturase when HUFA are low (Nakamura et al., 2004; Li et al., 2020).

As previously mentioned, only one Lxr subtype has been identified in fish, and shows structural similarity across fish species and other subtypes identified in amphibians, birds, and mammals. Mammalian LXRs α and β are well characterised for their ability to bind oxysterols as endogenous ligands and are reported to regulate diverse processes including bile acid synthesis and lipogenesis. Lxr heterodimerises with Rxr and binds to Lxr response elements (LREs) in the promoter regions of target genes (Figure 2.6). PUFA is thought to inhibit the prolipogenic actions of Lxr via various mechanisms. For instance, they may compete with oxysterols for binding to Lxr, thereby preventing oxysterol binding, and antagonising the induction of target genes, notably Srebp-1c, and consequently genes containing either LRE or SRE elements in their promoters. It has also been suggested that PUFA may inhibit Lxr through activation of Pparα and Pparγ. As previously noted, PUFAs act as ligand for activation of Ppars, and overexpression of Pparα and Pparγ inhibits srebp-1c promoter activity probably due to competition between Ppars and Lxr for the Rxr (Yoshikawa et al., 2003; Sampath and Ntambi, 2005).

In fish, Hnf4α is a highly conserved nuclear receptor that is homologous to HNF4 in mammals. It binds to DR1 elements as a homodimer and plays a key role in regulating the expression of genes involved in hepatocyte differentiation and hepatic functions such as cholesterol and lipoprotein secretion (Wang et al., 2018). Analysis of the hepatic transcriptome of Atlantic salmon indicated a relationship between the expression levels of hepatic lipid transport-related genes and the alterations of hnf4α expression. In rabbitfish, Δ6Δ5 fads2 and Δ4 fads2 expression levels were reported to be correlated with the expression levels of Hnf4α (Xie et al., 2021). Unlike Ppar which binds free fatty acids, hnf4α binds fatty acyl CoA esters. Binding of saturated fatty acids such as palmitoyl CoA and myristoyl-CoA results in activation of HNF4α while the binding of PUFAs like α-linolenic acid, EPA and DHA suppresses HNF4α in mammals (Sampath and Ntambi, 2005).

2.6.8. Antioxidant defence system in fish

Reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-•), hydroxyl radicals (HO•), and hydrogen peroxide (H_2O_2) are by-products of oxidative reactions in the mitochondria, endoplasmic reticulum, peroxisomes and plasma membrane. Superoxide anion radicals are mostly produced in the mitochondria as the first ROS during the chemical reduction of oxygen to water in the electron transport chain (ETC). In addition, hydrogen peroxide is also produced in the mitochondrial matrix during the reduction of oxygen to water (Filho et al., 1993; Hoseinifar et al., 2020). In order to keep the intracellular levels of ROS in a steady-state

and preserve cellular functioning, living organisms possess an antioxidant defence system, which falls into two distinct categories: (1) enzyme antioxidant system and (2) non-enzyme antioxidants. The enzyme antioxidant system provides the primary protection and includes superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT); while the non-enzymatic antioxidants include glutathione, thioredoxin, and vitamins C and E (Zhang et al., 2004; Deng et al., 2015; Hoseinifar et al., 2020). An imbalance between the production of ROS and the antioxidant defence system can have severe consequences, including DNA alterations, protein denaturation, lipid peroxidation, apoptosis, and cell damage (Figure 2.7). Lipid peroxidation results in the generation of malondialdehyde (MDA), capable of damaging cell structure and function (Bartoli et al., 2011; Cherian et al., 2019).

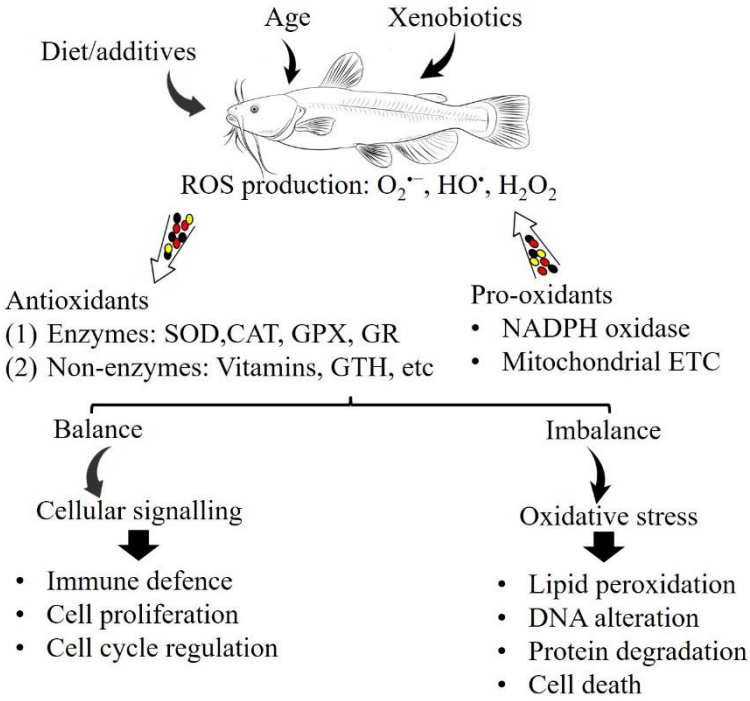

Figure 2.7. An illustration of the antioxidant defence system in fish. SOD, Superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase; GTH, glutathione; ETC, electron transport chain. Derived from Hoseinifar, et al., 2020.

The enzyme superoxide dismutase (SOD) which is located in the mitochondrial matrix and cytosol, catalyses the conversion of superoxide anion radicals to hydrogen peroxide and oxygen molecules, thereby eliminating superoxide radicals. In the absence of this reaction, the potentially reactive and most harmful hydroxyl radicals are formed, whose actions are only prevented through inhibition of their formation or repair of the resulting damage (Hoseinifar, et al., 2020; Azadmanesh et al., 2021). On the other hand, hydrogen peroxide can be eliminated by enzymes such as catalase (CAT) and glutathione peroxidase (GPx). Catalase catalyses hydrogen peroxide decomposition to water and molecular oxygen. Hydrogen peroxide is also neutralised by GPx in the presence of glutathione. The utilised glutathione is then replenished through glutathione reductase activity (Hoseinifar, et al., 2020). In aquaculture, different factors including age of fish, diet, and xenobiotics, influence the oxidative stress and antioxidant capacity in teleost fish. Thus, to improve the antioxidant defence capability in cultured fish, different approaches including the administration of synthetic antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene have been used. Due to the negative effects of synthetic antioxidants on the environment and fish, dietary approaches such as the application

of prebiotics, probiotics, and synbiotics are used to improve the antioxidant defence capability in cultured fish (Hoseinifar, et al., 2020).

2.7. Intestinal microbiota of fish

2.7.1. Composition of intestinal microbiota

The intestinal lining harbours a diverse and complex community of microbes (bacteria, viruses, archaea, and fungi) which are collectively known as microbiota. The interaction between fish and microbiota in their immediate environment starts early during development, and distinct microbiota communities occur at different ontogenetic stages (Burgos et al., 2018). This early interaction is vital as the microbiota educate the host immune system to recognise Microbe-Associated Molecular Patterns (MAMPs) and increase the tolerance to microbial immuno-determinants (Xu and Gordon, 2003). Proteobacteria (Gram-negative), Fusobacteriota (Gram-negative), Firmicutes (Gram-positive), Bacteroidota (Gram-negative), Actinobacteriota (Gram-positive), and Verrucomicrobiota (Gram-negative) make up the majority of the gut microbiota of fish (Ghanbari et al., 2015; Tarnecki et al., 2017; Talwar et al., 2018). For instance, the phyla Proteobacteria, Firmicutes, and Fusobacteriota account for over 75% of the total gut microbiota in carps (Tarnecki et al., 2017; Yukgehnaish et al., 2020). The genus *Cetobacterium* (phylum Fusobacteriota) has been identified as a common member of the microbiota of grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*), common carp (*Cyprinus carpio*), and crucian carp (*Carassius auratus*) and may be considered a core genus among carps (Tarnecki et al., 2017). In the Atlantic salmon (*Salmo salar*), Proteobacteria and Firmicutes largely dominate the gut microbiota in both freshwater and saltwater-raised fish (Tarnecki et al., 2017). Similarly, Proteobacteria, Firmicutes, and Fusobacteriota form dominant groups of bacteria in the gut of Nile tilapia (*Oreochromis niloticus*) (Standen et al., 2015; Zhang et al., 2016). In channel catfish (*Ictalurus punctatus*), the dominant phyla in the gut microbiome were Firmicutes, Proteobacteria, Fusobacteriota and Cyanobacteriota, with other bacteria such as Verrucomicrobiota, Bacteroidota and Tenericutes occurring in low abundance (Burgos et al., 2018).

2.7.2. Physiological and immune-modulation roles of intestinal microbiota

The intestinal microbiota is essential in the development of normal intestinal structure and functions, and the host and its associated microbiota can be best described as a single meta-organism or holobiont (Bates et al., 2006; Ghanbari et al., 2015; Tarnecki et al., 2017). In the vertebrates, the gut microbiota plays vital roles by boosting host immune system development, nutrient uptake, growth, and resistance against opportunistic and obligate pathogens (Burgos et al., 2018; Thejaswini et al., 2022). For instance, beneficial microorganisms such as the lactic acid bacteria (LAB) are capable of stimulating host gastrointestinal development, digestive function, immunological response and tolerance, and disease resistance (Ringø et al., 2018). Moreover, the microbiota status has been shown to strongly affect both lipid biosynthesis and lipid degradation in the liver (Costantini et al., 2017).

The role of microbiota in nutrient uptake can be seen in their ability to break down compounds such as cellulose in food into metabolites of nutritional value. Such bacteria are capable of producing digestive enzymes, including cellulase, which digests cellulose into metabolites that can be utilised as an energy source (Banerjee and Ray, 2017; Yukgehnaish et al., 2020). Intestinal bacteria capable of producing cellulase include *Citrobacter* sp., *Enterobacter* sp., *Bacillus coagulans*, *Bacillus cereus*, *Brochothrix* sp., *Brochothrix thermosphacta*, *Bacillus*

licheniformis, *Bacillus circulans* TM1, *Bacillus megaterium* CI3, and *Aeromonas* sp. These bacterial species have been isolated in *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Gadus morhua*, *Channa punctatus*, *Heteropneustes fossilis*, *Oreochromis mossambicus*, and *Ctenopharyngodon idella* (Banerjee and Ray, 2017). *Clostridia* (phylum Firmicutes), one of the major representatives in fish gut microbiota, is known for the synthesis of short-chain fatty acids (SCFAs) such as propionate and butyrate, which are supplied to the host for better growth (Yukgehnaish et al., 2020). Other members of the phylum Bacteroidota mainly produce acetate and propionate, while other bacteria such as *Pseudomonas fluorescens* and *P. putida* were established as xenobiotic degrading bacteria (Yukgehnaish et al., 2020). Some Fusobacteria, such as *Cetobacterium somerae*, are involved in the synthesis of vitamin B12 since their prevalence in the gut is known to be negatively correlated with dietary availability of vitamin B12 (Eichmiller et al., 2016).

Some bacteria that comprise the intestinal microbiota modulate fish innate immune system by interacting with host natural killer (NK) cells, neutrophils, and monocytes. In addition, the beneficial bacteria prevent pathogenic bacteria from causing harm to fish through competition for nutrients and adhesion sites and antagonise the pathogenic bacteria by secreting antimicrobial molecules, organic acids, siderophores, bacteriocins, and hydrogen peroxide (Merrifield and Rodiles, 2015; Yukgehnaish et al., 2020). Rainbow trout fed a diet containing probiotic strains of LAB such as *Lactococcus lactis* ssp. *lactis* CLFP 100, *Leuconostoc mesenteroides* CLFP 196, and *Lactobacillus sakei* CLFP 202 exhibited higher phagocytic activity of head kidney leukocytes and the alternative complement activity in serum and a higher survival rate (97.8% - 100%) when challenged by *Aeromonas salmonicida* ssp. salmonicida compared to the control (Balcázar et al., 2007). In addition, probiotic bacteria such as *V. alginolyticus* have been shown to be potent in the treatment of fish infections by *V. ordalii*, *V. anguilarrum* and *A. salmonicida*. Another important bacterium is *Pediococcus acidilactici*, a facultative anaerobe which also produces lactic acid and bacteriocins with adverse effects on a variety of pathogenic bacteria. Other strains, such as *Bacillus circulans*, have been reported to stimulate the immune response and growth performance in *Siberian sturgeon* (Yukgehnaish et al. 2020).

2.7.3. Effect of dietary fatty acids on intestinal microbiota composition

Dietary characteristics, including the quantity and quality of lipids (SFAs, MUFAs, and PUFAs), impact the composition and function of gut microbiota. A high SFA diet, for example, decreased microbial diversity and richness, Bacteroidota abundance, and increased Firmicutes abundance in mice (Costantini et al., 2019; Schoeler and Caesar, 2019; Mokkala et al., 2020). In humans, a high SFA intake has been related to an increase in Firmicutes and Proteobacteria. However, high gut microbiota diversity and low Firmicutes to Bacteroidota ratio are reported effects exerted by PUFA-rich diets (Costantini et al., 2019). Consumption of diets rich in n-3 LC-PUFA increased the numbers of Actinobacteriota, such as Bifidobacterota; LAB, such as Lactobacillus; Verrucomicrobota; Alphaproteobacteria; and Deltaproteobacteria in mice (Cani et al., 2007; Mokkala et al., 2020). Dietary fat may alter bacterial adherence to the host epithelium or change the characteristics of bacterial metabolites. For instance, the majority of bacteria utilise exogenously derived lipids, such as those coming from host diets, as components of their membranes, where the ratio of saturated to unsaturated fatty acids controls bacterial membrane fluidity (Mokkala et al., 2020).

3. MATERIALS AND METHODS

3.1. Fish Husbandry

The experiments were conducted in a recirculation aquaculture system (RAS), at the Research Centre for Aquaculture and Fisheries (HAKI), Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences. All procedures involving the handling of fish were conducted in line with the European Union Directive 2010/63/EU regarding the protection of animals for scientific purposes. The research was approved by the Ethical Committee of the Research Centre for Aquaculture and Fisheries (HAKI), Hungarian University of Agriculture and Life Sciences (license no. MATE-SZIC/2240-1/2022), established according to Hungarian State Law 9/1999 (I. 27) and operated according to the relevant Hungarian legislation concerning animal experiments, transportation of animals, and their welfare (40/2013. II. 14). Animal suffering during sampling was minimized by using an anaesthetic, Norcaicum/Tonogen (50 mL/100 L water).

3.2. Experimental animals

Two commercially important catfish—the African catfish (*Clarias gariepinus* × *Heterobranchus longifilis*) and the European catfish (*Silurus glanis)*—were studied. Fish were given time to acclimate to experimental conditions prior to the administration of the diets. During the acclimatisation period, the animals were initially fed a basal diet, followed by an incremental replacement of the basal diet with experimental diets until the basal diet was completely replaced by the experimental diets. The fish studied throughout the course of this study inevitably experienced stressful procedures, especially during handling, transport, weighing, and anaesthesia. However, care was taken to minimise the stress levels experienced by the fish, which can have detrimental effects on the animals' physiological and immune responses.

3.3. Feed ingredients, experimental diets, and feed production

Three oil sources, fish oil (FO), rapeseed oil (RO) and insect oil (IO), were used in the feed formulations. The oil (IO), which was produced by a defatting procedure using black soldier fly (*Hermetia illucens*) larvae (BSFL), was provided by Agroloop Ltd. (Budapest, Hungary). Three diets (one control: CTR, and two experimental diets: IO50 and IO100) were formulated for the two species, the main difference being the physical properties of the pellets (sinking feed for European catfish, floating feed for African catfish). For each species, the feeds (CTR, IO50 and IO100) were formulated to be isoproteic with a protein content of approximately 430 g/kg and the same fat content of approximately 110 g/kg (Table 3.1). The main difference between the diets (CTR, IO50 and IO100) was the oil source (FO, RO, and IO). So, we prepared a control diet that contained a mixture of fish oil and rapeseed oil (50:50) and two experimental diets, IO50, in which 50% of FO/RO blend was replaced with black soldier fly larvae oil (BSFLO), and IO100, in which insect oil was the only oil source (Table 3.1). Rapeseed oil has moderate levels of 18:2n-6 and 18:3n-3, with the ratio of 18:2n-6/18:3n-3 of 2:1, regarded as beneficial to fish and human health (Bell et al., 2003). The internal marker yttrium oxide was included in all the diets for the determination of the apparent digestibility coefficient (ADC) of the nutrients.

The three different feeds (CTR, IO50, and IO100) dedicated to each species feeding trial were produced at the Institute of Food Technology, University of Novi Sad (Novi Sad, Serbia). Briefly, the dry ingredients were ground in a hammer mill (ABC Inženjering, Pančevo, Serbia)

equipped with a 1 mm sieve and then mixed in a Muyang SLHSJ0.2A double-shaft pedal mixer (Muyang, Yangzhou, China). Fish oil, rapeseed oil, and insect oil, preheated at 50 °C, were added directly into the mixer according to the formulation (Table 3.1). The material was preconditioned by the direct addition of steam and water in the double-shaft mixer until the final temperature of the material reached 80 °C. The mixed preconditioned material was then processed into 3.0 mm pellets using a co-rotating twin-screw extruder (Bühler BTSK-30, 7 sections, length/diameter ratio = 28:1, Bühler, Uzwil, Switzerland) with a 3 mm die opening (die open area of 7.06 mm²). The produced pellets were subsequently dried in a vibrating dryer (FB 500x200, Amandus Kahl, Hamburg, Germany) at 80 °C until the product reached a final moisture content in the range of 100–120 g/kg.

Table 3.1. Ingredients and proximate composition (g/kg of diet) of the control (CTR) and experimental diets (IO50 and IO100).

	Diets		
Ingredient	**CTR**	**IO50**	**IO100**
Wheat[1]	250	250	250
Poultry meal (65% P)[2]	190	190	190
Fish meal[3]	110	110	110
Soybean flour (50% P)[4]	100	100	100
Soy protein concentrate[5]	100	100	100
Full fat soybean[1]	100	100	100
Wheat Gluten[6]	74	74	74
Vit-Min Premix[7]	15	15	15
Mono-Calcium Phosphate	10	10	10
Yttrium (III)-oxide[8]	1	1	1
Fish oil[9]	25	12.5	0
Rapeseed oil[10]	25	12.5	0
Insect oil (BSFLO)[11]	0	25	50
Proximate composition			
Dry matter	949.7	952.2	948.0
Crude protein	433.8	430.0	429.1
Crude lipid	108.0	107.8	109.9
Ash	80.4	78.5	83.5
Crude fiber	30.7	37.3	29.3
Gross energy (MJ/kg)	19.7	19.8	19.7

[1] Source: Nutricija DOO, Novi Sad.
[2] SONAC Poultry meal 65 Premium, Sonac Usnice SP. z.o.o., Usnice, Poland.
[3] 999 Fish meal LT, TripleNine Fish Protein A/S, Esbjerg, Denmark.
[4] SOPRO 200, Sojaprotein, Bečej, Serbia.
[5] Tradkon SPC500-F, Sojaprotein, Bečej, Serbia.
[6] Fidelinka Skrob, Subotica, Serbia.
[7] Cargill Ltd., Budapest, Hungary
[8] Sigma Aldrich, 99.99 %
[9] FF Skagen, Skagen, Denmark.
[10] Victoria oil, Šid, Serbia.
[11] Agroloop, Budapest, Hungary.

3.4. Fish feeding trials

The first trial (Trial 1) involved a total of 630 European catfish (*Silurus glanis* L) juveniles (average initial body weight of 28.1 ± 0.17 g) originating from a fish farm in Hungary. The fish were randomly distributed in a recirculation aquaculture system (RAS) equipped with nine 1 m³ fibreglass tanks (70 fish per tank) at HAKI (Figure 3.1) on 1st September 2022. A second trial (Trial 2) involving 900 juvenile African catfish hybrids (average initial weight: 29.1 ± 1.69 g), originating from the hatchery of the institute (HAKI), had a similar setup as the first trial, starting on 13th January 2023. For each trial, one control group (CTR) and two experimental groups (IO50 and IO100) were set up, randomly distributed in tank triplicates, and fed for 8 and 7 weeks, respectively. During the feeding period, fish were fed at a 3% feeding rate daily until apparent satiation using automatic feeders, and feed consumption was recorded for each tank, as well as care taken to avoid feed waste and to ensure all feed supplied was consumed. Water quality parameters such as temperature, dissolved oxygen (DO), and pH were measured regularly by using portable equipment, while ammonium (NH_4+), nitrites (NO_2-), and nitrates (NO_3-) were measured on a weekly basis in the accredited laboratory of the institute. The results of water quality measurements for the two trials are presented in Table 3.2.

Figure 3.1. Recirculating aquaculture system (RAS) used for fish feeding trials

Table 3.2. Physico-chemical parameters of water in RAS used in the two feeding trials

	Trial 1			Trial 2		
Parameter	Min.	Max.	Mean	Min.	Max.	Mean
Temperature (°C)	24.3	24.6	24.5±0.10	24.9	25.2	25.0±0.10
pH	8.25	8.79	8.55±0.14	7.80	8.73	8.34±0.24
DO (mg/L)	8.00	8.90	8.20±0.40	7.80	8.80	8.00±0.20
NO_2^- (mg/L)	<0.020	0.070	0.040±0.01	<0.020	0.030	0.003±0.00
NO_3^- (mg/L)	8.24	31.1	20.8±6.72	6.71	28.8	14.3±7.10
NH_4^+(mg/L)	<0.100	0.257	0.214±0.07	<0.100	0.200	0.130±0.03

3.5. Sample collection

Fish were fasted for 24 hours at the end of the experiment. Individual fish were weighed to determine the final body weights of the fish in each tank. In addition, 10 fish were randomly sampled from each replicate tank, and the total length was measured for each fish, followed by dissection to obtain the liver weight for the calculation of Fulton index (condition factor-CF) and hepatosomatic index (HSI), respectively. For whole-body analysis of proximate composition (dry matter, crude protein, crude fat, ash content, and gross energy) and fatty acid profiles, three (3) fish per tank (9 fish per treatment) were sampled, frozen and freeze-dried until analysis. Three additional fish per tank/replicate (9 fish per dietary group/treatment) were sampled and blood collected from the caudal vein using a heparinised syringe into heparinised microcentrifuge tubes and centrifuged at 1700 rpm (CAPP CR-1730R, Nordhausen, Germany) for 20 minutes at 4 °C to obtain plasma, which was stored at –20 °C until analysis of biochemical and immunological parameters. The same fish specimens were dissected, and the liver immediately frozen in liquid nitrogen and stored at –80 °C for analysis of antioxidative status. Similarly, the whole intestines were sampled and stored in 8% formalin solution until histological processing. Four additional fish per tank (12 fish per treatment) were dissected to obtain the spleen, head kidney, and liver, separately placed in 2.0 mL centrifuge tubes containing RNAlater, left overnight at 4°C and stored at –80°C for expression analysis of oxidative stress, immune, and lipid metabolism-related relative gene expression. The remaining liver tissue was frozen in liquid nitrogen for total lipids and fatty acid composition analysis.

To investigate the effect of diets on the gut microbiota, fish were fed the experimental diets for an additional week, followed by collecting faecal and mucus content of the intestine six hours after feeding. Prior to dissecting and obtaining intestinal content, each fish was wiped with 70% ethanol to avoid contamination from external body surface microbiota. The entire intestine was removed aseptically from each fish (three per replicate) using alcohol-disinfected instruments, and faecal material was collected by squeezing out and scraping the intestinal mucosa with a sterile spatula to collect luminal and mucosa-associated microbiota (Rimoldi et al., 2018). Samples obtained were placed in microcentrifuge tubes and immediately frozen, followed by storage at –80 ˚C until analysis (Goodrich et al., 2014; Rimoldi et al., 2018). An additional three fish per tank with full stomachs were sampled, collecting mid-intestine into liquid nitrogen for digestive enzyme activity assessment, and an additional five to eight fish/tank were sacrificed to collect faeces for digestibility studies. In this case, the whole intestines were removed, and the solid part of the faeces was collected as pooled samples per tank. The faecal samples were refrigerated, freeze-dried, and stored in an exicator until analysis. Figure 3.2 shows a schematic illustration of the two feeding trials, sample collection and the analyses performed.

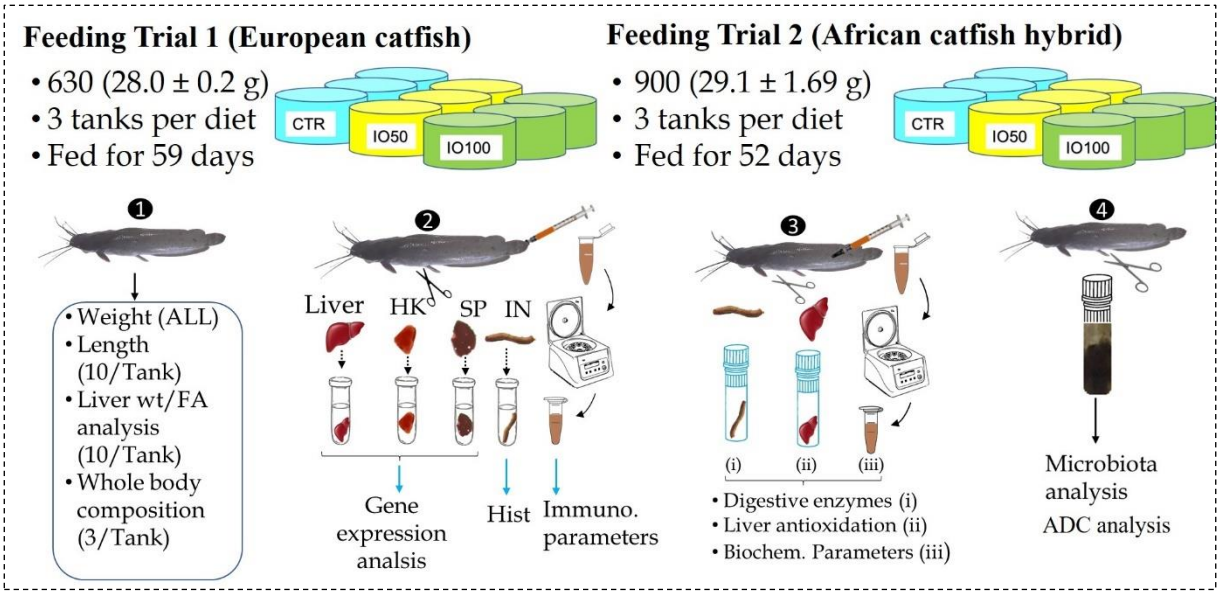

Figure 3.2. Schematic illustration of the two feeding trials, measurements taken, samples collected, and the analyses performed. HK-head kidney, SP-spleen, IN-intestine, FA-fatty acid, Hist-Histology, ADC-apparent digestibility coefficient

3.6. Biochemical analysis

The chemical composition of experimental feeds and the whole fish body were analysed for dry matter, crude protein, crude lipid, ash content, and gross energy as per standard methods of analysis (AOAC, 2000). Proximate and fatty acid profiles of whole fish body samples were analysed using finely ground, lyophilised, and homogenised dried samples, whereas the feeds were analysed on an "as is" basis. Each sample was analysed in triplicate.

3.6.1. Proximate composition analyses

Briefly, dry matter was determined by drying the samples (feed and lyophilised fish whole body samples) at 105°C for 4 h in a pre-weighed and dry dish and lid to a constant weight in an oven, according to AOAC (2000; method 950.46 for water). Ash content was determined by combusting a dry sample (2.0 g) placed in pre-weighed crucibles in a muffle furnace at 550 °C for 4 hours, then weighing it immediately after cooling to obtain its final weight (AOAC, 2000; method 942.05 for crude ash). Crude protein level was determined by the Kjeldahl method, using a digestion block (Kjeldatherm, Gerhardt, Germany) and a fully automatic distillation apparatus (Vapodest 450, Gerhardt, Germany). To do this, 0.5 g of dry samples were digested with 10 mL of concentrated H_2SO_4 and 10 mL of 30% H_2O_2. The generated ammonium sulphate was then distilled off by using 2% H_3BO_3, and measured by titration. The crude protein amount was then calculated as N × 6.25 (AOAC, 2000; method 954.01 for crude protein). Crude lipid content was determined by Soxhlet extraction using an automatic system (Soxtherm Unit SOX416, Gerhardt, Germany) with petroleum ether (40-60 ºC) as solvent, according to the AOAC (2000; 945.16 Soxhlet method). Crude fibre content was determined using an automatic analyser, the Gerhardt Fibretherm FT12 apparatus (Gerhardt GmbH & Co. KG, Germany), and the AOAC (2000; method 962.09 for crude fibre). Gross energy of the diets was measured using a bomb calorimeter (model 6400, Parr Instruments, Moline, IL, USA) in accordance with the manufacturer's instructions.

3.6.2. Analysis of fatty acid composition

To determine the fatty acid composition of the diets, homogenised and lyophilised whole fish and fresh fish liver samples, crude lipid was extracted from the samples using the non-destructive Folch method (Folch et al., 1957) and used in subsequent fatty acid analysis. Briefly, total lipids were extracted from 0.5 - 2 g of sample by homogenising in 20 volumes of ice-cold chloroform/methanol (2:1 v/v) according to Folch et al. (1957). For the insect oil, fatty acid determination was performed on an "as is" basis. Fatty acids were analysed via gas chromatography (Agilent 7890A GC System) as methyl esters of fatty acids, according to the ISO 12966-2:2017 standard's Rapid method. About 10 milligrams of fat were dissolved in 1 mL of isooctane in a 15-mL screw-top centrifuge tube. To methylate esterified components, 100 µL of 2 M potassium hydroxide (dissolved in methanol) was added and vigorously vortexed for 1 minute. After the reaction, the sample became opaque, which then cleared after 2 minutes of resting. Thereafter, 4 mL of saturated (40 g/100 mL) sodium chloride solution was added and vortexed. The two-phase solution formed was then centrifuged at 3700 × g for 10 minutes before transferring the upper phase to another vial already containing 0.5 g of Na_2SO_4 powder to remove excess moisture from samples.

Finally, an aliquot of the sample was pipetted into a GC vial for analysis. Samples were analysed using the GC-FID method, and the Agilent 7890A GC-FID system was used. For separation, a Phenomenex Zebron ZB-FAME (60 m, 0.25 mm, 0.20 µm) column with a cyanopropyl stationary phase and hydrogen gas (1.2 mL/min) mobile phase was used. Inlet temperature was 250°C, and detector temperature was 250°C. A split ratio of 50:1 and 1 µL injection volume were used. The temperature programme started from 100°C, which was maintained constant for 3 minutes. Then the column was heated at 20°C/min to reach 180°C, maintained for 10 minutes, and finally to 240°C at 10°C/min, maintained for 3 minutes. Identification was done using the Supelco 37-component FAME mixture, and results were expressed as a percentage of the area. Tables 3.3 and 3.4 show the fatty acid profiles of BSFLO and the diets (CTR, IO50 and IO100) used in European catfish and African catfish hybrid feeding trials, respectively.

Table 3.3. Fatty acid composition (w% of total FA) of BSFLO and the diets fed to European catfish

FA	BSFLO	Diets		
		CTR	IO50	IO100
8:0	0.06	-	-	-
10:0	1.22	-	0.26	0.53
11:0	0.01	-	-	-
12:0	52.38	0.31	11.75	23.67
13:0	0.02	-	-	-
14:0	12.46	2.34	4.32	6.47
14:1	0.35	-	-	-
15:0	0.24	0.20	-	-
15:1	0.12	-	-	-
16:0	15.16	13.67	14.36	15.25
16:1	0.00	2.83	2.68	2.58
17:0	0.27	-	-	0.19
17:1	0.17	-	-	-
18:0	0.00	3.50	3.74	3.91
18:1n-9t	0.16	-	-	-
18:1n-9c	0.511	31.89	24.33	17.18
18:2n-6	13.42	24.52	24.31	23.52
18:3n-3	0.02	3.69	3.00	2.19
19:0	0.04	-	-	-
20:0	0.38	0.34	0.27	0.20
20:1n-9	0.09	3.10	1.96	0.74
20:2	0.03	1.23	0.78	0.00
20:3n-6	0.02	0.00	0.00	0.77
20:4n-6 (ARA)	0.02	0.29	0.25	0.00
20:5n-3 (EPA)	0.03	2.07	1.39	0.54
22:0	0.02	0.20	0.16	0.00
22:1n-9	0.02	0.34	0.19	0.00
22:6n-3 (DHA)	0.03	2.50	1.80	0.77
23:0	0.01	-	-	-
24:0	0.09	-	-	-
24:1n-9	0.08	0.42	0.269	-
∑SFA	82.35	20.55	34.86	50.22
∑MUFA	3.96	38.57	29.43	20.49
∑PUFA	13.68	34.31	31.53	27.80

Abbreviations: t/c trans and cis isomers of FA; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; PUFA, polyunsaturated fatty acid; BSFLO, black soldier fly larvae oil. Values are presented based on duplicate analyses.

Table 3.4. Fatty acid composition (w% of total FA) of BSFLO and the diets fed to African catfish hybrid.

		Diets		
FA	**BSFLO**	**CTR**	**IO50**	**IO100**
4:0	-	0.01	-	0.01
6:0	-	0.01	0.01	0.01
8:0	0.07	0.04	0.09	0.06
10:0	1.21	0.02	0.28	0.54
11:0	0.01	-	-	0.01
12:0	51.19	0.34	11.46	22.94
13:0	0.04	0.01	0.02	0.02
14:0	12.04	2.27	4.18	6.22
14:1n-5	0.46	0.08	0.19	0.26
15:0	0.32	0.22	0.22	0.24
15:1n-5	0.12	0.06	0.04	0.01
16:0	15.12	13.59	14.15	14.85
16:1n-7	2.76	2.86	2.75	2.68
17:0	0.27	0.16	0.18	0.21
17:1n-7	0.20	0.22	0.20	0.18
18:0	2.76	3.56	3.63	3.74
18:1n-9t	0.30	0.12	0.12	0.21
18:1n-9c	0.57	34.07	26.18	17.94
18:2n-6c	11.49	26.65	25.28	23.50
18:2n-6t	0.05	0.16	0.10	0.02
18:3n-6	0.01	0.08	0.06	0.04
18:3n-3	0.09	4.38	3.51	2.55
19:0	0.04	0.08	0.07	0.06
20:0	0.45	0.35	0.27	0.19
20:1n-9	0.08	3.04	1.94	0.83
20:2n-6	0.02	0.23	0.15	0.10
20:3n-6	0.02	0.06	0.05	0.04
20:4n-6 (ARA)	0.04	0.31	0.25	0.19
20:5n-3 (EPA)	0.03	2.56	1.63	0.73
22:0	0.02	0.25	0.19	0.13
22:2n-6	-	0.02	0.02	0.01
22:1n-9	0.02	0.50	0.32	0.14
22:6n-3 (DHA)	0.01	3.44	2.26	1.07
23:0	0.01	0.03	0.03	0.02
24:0	0.13	0.10	0.12	0.07
24:1n-9	0.07	0.12	0.06	0.19
∑SFA	83.68	21.03	34.90	49.30
∑MUFA	4.57	41.07	31.78	22.45
∑PUFA	11.75	37.89	33.32	28.25

Abbreviations: t/c trans and cis isomers of FA ; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; PUFA, polyunsaturated fatty acid; BSFLO, black soldier fly larvae oil. Values are presented based on duplicate analyses.

3.6.3. Analysis of amino acid composition of the diets

Amino acids were characterised by acidic hydrolysis and chromatographic analysis. Acid hydrolysis was carried out for amino acids using a microwave digestion system (Milestone Ethos One). Briefly, 25 mg of the samples were hydrolysed by 6 N HCl containing 1% of phenol in a Milestone Ethos One microwave digestion system. After complete hydrolysis and cooling down at room temperature, the hydrolysates were filled up to a total volume of 5 mL with 1 M borate buffer (pH 8.51). This was followed by the amino acid derivatisation with the Waters AccQ Fluor reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). The derivatised samples were separated using an Acquity UPLC system equipped with a photodiode array detector, UPLC-PDA (Waters Acquity UPLC H-Class, Milford, USA), using an AccQ UPLC peptide BEH C18 (2*:*1 × 100 mm, 1.7 µm) column (Waters), and AccQ Tag Ultra Eluents A, B, and water in the gradient mode, at a flow rate of 0.7 mL/min. The chromatograms were evaluated at 260 nm, using amino acid standards. The results obtained are presented in Table S1.

3.6.4. Digestibility

Yttrium, dry matter, and crude protein content were determined in the feeds and lyophilised faeces. Yttrium content was analysed by the Inductively Coupled Plasma (ICP) method (Thermo Scientific 6500 ICP-OES, Massachusetts, USA) via digestion with mixtures of nitric acid (R.G. 65%) and hydrogen peroxide (R.G. 30%) followed by extraction using the microwave digestion under high pressure (Milestone Ethos Plus, Sorisole, Italy). The protein and dry matter content of the faeces samples were determined similarly as previously mentioned in the rest of the samples (sub section 3.6.1).

3.6.5. Plasma biochemical parameters

In order to assess the physiological state of experimental fish as a result of feeding on the formulated diets, the plasma samples were assayed for the following biochemical parameters: alanine aminotransferase (ALT), alkaline phosphatase (ALP), as well as the contents of glucose (GLU), total protein (TP), total cholesterol (CHOL), albumin (ALB), globulin (GLOB) = total protein – albumin, ratio between albumin and globulins (A/G), lipase (LIPA), amylase (AMY), creatinine (CREA), calcium (CA), total bilirubin (TBIL), and phosphorus (PHOS). Analysis was performed using a Samsung PT10V blood analyser and the Comprehensive Plus test assays (Samsung, Seoul, Republic of Korea).

3.6.6. Plasma immunological parameters

Lysozyme activity was determined based on the lysis of *Micrococcus lysodeikticus* bacterial cells (Sigma, ATCC 4698) as a substrate for the lysozyme, according to Ellis (1990) with modifications. The dilutions of hen egg white lysozyme (Sigma, USA) ranging from 0 to 20 µg/mL in phosphate buffer (0.05 M KH_2PO_4 and Na_2HPO_4, pH = 6.24) were taken as the standard. This, along with the undiluted blood plasma sample (50 µL), was placed into the wells of a 96-well plate in triplicate, followed by the addition of 250 µL of *M. lysodeikticus* suspension prepared in the same buffer (75 mg/100 mL). Optical densities were measured on a spectrophotometer (Multiskan Sky, Thermo Fisher Scientific, Vantaa, Finland) against a blank of potassium phosphate buffer after shaking for 30 seconds and after 20 minutes at 531 nm at room temperature. The lytic activity of lysozyme was calculated by comparison with the results of a standard curve obtained using pre-determined concentrations of chicken egg white

lysozyme. The activity of plasma lysozyme was then expressed as μg lysozyme/mg protein. To determine the protein concentration of plasma, a colourimetric assay based on the biuret reaction using a protein diagnostical reagent kit (Fluitest TP, Analyticon Biotechnologies AG, Lichtenfels, Germany) was used according to the manufacturer's instructions.

Plasma total immunoglobulin levels were measured according to the method described by Sharma et al. (2010) with modifications. Initially, the total protein (TP) concentration in blood plasma was determined via a colourimetric assay (Fluitest TP, Analyticon Biotechnologies AG, Lichtenfels, Germany), according to the manufacturer's instructions. Thereafter, 50 μL of plasma were mixed with 50 μL of 12% polyethylene glycol (PEG) that had been suspended in deionised water, and the mixture was incubated at room temperature for 2 h under constant mixing. Following centrifugation at 4000 ×g for 15 min, the supernatant was collected and the protein concentration determined as before. The protein amount of the supernatant is the protein without the IG, conjugated to PEG. Total immunoglobulin (mg/mL) was determined from the expression: total immunoglobulin = TP in individual sample plasma − TP in supernatant.

Myeloperoxidase (MPO) activity in blood plasma was measured as described in Kokou et al. (2012) with modifications. Briefly, 15 μL of plasma was mixed with 135 μL of Hank's balanced salt solution (HBSS) in a microtiter plate, followed by the addition of 25 μL of tetramethylbenzidine dihydrochloride (TMB) solution and 25 μL of 5 mM hydrogen peroxide solution. After 5 min reaction time, 50 μl of 4M sulphuric acid (H_2SO_4) was added to stop the reaction, followed by centrifugation at 6500 rpm for 10 min. The supernatant (150 μl) was transferred to flat-bottom 96-well plates, and absorbance was measured at 450 nm in a plate reader (Multiskan Sky, Thermo Fisher Scientific, Vantaa, Finland). Wells without plasma were used as blanks.

3.6.7. Digestive enzyme activities

Intestine extracts were prepared by homogenising the samples in 50 mM Tris buffer containing 200 mM NaCl, pH 7.6, in a sample-to-buffer ratio of 1:9 (w/v), using mortar and pestle. The homogenates were centrifuged at 13,000×g for 10 min, supernatants were aliquoted four times and then preserved at -80 °C until analysis.

Lipase activity was assayed according to the method of Winkler and Stuckman (1979), following the release of ρ- nitrophenol (ρ-NP) from ρ-nitrophenyl palmitate (ρ-NPP). Briefly, 2.0 mL of freshly prepared substrate (30 mg of p-nitrophenyl palmitate in 10 mL of isopropanol (Chem-Lab NV, Belgium) mixed with 90 ml of 50 mM Tris buffer, pH 7.5, 1mM $CaCl_2$, and 0.3% Triton x-100) was pre-warmed at 37°C and then mixed with 0.1mL of sample extract. The mixture was incubated for 15 min at 37°C for the reaction to take place, after which the reaction was stopped with 2 M sodium carbonate. The absorbance was then recorded at 410 nm against enzyme-free control. Under the conditions described the extinction coefficient of p-nitrophenol is 15,000 $M^{-1}.cm^{-1}$. Enzyme specific activities are reported as unit of activity per milligram of protein, with one enzyme unit representing 1 mmol of ρ-nitrophenol liberated in 1 minute of hydrolysis using the following equation:

$$\text{Activity (units)} = \frac{(\text{Absorbance at 410 nm-blank}) \times \text{total volume of reaction mixture}}{15{,}000 \times \text{Time (min)} \times \text{volume of enzyme} \times \text{mg protein in reaction mixture}}$$

Trypsin activity was determined using benzoyl-$_{DL}$-arginine-p-nitroanilide (BAPNA) as a substrate as described by Erlanger et al. (1961). Briefly, BAPNA (43.5mg) (Sigma) was

dissolved in 1 mL of dimethylsulfoxide (DMSO) and the resulting solution made up to 100 mL with 0.05M Tris-HCl buffer containing 0.02M $CaCl_2.2H_2O$, pH 8.2. A total of 1 ml of freshly prepared BAPNA substrate was mixed with 180 µl of water and allowed to stand for 5 min at 25°C, followed by addition of 20 µl of enzyme extract, mixed thoroughly, and the mixture left to stand for further 10 minutes for the reaction to take place, before adding 200 µl of 30% acetic acid solution to stop the reaction. The absorbance of the resulting mixture was then determined at 410 nm with spectrophotometer followed by calculation of trypsin activity (BAPNA/mg protein) using the following formula:

$$\text{Activity (units)} = \frac{(\text{Absorbance at 410 nm-blank}) \times 1000 \times \text{total volume of reaction mixture}}{8{,}800 \times \text{Time (min)} \times \text{volume of enzyme} \times \text{mg protein in reaction mixture}}$$

where 8,800/M·cm is the extinction coefficient of *p*-nitroaniline. One unit of enzymatic activity was defined as the amount of enzymatic extract required to release 1µmol of *p*-nitroaniline per minute. The reaction was run in triplicates for each sample. To determine specific activity, the protein concentrations of the extracts were determined as previously described. The specific enzyme activity was then expressed as milliunit per milligram of protein (mUnit/mg protein).

The activity of amylase was determined using starch (1% w/v) as substrate, prepared in phosphate buffer (pH 6.9) as described by Rick and Stegbauer (1974). Briefly, 50 µL of starch and 50 µL of enzyme extract were mixed and incubated at 25°C for 15 min, followed by addition of 50 µL of the stop reagent comprising a mixture of potassium-sodium tartrate and 3,5-dinitrosalicylic acid (DNSA) to stop the reaction. The mixture was incubated in a boiling water bath for 15 min. After cooling, the reaction mixture was diluted with 450 µL of distilled water and absorbance recorded at 540 nm in a microplate reader. A blank control was set by mixing 50 µL of starch and 50 µL of the stop reagent and the mixture incubated in a boiling water bath for 15 min, before adding 50 µL of the enzyme extract. A standard curve was prepared using maltose monohydrate standard and the activity determined from the maltose standard curve. The protein concentration of the samples was determined as previously described and specific activity calculated accordingly. One unit of amylase-specific activity was defined as the amount in µmole of maltose released per minute per mg protein.

3.6.8. Antioxidant enzyme activities and lipid peroxidation analysis

Liver samples stored at –80 °C were homogenised and the crude extracts assayed for superoxide dismutase (SOD) activity, malondialdehyde (MDA), total glutathione (GSH), and total antioxidant capacity (TAOC), using commercial assay kits, according to the manufacturer's instructions. The SOD activity was determined using the SOD assay kit (CS0009), which provides a simple and sensitive procedure for measuring SOD enzymatic activity in various sample types, including serum, plasma, tissue homogenates, and cell lysates. The sample SOD activity was determined by measuring the decrease in superoxide anions generated by the enzyme xanthine oxidase provided in the assay kit. The decrease in the superoxide anion was measured as a colour signal at 450 nm and was considered to be proportional to the SOD inhibition activity.

The content of MDA, which is an end product of lipid peroxidation, was determined using the MDA assay kit (MAK085). The kit determines lipid peroxidation by the reaction of MDA with thiobarbituric acid to form a colourimetric (532 nm) product proportional to the MDA present. Total glutathione was determined using Abbexa's Glutathione Assay Kit (abx096005) designed to directly measure the concentration of glutathione in the samples. In this assay, glutathione reductase reduces glutathione disulphide (GSSG) to glutathione (GSH), which then reacts with

the chromogenic reagent to produce GSSG and yellow TNB. The total glutathione content was then determined by measuring the absorbance at 412 nm.

The TAOC was determined using the TAOC assay kit (MAK187). Briefly, 100 mL of Cu^{2+} working solution was added to all standard and sample wells and mixed well using a horizontal shaker, followed by incubation for 90 minutes at room temperature, after which the absorbance was measured at 570 nm. All the absorbance measurements were taken using a multiplate reader (Multiskan Sky, Thermo Fisher Scientific, Vantaa, Finland). In addition, apart from the GSH kit (abx096005) procured from Abbexa (UK), all the other assay kits – the SOD assay kit (CS0009), the MDA assay kit (MAK085), and the TAOC assay kit (MAK187) – were procured from Merck.

3.7. Expression of genes involved in lipid metabolism, antioxidation and immune responses

3.7.1. RNA extraction and cDNA synthesis

To study the effect of dietary treatments on the expression of genes, total RNA was extracted from the spleen, head kidney, and liver samples of each catfish species using the SV total RNA Isolation system (Promega, Madison, WI, USA), according to manufacturer's instructions. The quantity of RNA was determined using a Nano-Drop spectrophotometer (NANODROP 2000, Thermo Fisher Scientific, Waltham, MA, USA). The RNA quality (integrity) was checked on 1% denaturing gel electrophoresis and its purity determined by measuring the ratio of OD at 260 nm to that at 280 nm. The cDNA was then generated from 400 ng of total RNA using LunaScript® RT SuperMix Kit (New England Biolabs, Ipswich, MA, USA), following the manufacturer's protocol. Briefly, a total of 20 µL of reaction mixture was prepared containing 4 µL of 5× Luna Script RT Super Mix, the purified RNA template, and nuclease-free water. Two control reactions (no-RT and no template controls) were included in the reactions as per the manufacturer's instructions. These were then placed in a thermocycler set at 25 ºC for 2 min for annealing, 55 ºC for 10 min for reverse transcription and 95 ºC for 1 min for heat inactivation of the reverse transcriptase, after which the resultant cDNA was stored at -20 ºC until further use in qPCR.

3.7.2. Gene Expression Analysis

The expression levels of immune, antioxidant defence system and lipid metabolism-related genes were determined by qPCR, using the synthesised cDNA template and the primers (Tables 3.5 and 3.6). The qPCR amplifications of samples were carried out in triplicate using a LightCycler 96 instrument and the FastStart Essential DNA Green Master qPCR kit (Roche, Switzerland). The qPCR reactions were performed in a final volume of 20 µL consisting of 5 µL diluted (1/20) cDNA, 1 µL (10 µM) of each primer, 3 µL nuclease-free water and 10 µL qPCR master mix (2×, containing Taq DNA polymerase, uracil-DNA glycosylase and dNTPs in an optimised PCR buffer). The qPCR conditions were: 95°C for 10 min followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. Negative controls containing no cDNA (no template) were systematically run. The specificity of the qPCR reactions was checked via a dissociation (melting) curve analysis, enabling confirmation of a single product in each reaction. After carrying out the melting curve analysis, the mean threshold cycle (Ct) values were calculated, and the comparative CT method ($2^{-\Delta\Delta CT}$ method) was used to calculate the relative expression of genes (Livak and Schmittgen, 2001). The efficiencies of qPCR reactions were determined using standard curves and the serial dilutions of cDNAs. Standard curves were drawn for each primer pair by plotting Ct values

against the log10 of different dilutions of cDNA sample solutions. Efficiencies (E) were calculated from the slopes of the standard curves using the equation: Efficiency = $(10^{(-1/slope)}-1) \times 100$.

Table 3.5. List of primers designed for quantitative real-time PCR (qPCR) analysis of the expression of genes involved in oxidative stress, immune and lipid metabolism in *S. glanis*

Gene	Forward (5'-3')	Reverse (5'-3')	Accession no.
Pparα	ATTGCCGTTTCCACAAGTGC	TTCGGACTGTGGCATCCTTC	XM_046873100.1
Pparγ	CCATCGGACGATAAAACCGC	CCGTGTAACAGTCCGTGTTG	XM_046875056.1
Srebp-1	CTGGGTCATCGCTTCTTTGTG	AGTCACCTGAGCCAGAGGAT	JX992742.1
hnf4α	TCTACCTCTCTAAGGGGGCG	CACTGGGGGCAAAGACTTGT	XM_046869234.1
g6pd	AATTCGCAGTGCCAGAGGAA	CTGCTACGGTACGTCAGGTC	XM_046869516.1
6pgd	GCTCTGATGTGGCGAGGTGG	CGTAGAAGGACAGTGCAGTGGTAAA	JX992745.1
Fas	TTGGAGCACACCCCAGTTAC	CTCAAAACGGTTGGTGGCAG	XM_046854194.1
Hadh	TTATGGAGGCTGTGCGACTG	CATGGGATAACCGGCTCCAA	XM_046837878.1
cpt1a	CGCATGTTCAACACCAGTCG	AGCAGACGTCCGTCGTAAAA	XM_046840168.1
Lpl	CGCCAAGGAAGGTGAGTTCT	GCTTGACTGGGCTTCTTTGC	XM_046858064.1
elovl2	TTGGACCCACTCTGAACAGC	CAGCTGGGCCTGTGTAAGAT	XM_046833984.1
fads2	TCGAACGATCCGTTTGGAACT	AGCCATTGATCACCCCTGTG	XM_046860054.1
sod1	AGGGGCCTGTAGAGTTCAGT	CCTTTGAGGACGCAAACTGC	XM_046861849.1
rag1	GCTTTCTGGATGGACTGCCT	TGCAGGTGCTGTCATCCAAT	AY552051.1
tlr-5	GAGCAGGTCATTGCGAAACC	CGCACTGAATGGCGTAATCG	XM_046866259.1
IL-8	AAAGCAGCTTGTTCGTCCCA	TCTGTGGTGGTTAGACAGCG	XM_046842083.1
tgf-β1	ACCTTACCAATCGGCACCTG	TGGCCTCAATTCGCTTCCTT	XM_046876053.1
irf 1	GGTTTGCCTGCATTACGACC	CTCGGGCGTCGTCCTTATAC	XM_046843029.1
β-actin	GCAGGAGTACGATGAGTCCG	CCATGCCCGTGTGGTCTTAT	XM_046837080.1

pparα, peroxisome proliferators-activated receptor alpha*; pparγ*, peroxisome proliferators-activated receptor gamma; *srebp-1*, sterol regulatory element-binding protein-1; *hnf4α*, hepatocyte nuclear factor 4α ; *g6pd*, glucose 6-phosphate dehydrogenase*; 6pgd*, 6-phosphogluconate dehydrogenase*; fas*, fatty acid synthase; *hadh*, hydroxyacyl-CoA dehydrogenase*; cpt1a*, carnitine palmitoyltransferase 1A*; lpl*, lipoprotein lipase*; elovl2*, elongase 2*; fads2*, fatty acyl desaturase 2*; sod1*, Superoxide dismutase 1; *rag-1*, recombination activating genes-1; *tlr-5*, Toll-like receptor 5; *IL-8*, interleukin 8; *tgf-β1*, Transforming Growth Factor beta 1; *irf 1*, interferon regulatory factor 1a; *β-actin*, beta actin.

Table 3.6. List of primers for quantitative real-time PCR (qPCR) analysis of the expression of genes involved in oxidative stress, immune, and lipid metabolism in African catfish hybrid

Gene	Forward (5'-3')	Reverse (5'-3')	Accession no./reference
pparα	TATTCCAAGGGTCGTTGGCG	CTGGATCACTCATTGGACTCAG	XM_053508188.1
srebp-1	CCTGGAGGTAAAATCGAAGAGTG	CGCTTGTCCCCTAGCTTCTC	XM_053514460.1
hnf4α	GTCAGGTAGCTGAGAATGCGT	CTGGCGAGGAGTCTGTGCTC	XM_053483068.1
g6pd	AGATGTGGGAAAGCGCTGAA	GACACGTACCACCAGTTCGT	XM_053482785.1
6pgd	CTTTACCACCGCACTGTCCT	CTGGCCAGGATTTGAGAGCA	XM_053482564.1
fas	TGACGGCTACACCCCATCTA	TCCCATCACACACCTCATGC	XM_053511065.1
elovl2	GCAGTACTCTGGGCATTTGTC	GGGACATTGGCGAAAAAGTA	Oboh et al. 2016
elovl5	ACTCACAGTGGAGGAGAGC	GGAATGGTGGTAAACGTGCA	Oboh et al. 2016
fads2	TCCTATATGCTGGAACTAATGTGG	AGGATGTAACCAACAGCATGG	Oboh et al. 2016
hadh	GGCTCCAGGAACAAGTCAGG	TTTGGAACCATGACCTCGCT	XM_053490732.1
cpt1a	CGCATGTTCAACACCAGTCG	AGCAGGCGTCCGTCATAAAA	XM_053493295.1
lpl	GCGAGACACAAACCAGGGTA	AGCTGCCGTGCATTTTAAGC	XM_053486752.1
sod1	GGCCTAGTGCCTGGTTTACA	CAGATCTCCAACATGCCTGA	XM_053516133.1
tgf-β1	TGGGCTCATGTGCCTACATC	GGACAGCTGTTCCACCTTGT	XM_053486364.1
tlr-5	TCAGCACCGTGACCACATTA	TTCTGTCCCTGCACAGCTTAG	XM_053514825.1
elf1a	CCTTCAACGCTCAGGTCATC	TGTGGGCAGTGTGGCAATC	Gebremichael et al., 2023

pparα, peroxisome proliferators-activated receptor alpha*; srebp-1*, sterol regulatory element-binding protein-1; *hnf4α*, hepatocyte nuclear factor 4α ; *g6pd*, glucose 6-phosphate dehydrogenase*; 6pgd*, 6-phosphogluconate dehydrogenase*; fas*, fatty acid synthase; *hadh*, hydroxyacyl-CoA dehydrogenase*; cpt1a*, carnitine palmitoyltransferase 1A*; lpl*, lipoprotein lipase*; elovl2*, elongase 2*; fads2*, fatty acyl desaturase 2*; sod1*, Superoxide dismutase 1; *tlr-5*, Toll-like receptor 5; *tgf-β1*, Transforming Growth Factor beta 1; beta 1*β-actin*, beta actin.

3.8. Intestinal histology

Intestinal samples initially fixed in 8% formaldehyde solution were washed under running tap water, followed by dehydration in graded ethanol series (70–90%), and washed in xylene and soaked in liquid paraffin wax in an automatic tissue processor (Shandon; Citadel 2000 LE11 5RG, Thermo Fisher Scientific, Waltham, Massachusetts, United States). The intestine parts were then embedded in paraffin blocks using Leica HistoCore Arcadia H (Leica Biosystems, Wetzlar, Germany) equipment. Sections (5 μm) of the intestines were cut using a microtome (Leica RM 2245, Leica Biosystems, Wetzlar, Germany), and 2 sections were placed on slides and fixed in a water bath at a temperature of 42–44 °C (Kunz Instruments HP-3, Kunz Instruments Ab, Nynashamn, Sweden). Slides were then stained using the standard haematoxylin and eosin (H&E) staining technique (Shandon Varistain 24–4, Thermo Fisher Scientific, Waltham, Massachusetts, United States) and examined under a microscope (Nikon Eclipse 600, Auroscience Consulting Ltd., Budapest, Hungary). The length of the intestinal epithelial cells in each sample was measured in μm using ImageJ software. To obtain images on the slide, photographs were captured using a camera (QImaging Micro Publisher 3.3, QImaging, Surrey, Canada) connected to the microscope.

3.9. Gut microbiota structure

3.9.1. DNA extraction, amplification and sequencing

Total genomic DNA of intestinal microbiota was extracted from the faecal samples using the QIAamp Fast DNA Stool mini kit (Qiagen) and the modified protocol by Knudsen et al. (2016). A major modification in the protocol is the addition of a bead-beating step at the beginning of DNA extraction to increase cell lysis. Prior to DNA extraction, faecal samples were gently thawed on ice. In addition, following the addition of InhibitEX buffer and vortexing, samples were treated in the MagNA Lyser instrument three times, with cooling after each treatment (2000 rpm for 25 seconds). The elution of the sample was performed twice in 50 μL buffer ATE. The extracted DNA was checked for purity (quality) and concentration (quantity) using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). In order to monitor and mitigate contamination issues, two negative controls involving the use of nuclease-free water were included and processed similarly to the samples during DNA extraction. The DNA samples were stored at −20 °C until further use. The V3 and V4 variable regions of the 16S rRNA gene were amplified using the primers (Forward: 5'-CCTACGGGNGGCWGCAG-3', Reverse: 5'-GACTACHVGGGTATCTAATCC-3') recommended by Klindworth et al. (2013). PCR amplification was carried out using the KAPA HiFi HotStart Ready Mix (KAPA Biosystems) following the 16S metagenomics sequencing library preparation guide of Illumina. Sequencing was performed using an Illumina MiSeq sequencer using a MiSeq Reagent Kit v2 (500 cycles) by 2x250 paired-end reads. Primary data analysis (base-calling) was conducted using Bbcl2fastq^ software (v2.17.1.14, Illumina). The raw 16S rRNA gene sequence files and metadata are deposited at the NCBI SRA database under the BioProject PRJNA1222409.

3.9.2. Analysis of sequence raw data

The demultiplexed next-generation sequence raw data from both African catfish and European catfish digesta were separately processed in R 4.2.1 software (R Core Team, 2018) using the Divisive Amplicon Denoising Algorithm 2 (DADA2) 1.26.0 package to generate amplicon sequence variants (ASVs) (Callahan et al., 2016). A total of 1,113,284 and 1,475,331 raw reads were generated for digesta samples of African hybrid and European catfish, respectively. In addition, 848,201 raw reads were generated from the feed samples. The median of raw reads per sample was 148,073 for African catfish hybrid, 105,342 for European catfish and 141,755 for the feed samples. The minimum reads per sample were 91,392 for African catfish hybrid, 47,999 for European catfish and 133,098 for the feeds, while the maximum number of reads was 162,780 for African catfish hybrid, 148,515 for European catfish and 146,801 for the feeds. The primer sequences and low-quality reads were trimmed and filtered out from the demultiplexed paired-end reads. A model of error rates was developed, and error sequences were removed. The forward and reverse reads from each sample were merged (with 20 bp overlap), the ASV table was constructed, and chimeric sequences were removed from the ASV table. A total number of 211 and 432 unique ASVs for African catfish hybrid and European catfish, respectively, as well as 272 for the feed samples, were generated after the sequence denoising and ASVs filtering for chimeric sequences. The resulted ASVs were annotated using the reference database, Silva_nr99_v138_train_set (Quast et al., 2013; Yilmaz et al., 2014). The ASVs identified as chloroplasts and mitochondria as well as ASVs with no phylum-level taxonomic assignment were removed from the ASV table in order to generate a final dataset. The resulting ASV tables contained 194, 197 and 152 unique ASVs for African catfish hybrid, European catfish, and feed samples, respectively. The final ASV tables, taxonomy tables, and sample metadata were then used for further analyses of microbial diversity and composition.

3.9.3. Predictive functional analysis

Tax4Fun2 was used to predict the functional profile of bacterial communities based on the 16S rRNA gene amplicon data. Analysis was performed in R following the developer's recommendation and default functions (Wemheuer et al., 2020). The default reference database and the runRefBlast function were used with the database mode set to 'Ref99NR' and the path_to_otus argument set to the representative sequences file generated. Afterwards, the makeFunctionalPrediction argument was used with the path_to_otu_table argument set to OTU table and the min_identity_to_reference argument set at 0.97, using the Tax4Fun2 package (v1.1.5) in R. The functional annotations of Tax4Fun2 predictions were obtained based on the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database. A table of KEGG orthologs (KOs) and metabolic pathways at levels 1, 2, and 3 was produced for each dataset.

3.10. Calculations and statistical analyses

3.10.1. Calculations of growth performance, nutrient utilization, and body indices of fish

Growth performance, feed efficiency, and body indices were calculated using the following formulae (Ridha and Cruz, 2001; Li et al., 2017):

Survival rate (%)	=	100× (number of survived fish/numbers of stocked fish)
Weight gain (%)	=	100 × (final weight (g) - initial weight(g))/initial weight (g)

Specific growth rate (%/day)	=	100× [ln (final body weight) - ln (initial body weight)]/number of feeding days
Feed conversion ratio (FCR)	=	total weight of dry feed given (g)/total wet weight gain (g)
Protein retention (%)	=	100× (final protein content of fish biomass – initial protein content of fish biomass) /protein intake
Protein efficiency ratio (PER)	=	(final weight (g)-initial weight (g)) / protein intake (g)
Fat Retention (%)	=	100 × (final fat content of fish biomass – initial fat content of fish biomass)/fat intake
Condition factor (g/cm^3)	=	100 × fish weight (g)/[body length (cm)]^3
Hepatosomatic index (%)	=	100× (liver weight/body weight)

The lipid quality indices such as antherogenic, thrombogenic, and polyene indices and the apparent digestibility index of fish were determined using the following formulae (Lubis and Buckle,1990; Ulbricht and Southgate,1991):

Atherogenic index	=	[C12:0 + (4 × C14:0) + C16:0)]/ (Total n-6 PUFA + Total n-3 PUFA + Total MUFA)
Thrombogenic index	=	(C14:0 + C16:0 + C18)/ [(0.5 × Total MUFA) + (0.5 × Total n-6 PUFA) + (3 × Total n-3 PUFA) + (Total n-3 PUFA/Total n-6 PUFA)]
Polyene index (PI)	=	(C20:5+C22:6)/C16:0
ADC diet	=	[1 – ([Ydiet/ Yfaeces] × [Dfaeces / Ddiet])] x 100. Where Ydiet is the dietary yttrium level, Yfaeces is the faeces yttrium level, Ddiet is the dietary nutrient level and Dfaeces is the faeces nutrient level.

3.10.2. Statistical analysis

Prior to any statistical analysis, data were first checked for normality by the Shapiro–Wilk test and histogram plots and for homoscedasticity by Levene's test and residual plots, and data were normalised when necessary. One-way ANOVA was used to assess the differences in the measured parameters between the dietary treatments (CTR, IO50, and IO100). For parameters that significantly differed between the treatments, Tukey's multiple range test was used to indicate the magnitude of differences between the means. 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 intestinal microbiota between the dietary groups were evaluated using the Kruskal–Wallis test, followed by multiple comparisons using the Wilcox pairwise comparison test. The differences in beta-diversity were evaluated by performing permutation multivariate analysis of variance (PERMANOVA) with 999 permutations, followed by a pairwise comparison. Pearson's correlations were calculated in order to relate the abundance of microbiota and the levels of liver antioxidation parameters for each fish species. In this case, pooled data from all the dietary groups for a particular fish species was used. All figure generation and statistical testing were performed in R software (v 4.2.1), and results were considered significant at 95%.

4. RESULTS

4.1 Fish production and nutrient utilisation

4.1.1 Growth, nutrient utilisation and body indices

The growth performance, nutrient utilisation, and body indices of European catfish and African catfish hybrid are presented in Tables 4.1 and 4.2, respectively. In all the experimental trials, fish grew over four-fold of the initial body weight, with mortality only observed with African catfish hybrid and not European catfish. For instance, European catfish grew from an initial average body weight of 27.98±0.20 g to a final average body weight of 199.52±1.94 g during the eight weeks of feeding, while African catfish hybrid grew from an initial average body weight of 29.13±0.86 g to a final average body weight of 159.54±3.08 g during the seven-week feeding period. There were no statistical differences in growth performance (final weight, weight gain, and specific growth rate), nutrient utilisation (feed conversion ratio, protein efficiency ratio, protein retention, and fat retention), body indices (condition factor and hepatosomatic index), and survival of fish fed the control (CTR) and experimental diets (IO50 and IO100) at the end of the feeding periods. Biweekly measurements of fish weights revealed a similar pattern of weight increases across the fish groups fed control and experimental diets as observed with European catfish (Figure 4.1).

Table 4.1. Growth performance, nutrient utilisation, and body indices of European catfish (*Silurus glanis*) fed the CTR and experimental diets (IO50 and IO100) for eight weeks. Data expressed as mean ± standard deviation (SD)

Parameters	Dietary groups			ANOVA	Polynomial contrast	
	CTR	IO50	IO100	*p*-value	Linear *p*-value	Quadratic *p*-value
IBW (g)	27.94±0.24	27.89±0.09	28.13±0.34	0.505	0.393	0.438
FBW (g)	197.58±2.76	202.18±0.54	198.82±5.88	0.364	0.699	0.185
WG (%)	607.15±8.93	624.98±3.97	606.79±12.64	0.085	0.963	0.033
SGR (%/day)	3.31±0.03	3.35±0.01	3.32±0.03	0.167	0.758	0.071
FCR	0.60±0.01	0.60±0.01	0.60±0.01	0.948	0.815	0.832
PER	3.83±0.04	3.87±0.06	3.86±0.07	0.639	0.523	0.504
CF (%g cm^{-3})	0.55±0.02	0.55±0.05	0.54±0.02	0.583	0.357	0.681
HSI (%)	1.99± 0.19	2.07± 0.24	2.10± 0.25	0.446	0.236	0.739
FR (%)	78.32 ± 6.42	78.98 ± 11.78	72.63 ± 12.24	0.729	0.530	0.655
PR (%)	52.88±1.89	53.56±0.98	53.53±1.35	0.819	0.743	0.607
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; HSI, hepatosomatic index; SR, survival rate

Table 4.2. Growth performance, nutrient utilisation, and body indices of African catfish hybrid fed the CTR and experimental diets (IO50 and IO100) for seven weeks. Data expressed as mean ± SD

Parameters	Dietary groups			ANOVA	Polynomial contrast	
	CTR	IO50	IO100	*p*-value	Linear *p*-value	Quadratic *p*-value
IBW (g)	28.81±1.26	30.31±2.27	28.27±1.78	0.574	0.786	0.344
FBW (g)	155.27±4.45	162.47±0.19	160.87±3.78	0.229	0.195	0.229
WG (%)	439.11±8.11	437.61±39.63	469.77±22.53	0.488	0.335	0.520
SGR (%/day)	3.56±0.04	3.66±0.14	3.64±0.03	0.493	0.345	0.511
FCR	0.75±0.02	0.74±0.03	0.73±0.01	0.836	0.586	0.923
PER	3.10±0.10	3.15±0.14	3.17±0.07	0.786	0.535	0.868
CF (%g cm^{-3})	0.81±0.00	0.83±0.01	0.81±0.01	0.266	0.994	0.131
HSI (%)	1.04±0.05	1.04±0.14	1.13±0.14	0.702	0.504	0.668
FR (%)	122.03±8.04	119.11±13.89	111.72±8.43	0.503	0.273	0.773
PR (%)	46.28±1.73	46.18±1.60	46.62±0.95	0.924	0.768	0.625
SR (%)	91.5±3.50	89.5±1.50	89.0±0.00	0.960	0.735	0.406

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; HSI, hepatosomatic index; SR, survival rate

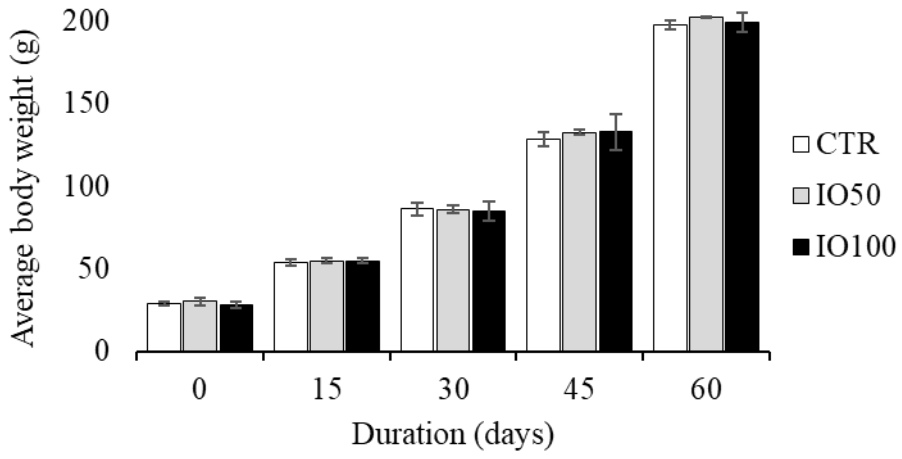

Figure 4.1. Changes in biweekly weights (mean ±SD) of European catfish (*Silurus glanis*) fed control (CTR) and experimental diets (IO50 and IO100).

The activities of three intestinal digestive enzymes – amylase, lipase, and trypsin – were not statistically different between the dietary groups in both European catfish and African catfish hybrid (Figure 4.2A). However, for the European catfish, the apparent digestibility coefficients (ADCs) of dry matter and crude protein were significantly different between the dietary groups (Figure 4.3). Similar results in the activities of the three digestive enzymes were observed for the African catfish hybrid (Figure 4.2 B). In addition, there were no significant differences in the ADCs of dry matter and crude protein between the dietary groups of African catfish hybrid (Figure 4.3).

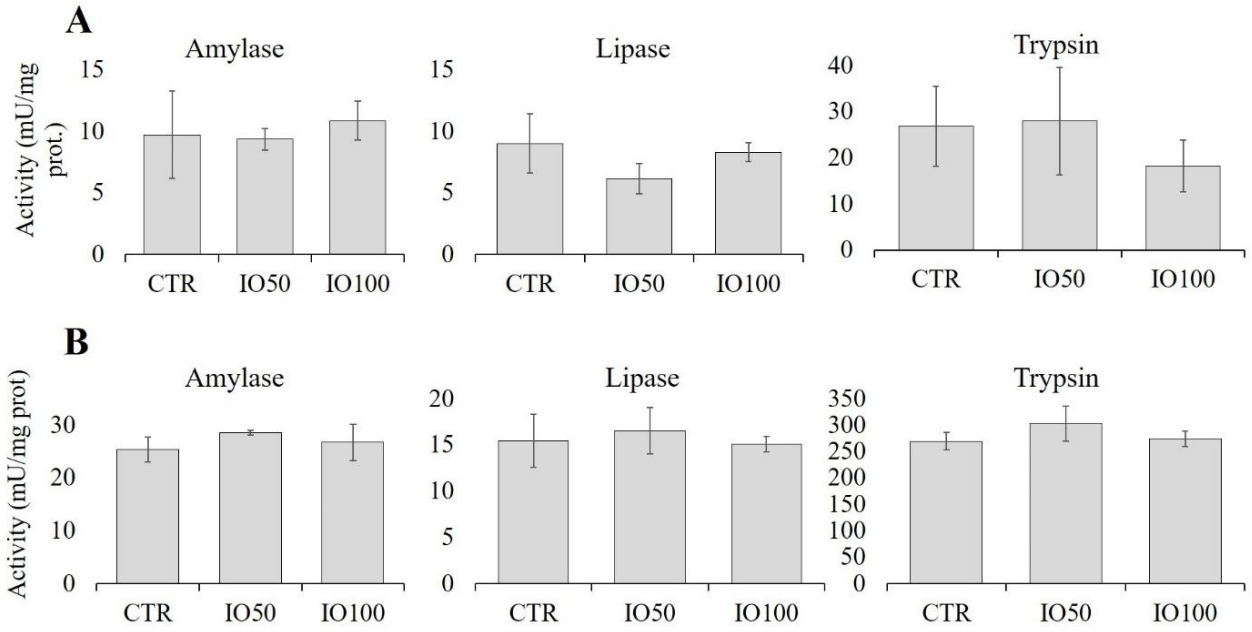

Figure 4.2. Digestive enzyme activity (mU/mg prot.) of European catfish (A) and African catfish hybrid (B) juveniles fed different oil-based diets.

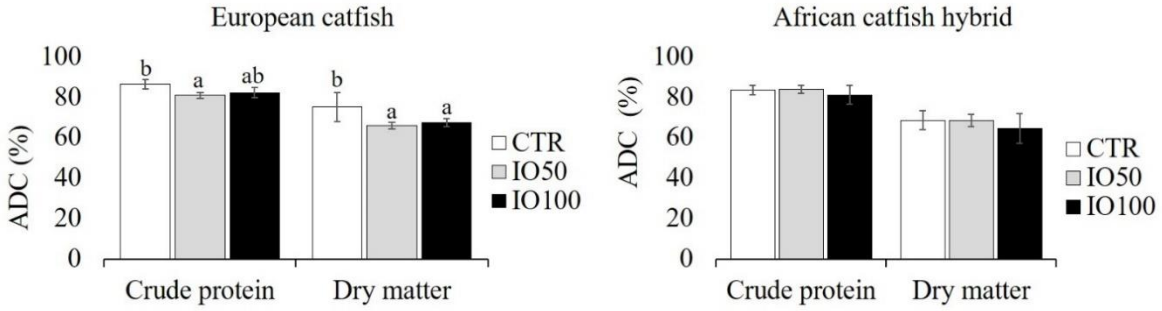

Figure 4.3. Apparent digestibility coefficients (ADCs) for protein and dry matter of the diets.

4.1.2 Whole body proximate composition and fatty acid profile

The whole-body proximate compositions of fish fed the CTR and experimental diets with different lipid sources are presented in Tables 4.3 and 4.4. There were no statistical differences in the whole-body moisture, crude protein, crude lipid, and ash between the dietary groups of European catfish (Table 4.3). For the African catfish hybrid, statistical differences were also not observed in the whole-body crude protein, crude lipid, and ash between the dietary groups, but there were significant differences for whole-body moisture content, which was lowest in the CTR group of fish (Table 4.4).

Table 4.3. Whole-body proximate composition (% wet weight) of European catfish fed experimental diets (means ± SD, n = 3).

		Dietary groups				Polynomial contrast	
	Initial	CTR	IO50	IO100	ANOVA *p* -value	Linear *p*-value	Quadratic *p*-value
Moisture	80.71	77.82±0.59	78.00±0.95	78.21±0.85	0.849	0.566	0.911
Crude protein	12.00	13.55±0.32	13.58±0.30	13.60±0.24	0.978	0.838	0.994
Crude lipid	4.02	4.86±0.27	4.96±0.67	4.67±0.65	0.824	0.698	0.649
Ash	2.08	2.11±0.11	2.18±0.02	2.21±0.10	0.394	0.210	0.718

Table 4.4. Whole-body proximate composition (% wet weight) of African catfish hybrid fed experimental diets (means ± SD, n = 3).

		Dietary groups				Polynomial contrast	
	Initial	CTR	IO50	IO100	ANOVA *p* -value	Linear *p*-value	Quadratic *p*-value
Moisture	77.01	72.43±0.61	73.47±0.33	73.29±0.16	**0.046**	**0.045**	0.083
Crude protein	13.55	14.66±0.07	14.42±0.13	14.51±0.10	0.066	0.114	0.059
Crude fat	6.51	8.92±0.30	8.90±0.70	8.57±0.58	0.703	0.467	0.718
Ash	1.87	2.34±0.15	2.46±0.17	2.59±0.26	0.368	0.174	0.975

Statistical *p*-values refer to differences between dietary groups (CTR, IO50 and IO100). Values in the same line with different superscript letters are significantly different ($p < 0.05$).

The fatty acid profile of whole body of European catfish fed insect oil-based diets (IO50 and IO100) indicated a higher total saturated fatty acid (SFA) than observed in the whole body of fish fed the control diet (Table 4.5). For African catfish hybrid, total SFA did not show significant statistical difference between the dietary groups (Table 4.6). Among SFAs, palmitic acid (16:0) was the most abundant in whole body of fish, regardless of the dietary grouping and species examined. While a linear increase in the w% of 16:0 with an increase in insect oil fraction in the diets was observed in European catfish, such observation was not recorded in African catfish hybrid (Table 4.6). Other whole body SFAs in European catfish showing a linear increase with insect oil inclusion in the diets were lauric acid (12:0), myristic acid (14:0), margaric acid (17:0), and stearic acid (18:0), while whole body arachidic acid (20:0) and behenic acid (22:0) linearly decreased with insect oil inclusion (Table 4.5). Similar trends in the w% of lauric acid (12:0), myristic acid (14:0), arachidic acid (20:0) and behenic acid (22:0) with increasing dietary insect oil fraction were recorded for African catfish hybrid (Table 4.6). In both the European catfish and African catfish hybrid, whole-body w% of MUFA, EPA, DHA, and total n-3 PUFA significantly decreased with an increase in dietary insect oil fraction. Across dietary fish groups, whole-body MUFA content was dominated by oleic acid (18:1n-9), which largely determined the trend in total MUFA content. The decrease in w% of oleic acid followed a linear pattern (Tables 4.5 and 4.6).

The whole-body w% of total PUFA as well as n-6 PUFA indicated no statistical difference between the dietary groups of European catfish (Table 4.5). However, there was a significant difference in the whole-body w% of total PUFA and n-6 PUFA between the dietary groups of African catfish hybrid, with both the w% of total PUFA and n-6 PUFA linearly increasing with an increase in dietary insect oil fraction (Table 4.6). Among the n-6 PUFAs, the w% of linoleic acid (LA, 18:2n-6) was similar across the dietary groups of European catfish, although it indicated a linear decrease with an increase in insect oil fraction in the diets. On the contrary, the w% of linoleic acid in the whole body of African catfish hybrid, significantly differed between the dietary groups, and linearly increased with an increase in insect oil fraction in the diets. The rest of the n-6 PUFAs, including γ-linolenic acid (GLNA, 18:3n-6), dihomo-γ-linolenic acid (DGLNA, 20:3n-6), and arachidonic acid (ARA, 20:4n-6), significantly differed between the dietary groups and linearly increased with an increase in the insect oil fraction of the diets. The trend in whole-body n-3 PUFA of African catfish hybrid was similar to what was observed in the whole body of European catfish, showing a significant difference between the dietary groups and linearly decreasing with an increase in insect oil fraction in the diets (Tables 4.5 and 4.6). In all the dietary groups of each species, DHA was the most predominant LC-HUFA in the whole body.

Table 4.5. Whole body fatty acid composition (% of total fatty acids) and lipid quality indices of European catfish fed different experimental diets (mean ± SD, n=3)

		Dietary groups				Polynomial contrast	
FA	Initial	CTR	IO50	IO100	ANOVA *p*-value	Linear *p*-value	Quadratic *p*-value
12:0	0.12	0.17±0.01^{a}	5.29±0.22^{b}	9.50±0.20^{c}	**<0.001**	**<0.001**	**0.017**
13:0	0.01	0.01±0.00	0.01±0.01	0.01±0.01	0.209	0.150	0.990
14:0	1.96	2.06±0.04^{a}	3.69±0.06^{b}	5.07±0.09^{c}	**<0.001**	**<0.001**	0.054
14:1n-5	0.04	0.07±0.02^{a}	0.14±0.00^{b}	0.18±0.01^{c}	**<0.001**	**<0.001**	0.077
15:0	0.24	0.27±0.01	0.26±0.01	0.25±0.01	0.197	0.088	0.708
15:1n-5	0.04	0.01±0.00	0.03±0.02	0.06±0.03	0.070	**0.030**	1.000
16:0	12.32	15.06±0.51^{a}	15.85±0.41^{ab}	16.68±0.21^{b}	**0.009**	**0.004**	0.950
16:1n-7	3.31	4.11±0.33	4.29±0.34	4.39±0.34	0.595	0.383	0.875
17:0	0.20	0.19±0.00^{a}	0.21±0.01^{b}	0.23±0.00^{b}	**0.002**	**<0.001**	0.736
17:1n-7	0.24	0.28±0.01	0.26±0.00	0.26±0.01	0.068	**0.030**	0.445
18:0	4.87	4.27±0.08^{a}	4.65±0.13^{b}	5.05±0.13^{c}	**<0.001**	**<0.001**	0.903
18:1n-9	40.14	36.56±1.08^{c}	30.77±0.36^{b}	26.82±0.91^{a}	**<0.001**	**<0.001**	0.216
18:2n-6	16.47	20.93±0.61	20.39±0.52	19.44±0.56	0.068	**0.027**	0.674
18:3n-6	0.46	0.48±0.04^{a}	0.60±0.04^{b}	0.62±0.01^{b}	**0.002**	**0.003**	0.095
18:3n-3	2.94	2.62±0.18^{c}	2.10±0.11^{b}	1.59±0.15^{a}	**<0.001**	**<0.001**	1.000
19:0	0.13	0.12±0.00	0.13±0.01	0.12±0.00	0.573	0.563	0.128
20:0	0.32	0.30±0.01^{c}	0.25±0.01^{b}	0.22±0.01^{a}	**<0.001**	**<0.001**	0.207
20:1n-9	3.00	3.39±0.07^{c}	2.43±0.04^{b}	1.82±0.06^{a}	**<0.001**	**<0.001**	0.009
20:2n-6	0.96	0.68±0.04^{a}	0.79±0.02^{b}	0.88±0.04^{b}	**0.003**	**0.001**	0.657
20:3n-6	0.98	0.85±0.09^{a}	1.20±0.06^{b}	1.41±0.08^{c}	**<0.001**	**<0.001**	0.344
20:4n-6	1.21	0.81±0.11^{a}	0.97±0.07^{ab}	1.07±0.06^{b}	**0.025**	**0.015**	0.600
20:5n-3	1.52	1.21±0.15^{c}	0.77±0.03^{b}	0.47±0.04^{a}	**<0.001**	**<0.001**	0.380
22:0	0.15	0.15±0.01^{b}	0.13±0.01^{a}	0.12±0.01^{a}	**0.001**	**<0.001**	**0.024**
22:1n-9	0.83	0.68±0.03^{c}	0.54±0.02^{b}	0.37±0.01^{a}	**<0.001**	**<0.001**	0.601
22:2n-6	0.12	0.07±0.00^{a}	0.08±0.00^{a}	0.12±0.04^{b}	**0.003**	**0.021**	0.258
22:6n-3	6.39	4.60±0.92^{c}	3.47±0.34^{b}	2.63±0.14^{a}	**<0.001**	**0.003**	0.743
23:0	0.02	0.02±0.00	0.02±0.00	0.02±0.00	0.218	0.267	0.506
24:0	0.15	0.12±0.09	0.09±0.03	0.12±0.13	0.987	0.886	0.604
24:1n-9	0.44	0.24±0.11	0.27±0.19	0.21±0.29	0.642	0.892	0.769
SFA	20.58	22.76±0.64^{a}	30.67±0.31^{b}	37.48±0.23^{c}	**<0.001**	**<0.001**	0.072
MUFA	48.19	45.51±1.43^{c}	38.90± 0.74^{b}	34.28±1.04^{a}	**<0.001**	**<0.001**	0.367
PUFA	31.22	31.73±2.03	30.43± 0.79	28.24±1.09	0.082	**0.032**	0.885
n-6 PUFA	20.37	23.90±0.89	24.08± 0.55	23.56±0.76	0.825	0.631	0.719
n-3 PUFA	10.85	7.83±1.20^{b}	6.34±0.30^{ab}	4.68±0.33^{a}	**0.009**	**0.003**	0.926
PUFA/SFA	1.52	1.40±0.14^{c}	0.98±0.02^{b}	0.75±0.03^{a}	**<0.001**	**<0.001**	0.155
AI	0.26	0.30±0.01^{a}	0.52±0.01^{b}	0.74±0.01^{c}	**<0.001**	**<0.001**	0.855
TI	0.28	0.37±0.04^{a}	0.48±0.01^{b}	0.62±0.02^{c}	**<0.001**	**<0.001**	0.498
PI	0.64	0.35±0.09^{b}	0.25±0.01^{ab}	0.19±0.01^{a}	**0.026**	**0.009**	0.760

Statistical *p*-values refer to differences between dietary groups (CTR, IO50 and IO100). Values in same line with different superscript letters are significantly different ($p < 0.05$). Fatty acids 4:0, 6:0, 8:0, 10:0, and 11:0, were detected and used in the calculation of total SFA but not included in the table. AI-atherogenic index, TI-thrombogenic index, PI-polyene index.

Table 4.6. Whole body fatty acid composition (% of total fatty acids) and lipid quality indices of African catfish hybrid fed different experimental diets (mean ± SD, n=3).

		Dietary groups				Polynomial contrast	
	Initial	CTR	IO50	IO100	ANOVA *p*-value	Linear *p*-value	Quadratic *p*-value
12:0	0.13	0.46±0.35^{a}	3.40±0.20^{b}	5.58±0.16^{c}	**<0.001**	**<0.001**	0.074
14:0	1.97	1.34±0.14^{a}	2.04±0.20^{b}	2.44±0.22^{b}	**0.001**	**<0.001**	0.320
14:1n-5	0.04	0.03±0.00^{a}	0.05±0.00^{b}	0.06±0.00^{c}	**<0.001**	**<0.001**	0.225
15:0	0.17	0.15±0.01	0.12±0.03	0.11±0.02	0.117	0.052	0.549
16:0	9.76	20.46±0.17	12.26±7.71	12.87±4.76	0.278	0.128	0.790
16:1n-7	4.81	2.57±0.10^{b}	2.74±0.08^{b}	2.30±0.07^{a}	**0.001**	**0.006**	**0.002**
17:0	0.08	0.16±0.01	0.10±0.06	0.10±0.04	0.242	0.128	0.495
18:0	3.20	8.31±0.31	4.84±3.15	5.14±1.77	0.204	0.143	0.288
18:1n-9	42.87	37.91±0.52^{c}	34.53±1.05^{b}	25.23±1.70^{a}	**<0.001**	**<0.001**	**0.013**
18:2n-6	16.22	16.50±0.09^{a}	29.60±11.10^{ab}	38.96±6.57^{b}	**0.017**	**0.006**	0.410
18:3n-6	0.39	0.29±0.02^{a}	0.40±0.03^{b}	0.51±0.03^{c}	**<0.001**	**<0.001**	0.927
18:3n-3	3.33	1.94±0.03^{c}	1.77±0.09^{b}	1.11±0.04^{a}	**<0.001**	**<0.001**	**0.013**
20:0	0.13	0.29±0.00	0.16±0.09	0.12±0.04	0.051	**0.024**	0.336
20:1n-9	4.06	2.77±0.04^{c}	2.08±0.11^{b}	1.11±0.09^{a}	**<0.001**	**<0.001**	0.062
20:2n-6	0.32	0.29±0.02^{a}	0.28±0.02^{a}	0.21±0.01^{b}	**0.001**	**<0.001**	**0.032**
20:3n-6	0.83	0.66±0.04^{a}	0.83±0.05^{b}	0.85±0.06^{b}	**0.006**	**0.004**	0.071
20:4n-6	0.93	0.53±0.03^{a}	0.69±0.09^{b}	0.71±0.03^{b}	**0.016**	**0.009**	0.124
20:3n-3	0.12	0.07±0.01	0.06±0.01	0.04±0.00	**0.011**	**0.005**	0.184
20:5n-3	2.72	1.00±0.04^{c}	0.76±0.06^{b}	0.38±0.04^{a}	**<0.001**	**<0.001**	0.074
22:0	0.06	0.18±0.01^{b}	0.08±0.06^{ab}	0.05±0.02^{a}	**0.011**	**0.005**	0.276
22:1n-9	0.31	0.19±0.02^{c}	0.14±0.03^{b}	0.06±0.01^{a}	**<0.001**	**<0.001**	0.561
22:5n-3	1.31	0.55±0.04	0.61±0.11	0.57±0.37	0.948	0.889	0.778
22:6n-3	5.62	2.98±0.09^{c}	2.23±0.26^{b}	1.33±0.12^{a}	**<0.001**	**<0.001**	0.563
24:0	0.06	0.07±0.00^{b}	0.05±0.02^{ab}	0.04±0.01^{a}	**0.042**	**0.017**	0.478
24:1n-9	0.53	0.30±0.01^{c}	0.20±0.06^{b}	0.11±0.01^{a}	**0.002**	**<0.001**	0.837
SFA	15.57	31.43±0.31	23.04±11.42	26.45±6.90	0.343	0.214	0.453
MUFA	52.63	43.77±0.54^{c}	39.74±1.00^{b}	28.88±1.85^{a}	**<0.001**	**<0.001**	**0.008**
PUFA	31.80	24.80±0.23	37.22±11.28	44.67±6.84	**0.049**	**0.019**	0.661
n-6 PUFA	18.69	18.27±0.17^{a}	31.80±11.26^{b}	41.23±6.65^{c}	**0.017**	**0.006**	0.410
n-3 PUFA	13.11	6.53±0.14^{c}	5.42±0.19^{b}	3.44±0.26^{a}	**<0.001**	**<0.001**	**0.021**
PUFA/SFA	2.04	0.79±0.00	1.99±1.15	1.84±0.86	0.198	0.118	0.362
AI	0.21	0.38±0.02	0.33±0.18	0.39±0.11	0.782	0.946	0.502
TI	0.20	0.59±0.00	0.39±0.27	0.46±0.18	0.343	0.214	0.453
PI	0.85	0.19±0.01	0.30±0.13	0.15±0.06	0.167	0.266	0.115

Statistical *p*-values refer to differences between dietary groups (CTR, IO50 and IO100). Values in the same line with different superscript letters are significantly different ($p < 0.05$). The fatty acids 4:0, 6:0, 8:0, 10:0, and 11:0, were detected and used in the calculation of total SFA but not included in the table. AI-atherogenic index, TI-thrombogenic index, PI-polyene index.

4.1.3 Liver fatty acid profiles

In both the European catfish and African catfish hybrid, dietary inclusion of insect oil in the diets did not affect the total lipid content of the liver (Table 4.7 and 4.8). The contents of total SFA, MUFA, and PUFA were similar across the dietary groups of these species. However, the FA profiles of the liver were influenced by the diets. Among the SFAs, significant differences between the dietary groups were only observed in the w% of MCFAs 12:0, 14:0, and 15:0, in the livers of European catfish (Table 4.7). In the African catfish hybrid, only the w% of SFAs 12:0, 14:0 and 22:0 significantly differed between the dietary groups (Table 4.8). In all cases, higher values were recorded in fish fed the insect oil-based diets than in the group fed the CTR diet, and exhibited a linear increase with increasing dietary insect oil fraction. In European catfish, the w% of lauric acid (12:0) in the liver of the CTR group (mean value, 0.34% of total FA) was higher than that in the corresponding diet (0.31% of total FA), while the w% of lauric acid in the livers of fish fed insect oil-based diets (IO50, 0.44%, and IO100, 0.97%) were much lower than that of the corresponding diets (IO50 diet, 11.75%, and IO100 diet, 23.67%). For myristic acid (14:0), the w% in the liver was lower than that of the corresponding diets for all dietary fish groups of European catfish. Similar results were observed in African catfish hybrid – the w% of lauric acid (12:0) and myristic acid (C14:0) in the liver (Table 4.8) were lower than that of the corresponding diets (Table 3.4). However, the relative contents (w% total FA) of both 16:0 and 18:0 were higher in the liver (Tables 4.7 and 4.8) than in the corresponding diets (Table 3.3 and 3.4, respectively).

Among the MUFAs, significant differences between the dietary groups were recorded for 16:1n-7 (palmitoleic acid) and 20:1n-9 (gondoic acid) in European catfish and 14:1n-5 (myristoleic acid), 15:1n-5, 18:1n-9t (elaidic acid), 20:1n-9 (gondoic acid) and 22:1n-9 (erucic acid) in African catfish hybrid. A linear increase with increasing insect oil fraction was observed in the w% of 16:1n-7 in the European catfish and 14:1n-5, 15:1n-5 and 18:1n-9t in the African catfish hybrid. The w% of 20:1n-9 in both European and African catfish hybrid, and 22:1n-9 in African catfish hybrid, linearly decreased with increasing insect oil inclusion (Tables 4.7 and 4.8). A similar trend was also observed in the w% of oleic acid (18:1n-9c), which was also numerically lower in the livers of fish fed insect oil-based diets. However, statistical differences in w% of oleic acid between the dietary groups in each species were not observed. Among the PUFAs, the w% of 18:3n-6 (γ-linolenic acid, GLNA), 20:3n-6 (dihomo-γ-linolenic acid, DGLNA), and 20:4n-6 (arachidonic acid, ARA) were significantly higher in the livers of fish fed insect oil-based diets than in fish fed the control diet (Table 4.7 and 4.8). In addition, the content of these fatty acids (GLNA, DGLNA and ARA) also linearly increased in the direction of increasing insect oil inclusion in the diets. With the exception of EPA (20:5n-3), which significantly differed between the dietary groups of African catfish hybrid but not in the case of European catfish, the w% of the rest of the n-3 PUFAs in the livers of both European catfish and African catfish hybrid were similar between the dietary groups. However, in both species, there was a general tendency for the liver DHA content to increase with an increase in insect oil fraction in the diets (Table 4.7 and 4.8). Across the dietary groups, the PUFA content in the livers of European catfish and African catfish hybrid were represented by four predominant fatty acids: LA>DHA>ARA>DGLNA in European catfish and LA>DHA>DGLNA>ARA in African catfish hybrid. In all fish groups, DHA was the most predominant LC-HUFA in the liver.

Table 4.7. Liver fatty acid composition (w% of total FA), total lipid content (on wet weight basis, g/100g) and lipid quality of European catfish fed experimental diets (mean ±SD).

Fatty acid	Dietary groups			ANOVA	Polynomial contrast	
					Linear	Quadratic
	CTR (n=6)	IO50 (n=8)	IO100 (n=6)	*p*-value	*p*-value	*p*-value
Total lipid	1.36±0.49	1.56±0.371	1.19±0.41	0.205	0.417	0.113
12:0	0.34±0.24[a]	0.44±0.12[ab]	0.97±0.62[b]	**0.004**	**0.009**	0.207
14:0	1.20±0.32[a]	1.54±0.18[ab]	1.78±0.41[b]	**0.016**	**0.005**	0.756
14:1n-5	0.12±0.09	0.16±0.07	0.10±0.08	0.389	0.668	0.227
15:0	0.30±0.25[a]	1.00±0.50[ab]	1.13±0.78[b]	**0.035**	**0.018**	0.273
15:1n-5	0.10±0.18	0.21±0.17	0.23±0.08	0.265	0.137	0.520
16:0	18.07±3.09	18.00±1.70	17.24±0.56	0.728	0.485	0.717
16:1n-7	4.76±0.90[a]	5.92±1.42[ab]	7.26±1.85[b]	**0.027**	**0.008**	0.897
17:0	0.24±0.11	0.31±0.07	0.29±0.10	0.397	0.384	0.294
17:1n-7	0.26±0.06	0.21±0.15	0.31±0.10	0.288	0.497	0.147
18:0	10.76±2.57	9.75±2.73	10.91±1.60	0.618	0.909	0.338
18:1n-9	32.27±2.80	30.10±2.79	28.13±4.11	0.116	**0.041**	0.944
18:2n-6	12.09±5.53	9.52±3.05	7.63±1.91	0.103	0.054	0.847
18:3n-6	0.11±0.13[a]	0.30±0.17[ab]	0.44±0.08[b]	**0.003**	**<0.001**	0.673
18:3n-3	0.48±0.16	0.38±0.19	0.26±0.17	0.124	**0.042**	0.905
19:0	0.13±0.17	0.28±0.16	0.29±0.09	0.138	0.097	0.282
20:0	0.12±0.14	0.05±0.14	0.00±0.00	0.122	0.085	0.829
20:1n-9	3.06±0.78[a]	3.13±0.48[a]	2.18±0.48[b]	**0.017**	**0.019**	0.072
20:2n-6	0.86±0.35	1.07±0.30	0.88±0.22	0.361	0.901	0.156
20:3n-6	2.05±0.68[a]	2.97±0.78[ab]	3.00±0.40[b]	**0.032**	**0.023**	0.155
20:4n-6	2.23±0.74[a]	3.72±1.22[b]	4.75±0.54[b]	**<0.001**	**<0.001**	0.594
20:5n-3	0.58±0.29	0.53±0.14	0.40±0.01	0.369	0.107	0.658
22:0	0.46±0.23	0.46±0.26	0.83±0.70	0.439	0.166	0.361
22:1n-9	0.23±0.12	0.24±0.09	0.17±0.05	0.356	0.257	0.331
22:2n-6	0.71±1.14	0.26±0.14	0.32±0.11	0.795	0.292	0.384
22:6n-3	5.08±1.81	5.68±2.01	5.96±1.79	0.712	0.428	0.853
23:0	0.08±0.09	0.16±0.19	0.10±0.16	0.593	0.790	0.332
24:0	1.36±1.11	0.95±1.00	1.40±1.22	0.798	0.947	0.403
24:1n-9	0.61±0.40	1.12±0.69	1.36±1.16	0.280	0.124	0.711
SFA	33.52±6.98	33.70±3.47	35.32±2.19	0.749	0.503	0.734
MUFA	41.72±1.94	41.39±2.97	40.04±5.03	0.678	0.416	0.752
PUFA	24.76±6.17	24.91±2.35	24.64±3.69	0.993	0.961	0.915
PUFA n-6	18.62±5.09	18.31±1.46	18.01±2.74	0.949	0.749	0.999
PUFA n-3	6.14±2.12	6.59±2.28	6.63±1.89	0.902	0.693	0.833
PUFA/SFA	0.80±0.35	0.75±0.13	0.70±0.10	0.717	0.421	0.991
AI	0.36±0.11	0.37±0.03	0.39±0.04	0.675	0.386	0.912
TI	0.64±0.22	0.60±0.15	0.61±0.09	0.912	0.785	0.747
PI	0.33±0.13	0.35±0.13	0.37±0.11	0.838	0.560	0.942

Statistical *p*-values refer to differences between dietary groups. Values in the same line with different superscript letters are significantly different ($p < 0.05$). The fatty acids 4:0, 6:0, 8:0, 10:0, 11:0 and 13:0 detected and used in the calculation of total SFA. AI-atherogenic index, TI-thrombogenic index, PI-polyene index.

Table 4.8. Liver fatty acid composition (w% of total FA), total lipid content (on wet weight basis) and lipid quality indices of African catfish hybrid fed experimental diets (mean ±SD).

	Dietary groups				Polynomial contrast	
FA	CTR (n=3)	IO50 (n=3)	IO100 (n=3)	ANOVA *p*-value	Linear *p*-value	Quadratic *p*-value
Total lipid (g/100g)	8.18 ±0.66	9.11±2.59	8.00 ±0.19	0.967	0.909	0.823
12:0	0.09±0.03[a]	0.63±0.21[b]	1.42±0.28[c]	**<0.001**	**<0.001**	0.428
14:0	1.15±0.06[a]	1.61±0.08[b]	2.18±0.03[c]	**<0.001**	**<0.001**	0.198
14:1n-5	0.03±0.01[a]	0.04±0.00[ab]	0.06±0.00[b]	**0.005**	**0.002**	0.839
15:0	0.08±0.01	0.10±0.03	0.11±0.01	0.291	0.146	0.628
15:1n-5	0.01±0.00[a]	0.02±0.01[a]	0.03±0.01[b]	**0.005**	**0.002**	0.205
16:0	24.55±1.79	23.93±1.91	23.41±0.47	0.682	0.402	0.964
16:1n-7	3.33±0.24	3.34±0.23	3.26±0.03	0.863	0.678	0.749
17:0	0.14±0.01	0.15±0.01	0.17±0.01	0.056	0.022	0.723
17:1n-7	0.20±0.02	0.22±0.02	0.21±0.03	0.464	0.702	0.254
18:0	11.60±0.76	11.69±0.35	12.16±0.43	0.456	0.260	0.636
18:1n-9t	0.23±0.02[a]	0.27±0.01[b]	0.29±0.01[b]	**0.007**	**0.003**	0.394
18:1n-9c	42.42±2.16	41.50±0.90	39.21±0.22	0.066	**0.028**	0.502
18:2n-6t	0.24±0.04	0.26±0.02	0.40±0.22	0.477	0.245	0.888
18:2n-6c	6.01±0.40	6.87±1.21	6.38±0.60	0.482	0.571	0.299
18:3n-6	0.26±0.04[a]	0.34±0.02[b]	0.42±0.01[c]	**0.002**	**<0.001**	0.968
18:3n-3	0.46±0.01	0.41±0.22	0.31±0.05	0.423	0.215	0.799
19:0	0.08±0.02	0.07±0.01	0.07±0.02	0.761	0.677	0.560
20:0	0.20±0.02	0.25±0.11	0.19±0.01	0.967	0.886	0.615
20:1n-9	2.56±0.04[b]	2.32±0.16[ab]	2.03±0.11[a]	**0.004**	**0.001**	0.778
20:2n-6	0.56±0.05	0.63±0.10	0.56±0.03	0.422	0.981	0.207
20:3n-6	1.49±0.16[a]	1.90±0.15[b]	2.31±0.02[c]	**<0.001**	**<0.001**	0.996
20:4n-6	1.17±0.14[a]	1.56±0.18[b]	2.04±0.09[c]	**<0.001**	**<0.001**	0.675
20:5n-3	0.53±0.01[b]	0.46±0.07[ab]	0.29±0.01[a]	**0.001**	**<0.001**	0.201
22:0	0.06±0.01[b]	0.07±0.01[b]	0.10±0.00[a]	**0.001**	**<0.001**	0.076
22:1n-9	0.19±0.01[b]	0.17±0.04[b]	0.10±0.01[a]	**0.007**	**0.003**	0.234
22:2n-6	0.01±0.02	0.02±0.01	0.03±0.01	0.236	0.103	0.952
22:6n-3	1.65±1.30	0.82±1.18	1.82±0.05	0.377	0.476	0.236
23:0	0.03±0.03	0.05±0.02	0.03±0.00	0.650	0.957	0.374
24:0	0.06±0.01	0.08±0.01	0.08±0.01	0.053	0.022	0.465
24:1n-9	0.14±0.08	0.20±0.05	0.24±0.02	0.165	0.069	0.844
SFA	38.51±2.37	38.65±1.98	40.01±0.58	0.567	0.350	0.647
MUFA	49.11±2.48	48.08±1.10	45.44±0.09	0.067	**0.029**	0.493
PUFA	12.38±1.71	13.27±1.99	14.55±0.67	0.304	0.140	0.865
n-3 PUFA	2.64±1.32	1.68±1.19	2.42±0.02	0.526	0.805	0.287
n-6 PUFA	9.74±0.77	11.58±1.64	12.13±0.66	0.087	**0.040**	0.439
PUFA/SFA	0.32±0.06	0.35±0.07	0.36±0.02	0.645	0.365	1.000
AI	0.48±0.05	0.51±0.04	0.56±0.01	0.085	**0.031**	0.581
TI	1.00±0.11	1.07±0.14	1.04±0.03	0.701	0.602	0.521
PI	0.09±0.05	0.05±0.05	0.09±0.00	0.504	0.947	0.261

Statistical *p*-values refer to differences between dietary groups (CTR, IO50 and IO100). Values

in the same line with different superscript letters are significantly different ($p < 0.05$). The fatty acids 4:0, 6:0, 8:0, 10:0, and 11:0, were detected and used in the calculation of total SFA but not included in the table. AI-atherogenic index, TI-thrombogenic index, PI-polyene index.

4.1.4 Nutritional quality indices

The nutritional quality of the lipids in fish fed the experimental diets was assessed by calculating the Atherogenic Index (AI), Thrombogenic Index (TI), and Polyene Index (PI), an indicator of PUFA damage. In the whole body of European catfish, significant results between the dietary groups were obtained for AI (range: 0.296 to 0.752), TI (range: 0.341 to 0.637) and PI (range: 0.176 to 0.407), with both AI and TI linearly increasing with increasing insect oil fraction in the diets, while PI linearly decreased with an increase in insect oil fraction in the diets (Table 4.5). In the African catfish hybrid, the AI, TI and PI of the whole body were similar between the dietary groups (Table 4.6), ranging from 0.21 to 0.53, 0.20 to 0.70, and 0.11 to 0.85, respectively. In the liver, the AI, TI and PI were similar between the dietary groups of European catfish (Table 4.7) as well as between the dietary groups of African catfish hybrid (Table 4.8). However, in African catfish hybrid, AI of the liver increased linearly with an increase in the insect oil fraction of the diet (Table 4.8). In the liver of European catfish, AI, TI and PI ranged from 0.235 to 0.526, 0.427 to 1.017, and 0.132 to 0.558, respectively, while in the liver of African catfish hybrid, AI, TI and PI were in the ranges of 0.44 to 0.57, 0.87 to 1.23, and 0.02 to 0.12, respectively.

4.2 Plasma biochemistry

The contents of plasma cholesterol (CHOL), creatinine (CREAT), phosphorus (PHOS), total bilirubin (TBIL), total protein (TP), calcium (CA), and immunoglobulins (IG), were not affected by the inclusion of insect oil as a source of dietary lipids for both the European catfish (Table 4.9) and African catfish hybrid (Table 4.10). In addition, the activities of alanine transaminase (ALT), amylase (AMY), lysozyme (LZY), and myeloperoxidase (MPO), were not significantly affected by the insect oil inclusion in the diets. However, a linear increase in MPO activity with increase in dietary insect oil inclusion was observed in African catfish hybrid. There was a tendency for IG content to increase with insect oil inclusion in the diets in both European catfish and African catfish hybrid (Tables 4.9 and 4.10). The content of albumin (ALB), globulin (GLOB), as well as in the activities of alkaline phosphatase (ALP) and lipase (LIPA) were only significant between the dietary groups of European catfish while for African catfish hybrid, only glucose (GLU) differed significantly between the groups. For instance, compared to the control, plasma GLOB content was significantly lower in European catfish fed IO100 diet and highest in fish fed IO50 diet, with no significant variation between fish fed the CTR and IO50 diets. The ALB content as well as the albumin to globulin ratio (A/G) were significantly higher in European catfish fed IO100 diet than in the groups fed the CTR and IO50 diets. Both ALB and A/G linearly increased with insect oil fraction of the diet. The activity of ALP was significantly higher in the IO100 group than observed in the CTR and IO50 groups of European catfish while the activity of plasma lipase (LIPA) was significantly higher in fish fed IO50 diet and lower in fish fed IO100 diet (Table 4.9).

Principal component analysis demonstrated two obvious clusters based on the two species. Principal components 1 (PC1) and 2 (PC2) based on plasma TP, ALB, GLOB, TBIL, CREA, GLU, CHOL, IG, CA and PHOS (Figure 4.4) accounted for 53.26% of the cumulative variation in the dataset, mainly driven by ALB, PHOS, TP, GLOB, TBIL, CHOL, and GLU. The multivariate analysis of interdependence of the plasma substrate profile demonstrated that the

biomarker profile in the two species is significantly different (ANOSIM: R = 0.4072; p = 0.001). In addition, no significant differences were observed in biomarker distribution between dietary groups for African catfish hybrid (R = 0.006783; p = 0.393) but significant for European catfish (R=0.1315, p= 0.031), and nonsignificant with both species combined (R= 0.02243, p= 0.184). Results of principal component analysis and non-metric multidimensional scaling based on plasma enzyme biomarkers (AMY, ALP and ALT) are presented in Figure 4.5. Principal components 1 (PC1) and 2 (PC2) accounted for 89.4% of cumulative variation in the dataset. The multivariate analysis of interdependence of the plasma enzymes profile shows strongly significant differences between the two species (ANOSIM: R = 0.9998, p = 0.001). The profile of plasma enzymes was similar between the dietary groups of African catfish hybrid (ANOSIM: R = 0.058; p = 0.891) but different between the dietary groups of European catfish (ANOSIM: R = 0.169, p = 0.01).

Table 4.9. Plasma biochemical and immunological parameters (mean ± SD, n=9) of European catfish (*S. glanis*) fed the CTR and experimental diets (IO50 and IO100) for eight weeks.

	Dietary groups				Polynomial contrasts	
Parameter	CTR	IO50	IO100	ANOVA *p*-Value	Linear *p*-Value	Quadratic *p*-Value
ALT	15.56±6.06	13.22±3.23	15.00±4.50	0.706	0.965	0.409
ALP	137.44±10.22[a]	130.67±7.94[a]	147.44±6.54[b]	**0.001**	**0.018**	**0.002**
CHOL	121.67±14.29	116.00±11.79	123.89±19.55	0.548	0.764	0.296
LIPA	82.22±6.72[a]	93.00±9.23[b]	80.89±8.46[a]	**0.008**	0.733	**0.002**
AMY	15.89±7.39	16.56±5.96	15.00±5.15	0.800	0.960	0.509
GLU	150.00±31.55	144.00±29.11	147.89±20.15	0.895	0.871	0.662
CREA	0.19±0.06	0.18±0.08	0.18±0.08	0.649	0.723	0.395
CA	11.12±0.41	11.23±0.26	11.51±0.68	0.236	0.102	0.678
TBIL	0.12±0.03	0.12±0.06	0.13±0.02	0.284	0.411	0.175
PHOS	9.08±0.54	9.16±0.46	9.30±0.62	0.682	0.393	0.882
TP	2.52±0.19	2.64±0.34	2.43±0.34	0.335	0.532	0.182
ALB	0.23±0.10[a]	0.27±0.11[a]	0.42±0.12[b]	**0.003**	**0.001**	0.190
GLOB	2.29±0.18[b]	2.38±0.26[b]	2.01±0.24[a]	**0.007**	**0.017**	**0.023**
IG	1.11±0.23	1.19±0.18	1.20±0.12	0.524	0.340	0.547
LZY	0.38±0.11	0.37±0.10	0.36±0.07	0.826	0.706	0.629
MPO	0.39±0.19	0.39±0.18	0.38±0.17	0.978	0.846	0.934
A/G	0.10±0.05[a]	0.11±0.04[a]	0.21±0.05[b]	**<0.001**	**<0.001**	**0.021**

Alanine transaminase, ALT (U/L); alkaline phosphatase, ALP (U/L); cholesterol, CHOL (mg/dL); lipase, LIPA (U/L); amylase, AMY(U/L); glucose, GLU (mg/dL); creatinine, CREA (mg/dL); calcium, CA (mg/dL); total bilirubin, TBIL (mg/dL); phosphorus, PHOS (mg/dL); total protein, TP (g/dL); albumin, ALB (g/dL); globulin, GLOB (g/dL); immunoglobulin, IG (g/dL); lysozyme, LZY (µg/mg prot); Myeloperoxidase, MPO (OD_{450nm}); albumin to globulin ratio, A/G ratio.

Table 4.10. Plasma biochemical and immunological parameters (mean ± SD, n=9) of African catfish hybrid fed the CTR and experimental diets (IO50 and IO100) for seven weeks.

	Dietary groups				Polynomial contrasts	
Parameter	CTR	IO50	IO100	ANOVA *p*-Value	Linear *p*-Value	*p*-Value Quadratic
ALT	27.00±4.94	21.50±3.33	24.83±6.61	0.209	0.477	0.106
ALP	39.00±6.69	43.33±5.57	36.33±4.27	0.126	0.423	0.061
CHOL	129.50±14.07	125.00±11.17	116.50±15.03	0.270	0.117	0.771
LIPA	<20	≤20	<20	-	-	-
AMY	23.67±7.44	21.17±7.81	23.17±9.39	0.859	0.918	0.594
GLU	125.33±26.21^{ab}	147.67±26.63^{b}	103.50±17.26^{a}	**0.019**	0.132	**0.014**
CREA	0.27±0.10	0.33±0.19	0.33±0.14	0.667	0.442	0.655
CA	10.97±0.26	11.03±0.29	11.30±0.32	0.144	0.066	0.502
TBIL	0.21±0.05	0.22±0.07	0.21±0.03	0.931	0.873	0.736
PHOS	8.10±0.36	8.17±0.54	8.45±0.61	0.473	0.256	0.679
TP	2.45±0.16	2.33±0.23	2.36±0.12	0.524	0.433	0.416
ALB	<1.0	<1.0	<1.0	-	-	-
GLOB	2.32±0.19	2.30±0.26	2.28±0.11	0.959	0.776	1.000
IG	1.58±0.31	1.70±0.36	1.80±0.24	0.486	0.324	0.501
MPO	0.87±0.30	1.01±0.15	1.11±0.18	0.110	**0.039**	0.845

Alanine transaminase, ALT (U/L); alkaline phosphatase, ALP (U/L); cholesterol, CHOL (mg/dL); lipase, LIPA (U/L); amylase, AMY(U/L); glucose, GLU (mg/dL); creatinine, CREA (mg/dL); calcium, CA (mg/dL); total bilirubin, TBIL (mg/dL); phosphorus, PHOS (mg/dL); total protein, TP (g/dL); albumin, ALB (g/dL); globulin, GLOB (g/dL); immunoglobulin, IG (g/dL); Myeloperoxidase, MPO (OD450nm).

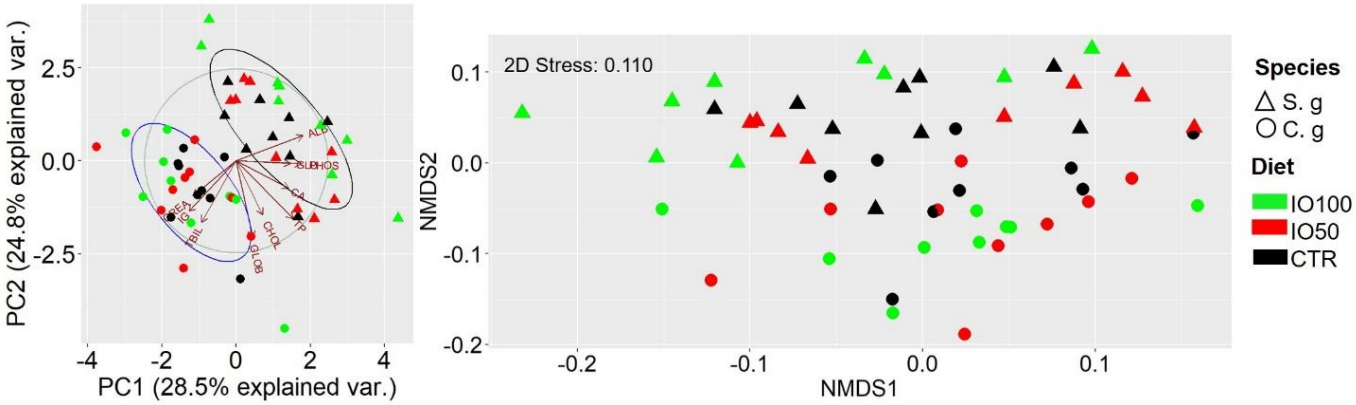

Figure 4.4. Principal component analysis (PCA, on the left) and non-Metric Multidimension Scaling (nMDS, on the right) ordination of plasma non-enzyme biomarkers (TP, ALB, GLOB, TBIL, CREA, CA, PHOS, GLU, CHOL, and IG) according to species and dietary groups. Δ *S.g - Silurus glanis*, ○ *C.g - Clarias gariepinus*

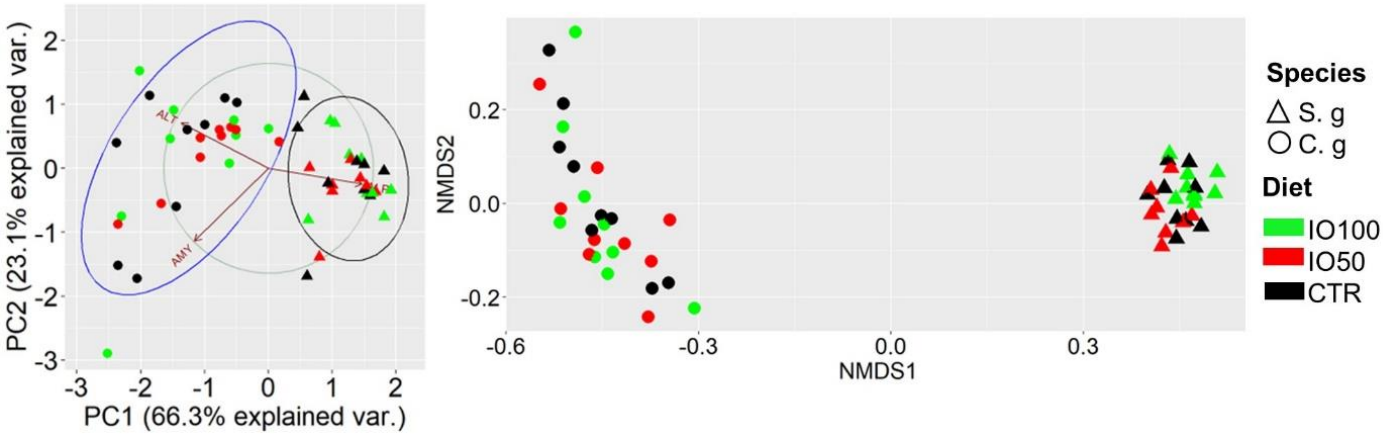

Figure 4.5. Principal component analysis (PCA, on the left) and non-Metric Multidimension Scaling (nMDS, on the right) ordination of plasma enzymes biomarkers (ALP, ALT, AMY). Δ *S.g - Silurus glanis,* ○ *C.g - Clarias gariepinus.*

4.3 Liver antioxidant capacity

The liver antioxidant indices of the two species studied are shown in Figure 4.6. In comparison to the CTR group, the activity of superoxide dismutase (SOD) enzyme used as a marker of oxidative stress, and total antioxidant capacity (TAOC), malondialdehyde (MDA) and reduced glutathione (GSH) were not significantly different between groups of European catfish fed insect oil-based diets (IO50 and IO100) and those fed the CTR diet. In addition, the hepatic expression of superoxide dismutase 1 gene (*sod1*) did not significantly differ between groups of fish fed the CTR diet and those fed the insect-based diets (IO50 and IO100). For African catfish hybrid, only the MDA content significantly varied between the dietary groups, with the lowest value recorded in fish fed IO50 diet. In general, there was a tendency for GSH, and TAOC to increase in fish fed insect oil-based diets while SOD activity and MDA content tended to decrease with insect oil inclusion in the diets of both European and African catfish hybrid. The hepatic expression of *sod1* gene in African catfish hybrid was also similar between the groups.

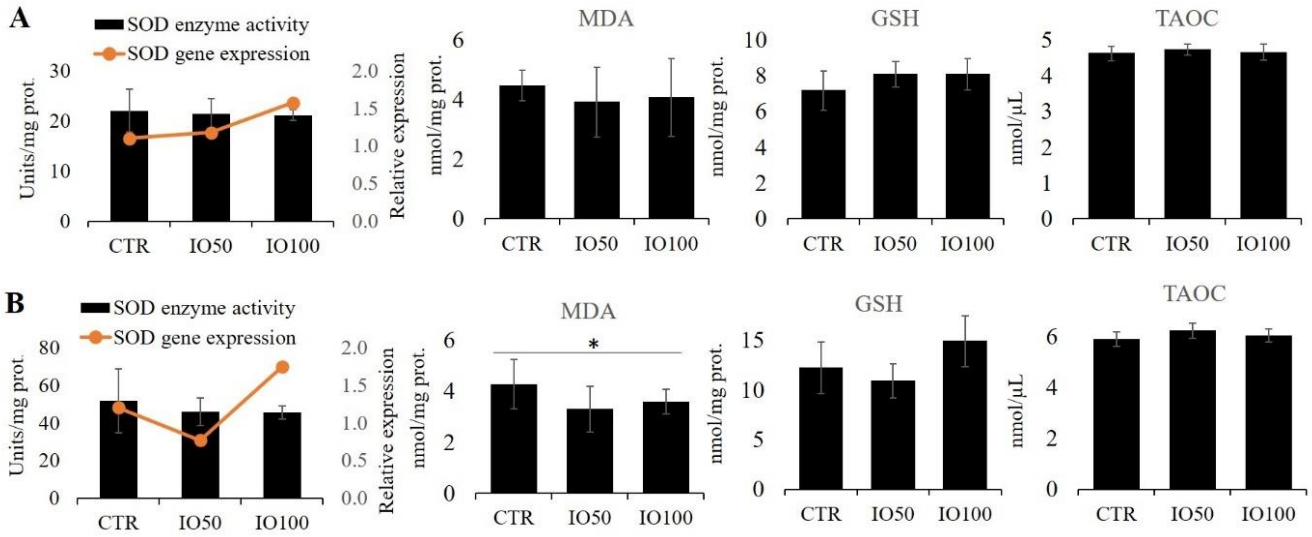

Figure 4.6. Effects of dietary black soldier fly larvae oil on the expression and activity of SOD enzyme, as well as on the contents of MDA, GSH and TAOC in the liver of European catfish (A) and African catfish hybrid (B). Data presented as mean ± SD. * indicates significant results.

4.5 Expression of lipid metabolism and immune response related genes

4.5.1. Hepatic expression of lipid metabolism genes

To estimate whether internal mechanisms might increase or suppress accumulation of dietary lipids, a number of genes involved in the biosynthesis, β-oxidation, and regulation of lipids were assessed in the liver samples of both African catfish hybrid and European catfish. Figures 4.7 and 4.8 present the results on relative expression 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 (TAG) metabolism (*lpl* gene), and transcription factors (*pparα*, *pparγ*, *srebp-1c* and *hnf4α*) regulating the expression of lipid metabolism genes in both species. There were no statistical differences in the relative expression of *fas, g6pd, 6pgd, fads2, elovl2,* and *elovl5*, between the dietary groups of European catfish and between groups of African catfish hybrid. In the European catfish, the relative expression of *cpt1a* linearly increased with insect oil fraction of the diets, being significantly higher in the group fed IO100 diet compared to the groups fed the CTR and IO50 (Figure 4.7). Although *cpt1a* expression was also highest in African catfish hybrid fed IO100, the results were statistically similar between the dietary groups of this species (Figure 4.8).

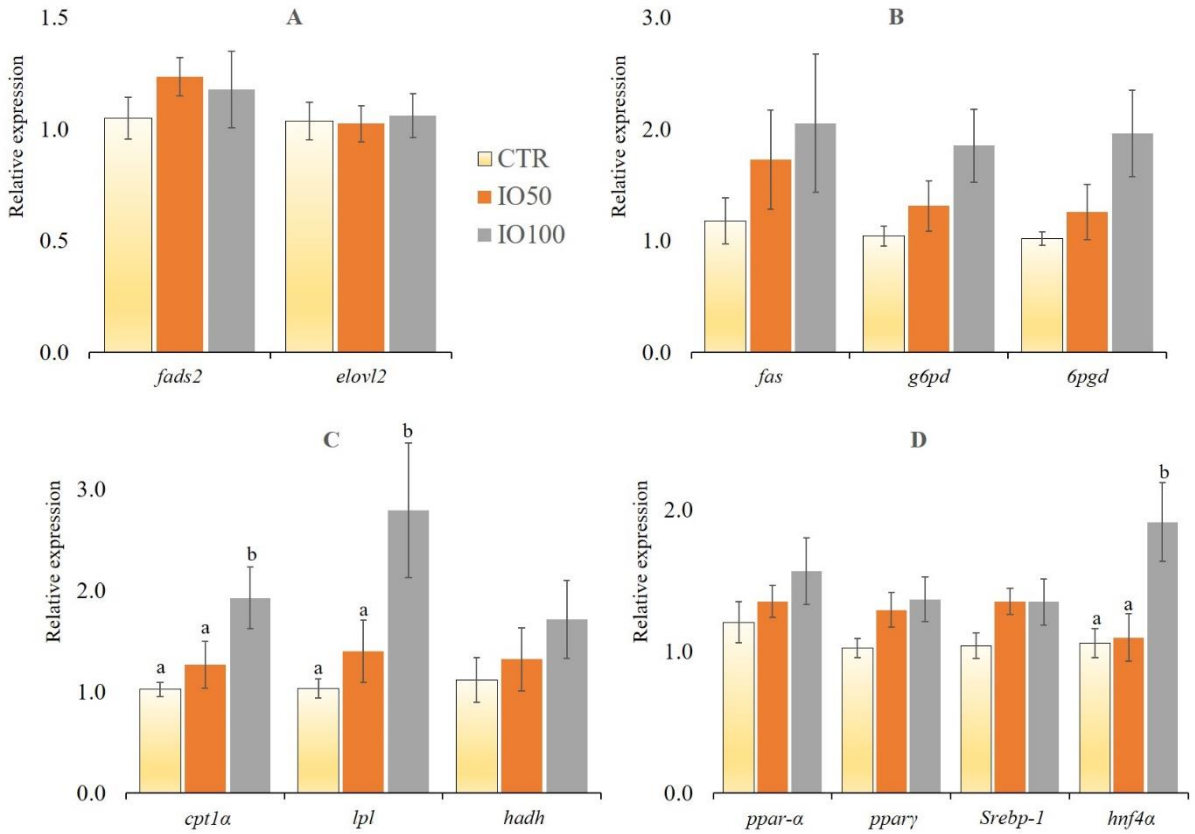

Figure 4.7. The relative expression level (mean ± SE, n=12) of genes involved elongation and desaturation (A), de novo fatty acid biosynthesis (B), β-oxidation (C), and transcriptional regulation, in the liver of European catfish juveniles fed the control (CTR) and experimental diets (IO50 and IO100). Different letters (a, b) denote significant differences between the dietary groups for any given gene.

The *lpl* gene involved in TAG hydrolysis was significantly upregulated in groups of European catfish and African catfish fed IO100 diet. However, a significant linear trend was only observed for *lpl* expression in European catfish. The gene *hadh* did not show any statistical difference between dietary groups of European catfish but significantly differed between the dietary groups of African catfish hybrid, being highest in fish fed IO100 diet and lowest in fish fed IO50 diet. Among the transcription factors (*pparα*, *pparγ*, *srebp-1* and *hnf4α*), only *hnf4α* was significantly expressed between the dietary groups of European catfish, being highest in the group fed IO100 when compared to the groups fed the CTR and IO100 diets (Figure 4.7). In the African catfish hybrid, groups fed IO50 indicated a significantly lower expression of *pparα* and *hnf4α* when compared to the group fed IO100 diet but not when compared to the CTR (Figure 4.8). The expression of *hnf4α* in European catfish exhibited a linear trend, but a quadratic trend was observed in the African catfish hybrid. Similar quadratic trends in African catfish hybrid were also observed in the relative expression of *pparα* and *hadh* genes, with no significant trends observed in the expression of these genes between the dietary groups of European catfish. There was no statistical difference in the expression of *srebp-1* between the dietary groups of African catfish hybrid, similar to observations on European catfish.

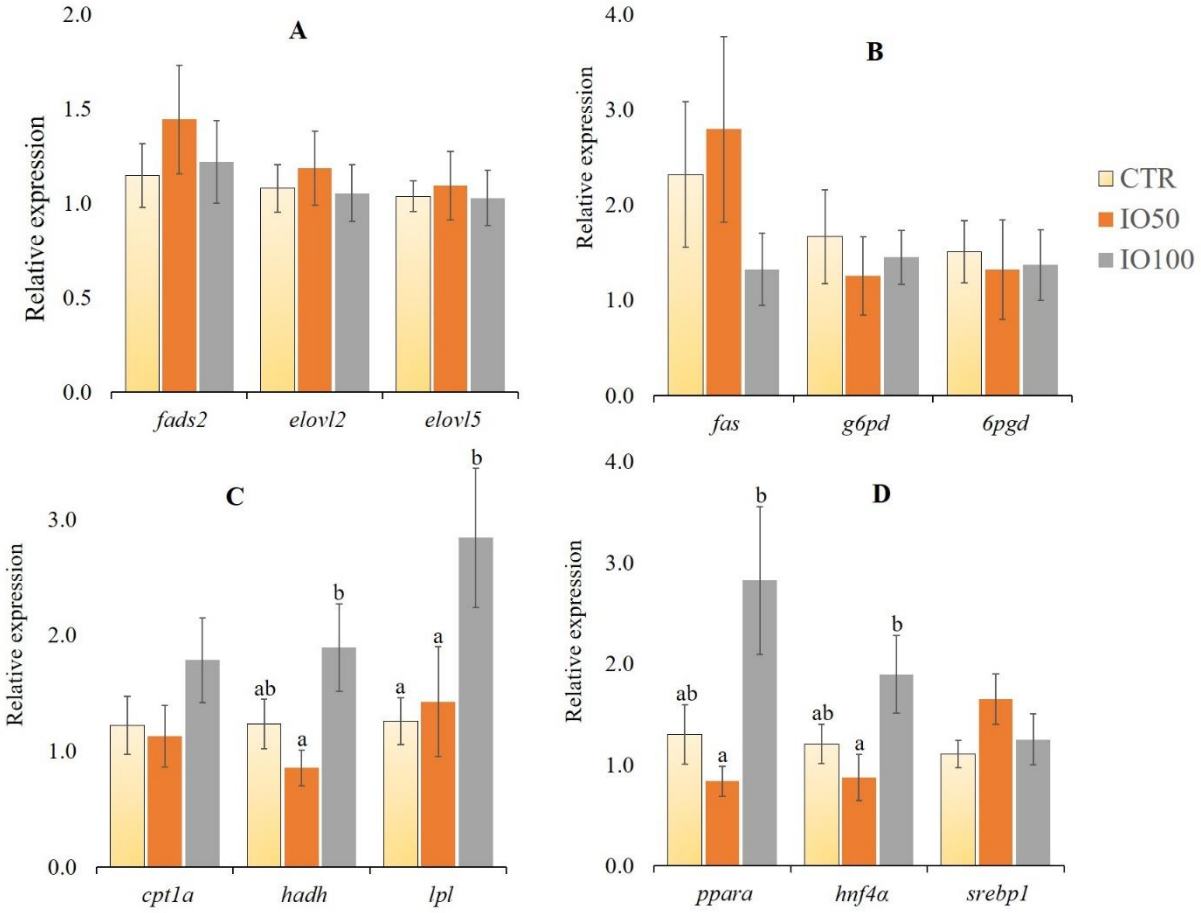

Figure 4.8. The relative expression level (mean ± SE, n=12) of genes involved elongation and desaturation (A), de novo fatty acid biosynthesis (B), β-oxidation (C), and transcriptional regulation, in the liver of African catfish hybrid juveniles fed the control (CTR) and experimental diets (IO50 and IO100). Different letters (a, b) denote significant differences between the dietary groups for any given gene.

4.5.2. Expression of immune related genes in spleen and head kidneys

The expression levels 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 of catfish (Figures 4.9 and 4.10). The expression of *tlr-5*, *il-8*, *irf-1*, *rag-1*, and *tgf-β1* exhibited a similar pattern in both the spleen and head kidney of European catfish (Figure 4.9). With the exception of *irf-1*, whose expression was significantly higher in the spleen of fish fed the IO100 diet than in fish fed the CTR diet, the relative expression of *tlr-5*, *il-8*, *rag-1* and *tgf-β1* did not differ significantly between the dietary groups of European catfish. In the head kidney of European catfish, all the genes indicated no major difference between fish fed the different diets. In the African catfish hybrid, the expression of immune-related genes was only evaluated for *tgf-β1* and *tlr-5* in the spleen samples (Figure 4.10). Similar to the European catfish, the expression of *tgf-β1* and *tlr-5* in the spleen of African catfish hybrid was similar between the dietary groups.

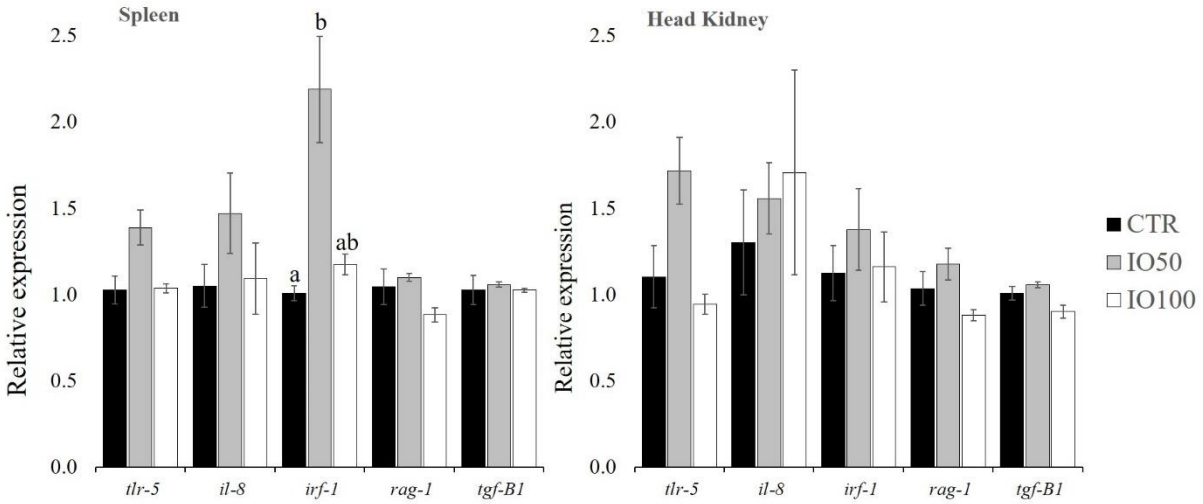

Figure 4.9. Relative expression levels (mean ± SE, n = 12) of genes involved in immune responses of European catfish juveniles fed the control (CTR) and experimental diets (IO50 and IO100). Different letters (a, b) denote significant differences between the dietary groups for any given gene.

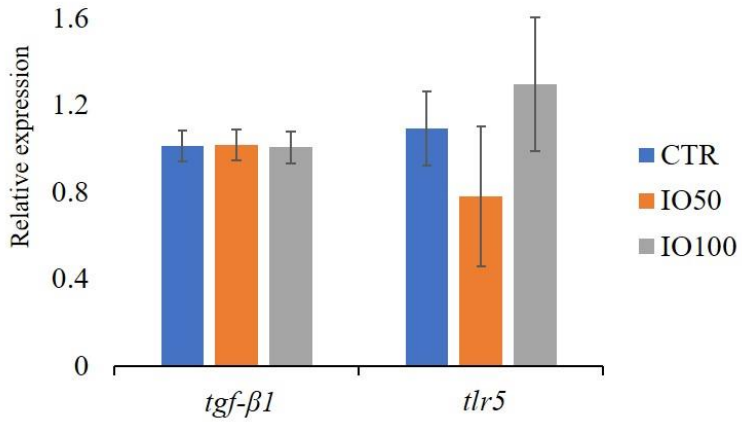

Figure 4.10. Relative expression levels of genes involved in immune responses in the spleen of African catfish juveniles fed the control (CTR) and experimental diets (IO50 and IO100).

4.6 Intestinal histomorphology

The intestinal wall shows an ordered arrangement of intestinal villi with densely and closely packed microvilli in all dietary groups of European catfish (Figure 4.11) and African catfish hybrid (Figure 4.12). There were no significant differences in the size (Figure S1) or number of villi between and within treatment groups. The intestinal epithelium was rich in large goblet cells which were evenly distributed, with no major variation in size or quantity between the dietary groups. The apical end of the intestinal follicles was regularly rounded, and no abnormal lesions or cell proliferations were observed. The lamina propria and epithelial layer showed no major separation and abnormal differences in the length of the cells of the epithelial layer were not observed. Any visible differences in the sections may be attributed to the characteristics of samples taken from different segments of the intestinal tract.

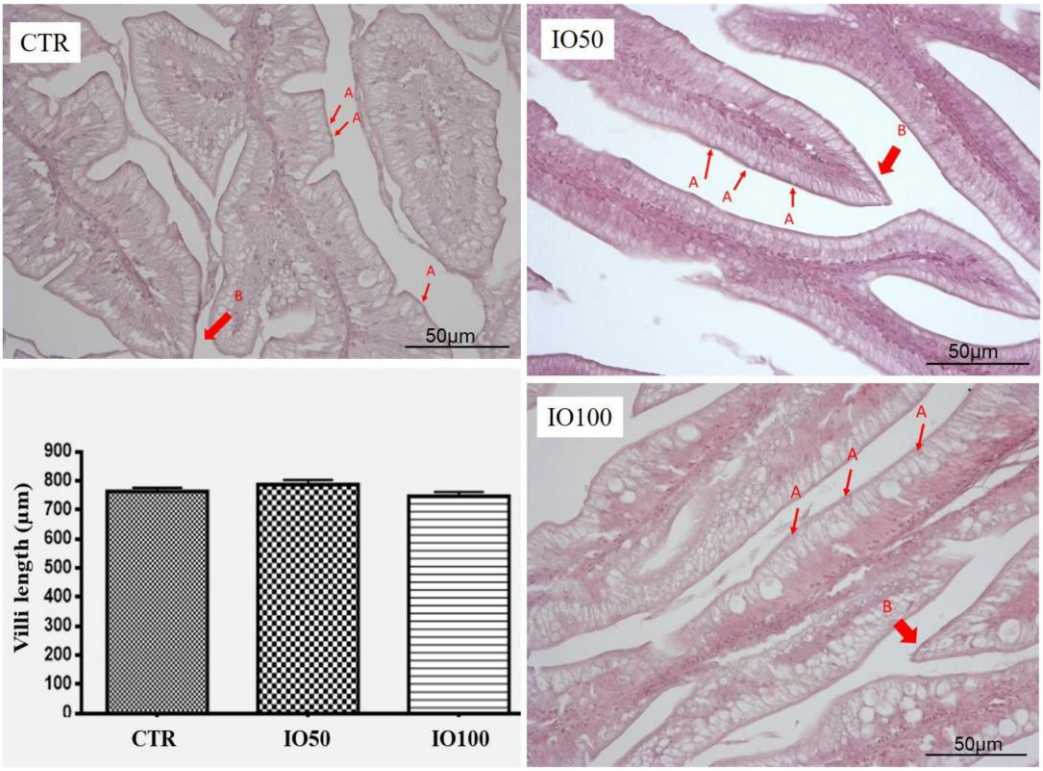

Figure 4.11. Histomorphology of intestinal villi of European catfish (*S. glanis*) fed a control diet (CTR) and insect oil-based diets (IO50 and IO100). A: goblet cells, B: apical end of the intestinal villus. All scales are 50 µm. Haematoxylin & eosin staining; × 200 magnification.

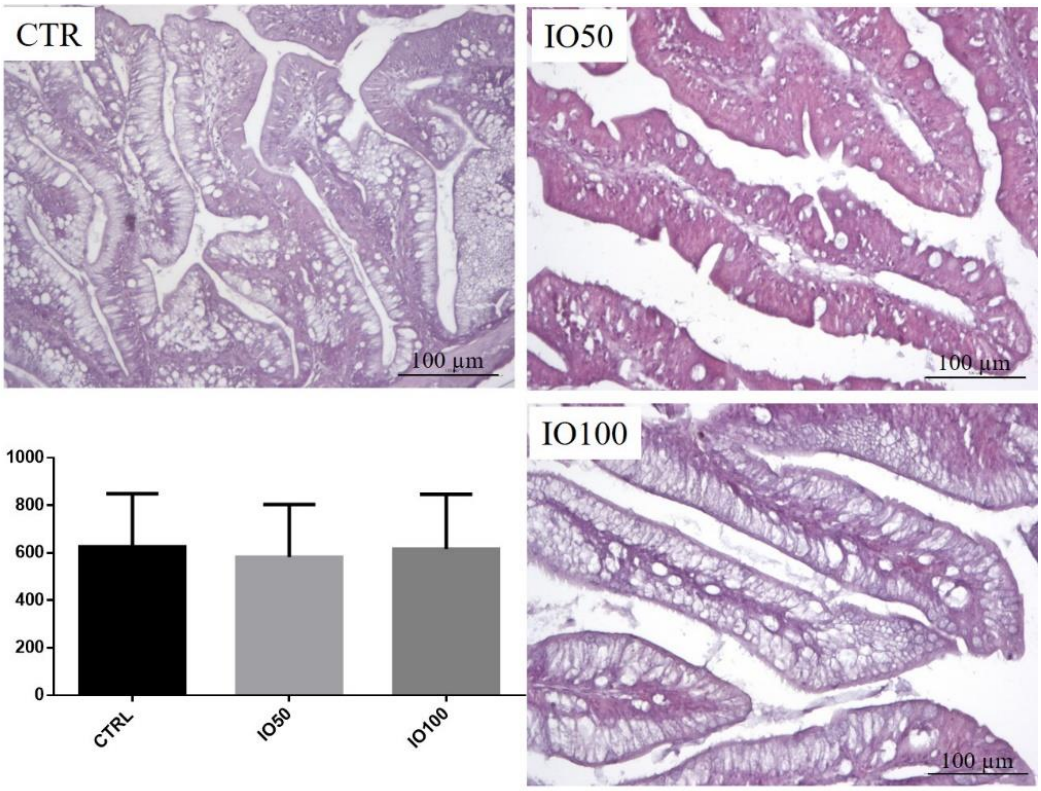

Figure 4.12. Histomorphology of intestinal villi of African catfish hybrid fed a control (CTR) diet and insect oil-based diets (IO50 and IO100). Haematoxylin & eosin staining; × 200 magnification.

4.7. Intestinal microbiota diversity and composition

4.7.1. Bacterial community composition

The intestinal microbiota of fish fed the CTR, and insect oil-based diets (IO50 and IO100) was analysed in order to assess whether groups fed insect oil were associated with alterations in the bacterial diversity, composition, and function. The alpha diversity (Shannon, Simpson, and Pielou) of intestinal microbiota of European catfish fed the CTR and insect oil-based diets (IO50 and IO100) was similar (Figure 4.13). In addition, there was no clear separation (PERMANOVA, $R^2 = 0.146$, $p = 0.447$) in the bacterial community structures between the three dietary groups of European catfish (Figure 4.14). Across the different dietary groups of European catfish juveniles, the bacterial community was dominated by the phyla Fusobacteriota and Spirochaetota, which together accounted for over 99% of the total bacterial abundance in the samples, with no significant difference in abundance between the different dietary groups. Other bacterial phyla which contributed <1% of the total bacterial abundance included Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota, Verrucomicrobiota, Patescibacteria, and Campylobacterota (Figure 4.15A). On average, Proteobacteria accounted for 0.16%, 0.20% and 0.30% of the total abundance of bacteria in the CTR, IO50 and IO100 fish groups respectively while Firmicutes accounted for 0.12%, 0.14% and 0.25% of the total bacterial abundance in the CTR, IO50 and IO100, respectively. The contributions of other notable phyla were as follows: Actinobacteriota (CTR, 0.09%; IO50, 0.07% and IO100, 0.13%), Bacteroidota (CTR, 0.04%; IO50, 0.03%; IO100, 0.05%) and Patescibacteria (CTR, 0.00012%; IO50, 0.0018%; IO100, 0.0085%). The ratio of Firmicutes to Bacteroidota increased with increase in insect oil fraction of the diets (CTR, 2.9; IO50, 11.5; and IO100, 14.8).

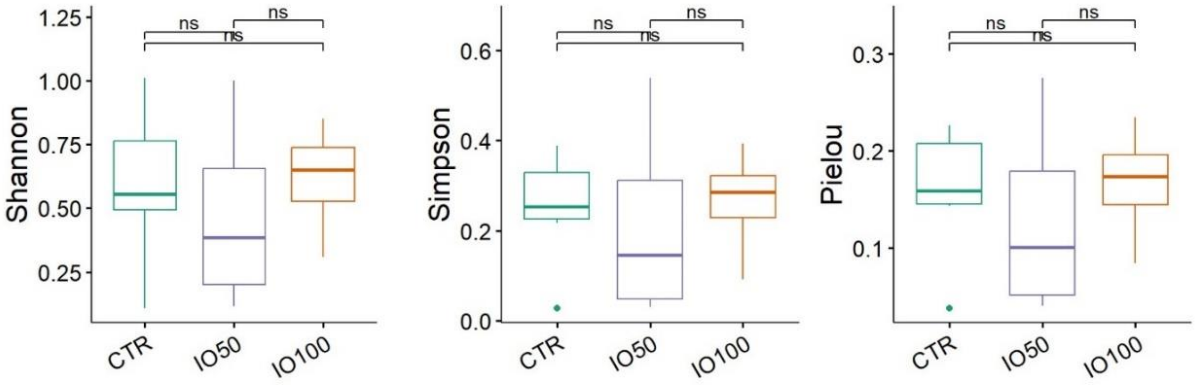

Figure 4.13. Alpha diversity indexes of the bacterial community in European catfish fed the CTR and insect oil-based diets (IO50 and IO100).

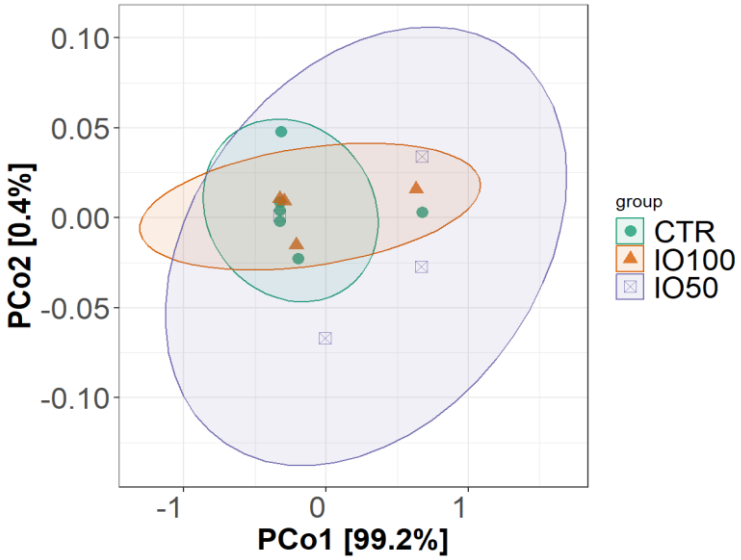

Figure 4.14. Comparisons of beta diversity of bacterial community among the three dietary groups of European catfish (CTR, IO50 and IO100) based on PCoA.

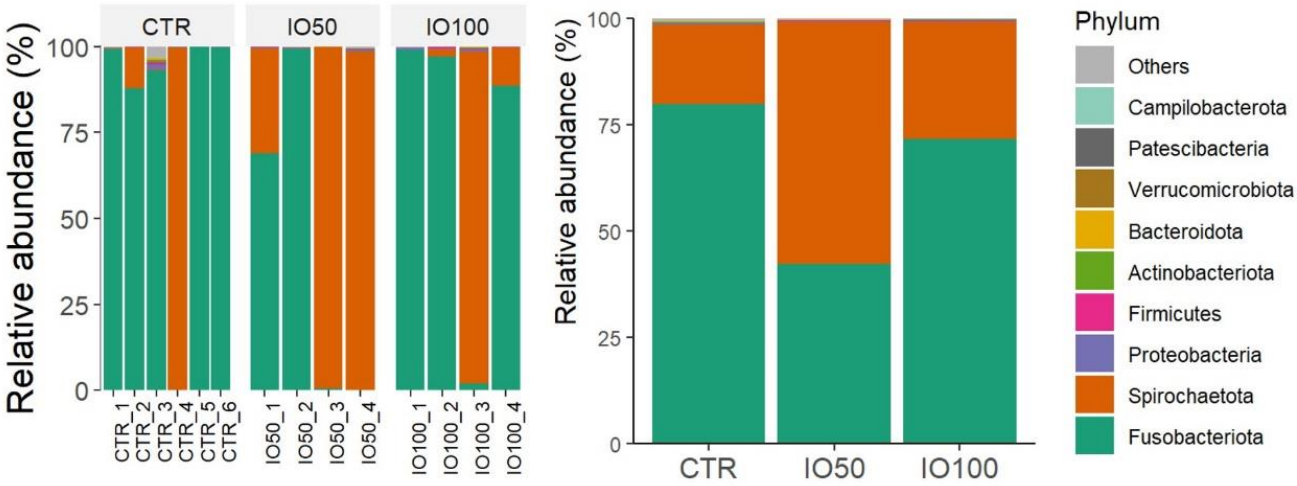

Figure 4.15A. Relative abundance of bacterial phyla in intestinal faecal samples of European catfish juveniles fed with the CTR, IO50 and IO100 diets.

Among the Fusobacteriota, only two genera, *Cetobacterium* and *Leptotrichia* were identified, with *Cetobacterium* occurring in all the samples while *Leptotrichia* was only identified in one fish sample fed IO100. The phylum Spirochaetota was only represented by the genus *Brevinema* which was identified in all the samples in varying abundance (Figure 4.15B). The genus *Brevinema* accounted for 7.1% of the total number of ASVs and relative abundances of 16.1% in the CTR, 54.3% in IO50 and 28.0% in IO100 fish groups, while *Cetobacterium* accounted for 15.2% of the total number of ASVs and average relative abundances of 83.6% in the CTR, 45.3% in IO50 and 71.2% in IO100 fish group, with no statistically significant differences between the fish groups. In a majority of the samples, *Cetobacterium* was the numerically dominant bacterial genus. Other notable genera ranked among the top 10 (Figure 4.15B) but with relative abundances less than 1% (Table S2) included: the Proteobacteria *Escherichia-Shigella*; the Firmicutes *Exiguobacterium*, *Aerosphaera* and *Bacillus*; and the Actinobacteria *Leifsonia*, *Corynebacterium*, and *Bifidobacterium*.

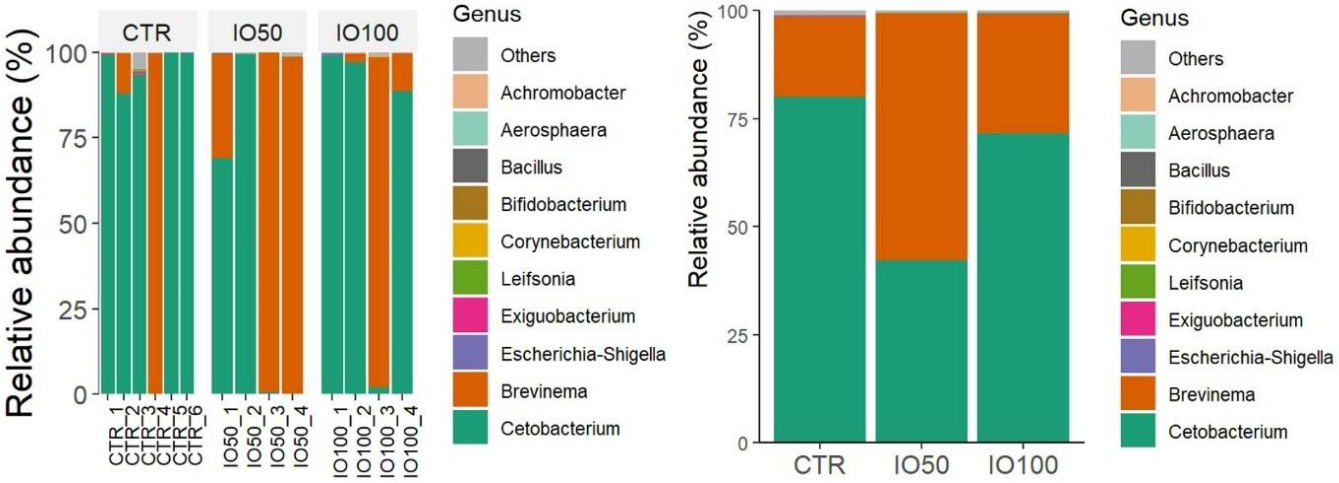

Figure 4.15B. Relative abundance of bacterial genera in intestinal faecal samples of European catfish juveniles fed with the CTR, IO50 and IO100 diets.

Across the different dietary groups of African catfish hybrid, the bacterial community in the intestinal faecal samples was dominated by the phyla Fusobacteriota, Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota, Verrucomicrobiota, Gemmatimonadota, Desulfobacterota, and Chlofroflexi (Figure 4.16). Like observed with European catfish, the Firmicutes to Bacteroidota ratio in African catfish hybrid was higher in fish fed insect oil-based diets than in those fed the control (CTR, 22.4; IO50, 969.6; and IO100, 208.1). The phylum Fusobacteriota accounted for over 75% of the total abundance of bacteria. Two genera of Fusobacteriota, *Fusobacterium* and *Cetobacterium* were identified in the samples, with *Cetobacterium* being the most dominant in all the fish samples analysed regardless of the dietary grouping (Figure 4.16, Table S3). *Fusobacterium* was only identified in one CTR sample. Other bacterial genera listed among the top 10 genera besides *Cetobacterium* included the seven Firmicutes: *Romboutsia*, *Turicibacter*, *Clostridium sensu stricto 1*, *Candidatus Arthromitus*, *Terrisporobacter*, *Epulopiscium* and *Staphylococcus*, as well as the Proteobacteria *Plesiomonas* and the Actinobacteriota, *Aurantimicrobium*, with most of the genera having abundances <1% (Table S3).

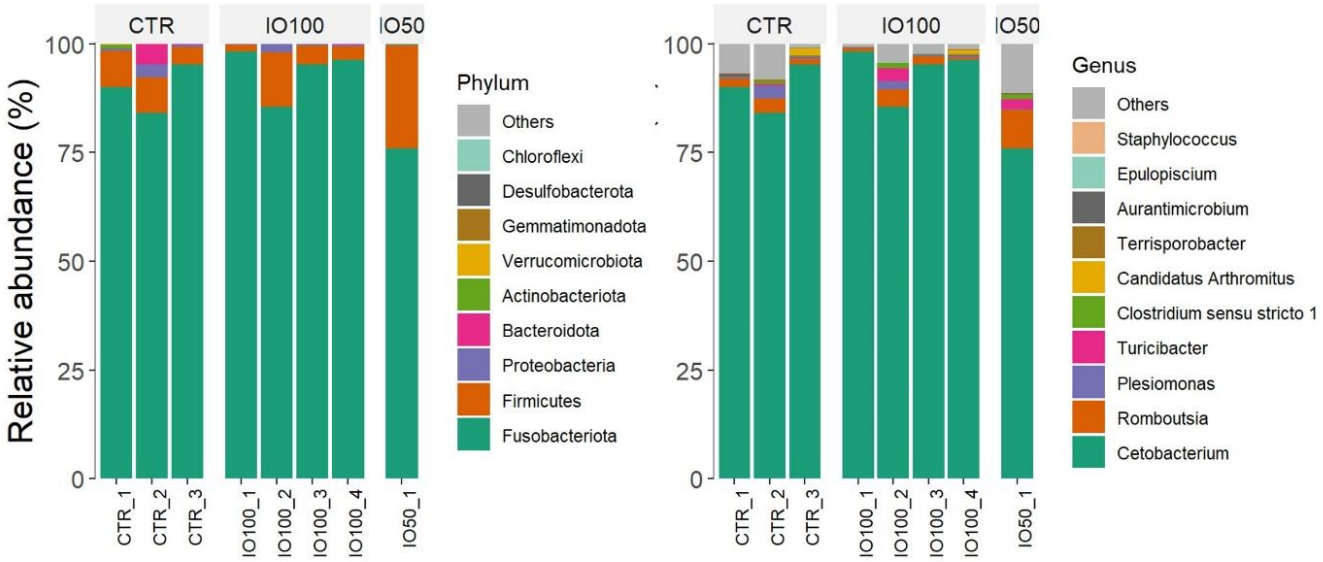

Figure 4.16. Relative abundance of bacterial phyla and genera in intestinal faecal samples of African catfish juveniles fed with the CTR, IO50 and IO100 diets.

There were no major differences in the bacterial community composition between the feeds. The bacterial phyla such as Firmicutes, Proteobacteria, Actinobacteriota, Fusobacteriota, and Bacteroidota, were still the most dominant. Other notable phyla recorded in the feeds were Myxococcota, Aquificota, Cyanobacteria, and Synergistota (Figure 4.17). Although most of these phyla were encountered in the fish faecal samples, the dominant genera in the feeds were different from those in the faecal material. For instance, while *Cetobacterium* and *Brevinema* were dominant in the fish digesta, the feed samples were dominated by the Firmicutes *Lactococcus* and *Leuconostoc* which accounted for over 42% of the bacterial abundance in the samples (Table S4). In addition, other notable genera such as *Pseudomonas* (Phylum Proteobacteria), *Aeromonas* (Phylum Proteobacteria), *Lactobacillus* (Phylum Proteobacteria), among others (Table S4), were common members of feed bacterial community.

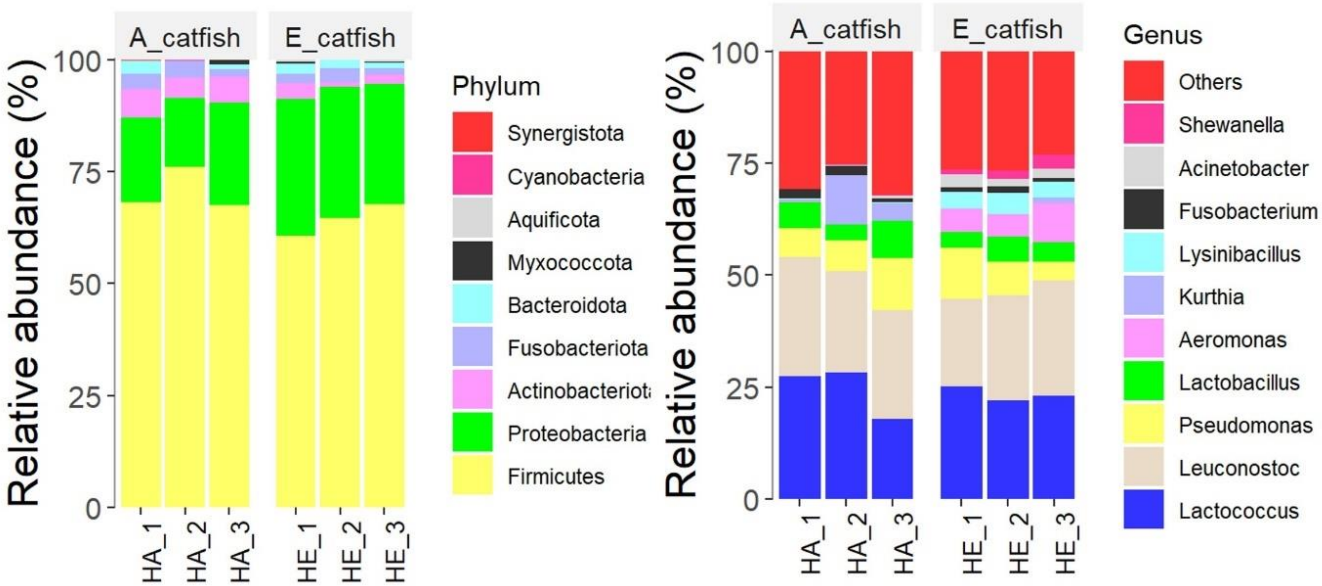

Figure 4.17. Relative abundance of bacterial phyla and genera in feed samples of African and European catfish juveniles. HA-1 (CTR feed), HA-2 (IO50) and HA-3 (IO100) refer to feeds for African catfish and HE-1 (CTR), HE-2 (IO50), HE-3 (IO100) for European catfish.

4.7.2. In silico prediction of the function of microbial community

In order to detect whether differences occurred in the inferred bacterial functions between the dietary fish groups, a Tax4fun2 analysis was performed. The relative abundances of bacterial pathways at three levels were statistically similar between the treatments in European catfish (PERMANOVA, $p>0.05$). Level 1 was dominated by metabolic functions (75.34±1.05%), followed by environmental information processing (10.76±0.88%), cellular processes (6.07±0.51%), genetic information processing (3.61±0.27%), human diseases (2.84±0.33%) and organismal systems (1.38±0.19%) (Figure 4.18). At level 2, in addition to global and overview maps (36.93±0.49%), 9 most abundant metabolic pathways included, carbohydrate metabolism (10.60±0.87%), amino acid metabolism (7.76±0.59%), membrane transport (6.91±0.96%), cellular community (4.40±0.33%), signal transduction (3.85±0.44%), lipid metabolism (3.44±0.46%), metabolism of cofactors and vitamins (3.20±0.10%), energy metabolism (3.19±0.22%) and xenobiotics biodegradation and metabolism. At level 3, the most abundant pathways were related to metabolic pathways (13.51±0.24%) and secondary metabolite biosynthesis (5.79±0.22%) (Figure 4.18).

In the African catfish hybrid, differences in the inferred bacterial functions between the dietary groups indicated statistically similar relative abundances of bacterial pathways (PERMANOVA, $p>0.05$). Similar to the European catfish juveniles, the bacterial functions at level 1 in the African catfish hybrid was dominated by metabolic functions (70.92±1.32%), followed by environmental information processing (12.34±1.43%), cellular processes (6.55±0.60%), genetic information processing (5.72±0.36%), human diseases (3.17±0.11%) and organismal systems (1.30±0.04%) (Figure 4.19). At level 2, in addition to global and overview maps (35.43±4.23%), 9 most abundant pathways included carbohydrate metabolism (10.92±0.51%), membrane transport (7.19±2.24%), amino acid metabolism (6.38±0.19%), signal transduction (5.13±1.11%), cellular community (4.24±0.98%), energy metabolism (3.47±0.23%), metabolism of cofactors and vitamins (3.40±0.18%), and nucleotide metabolism (2.99±0.21) and translation (2.21±0.66%). At level 3, the most abundant pathways were related to metabolic pathways (13.83±0.34%) followed by secondary metabolite biosynthesis (5.91±0.35%) (Figure 4.19).

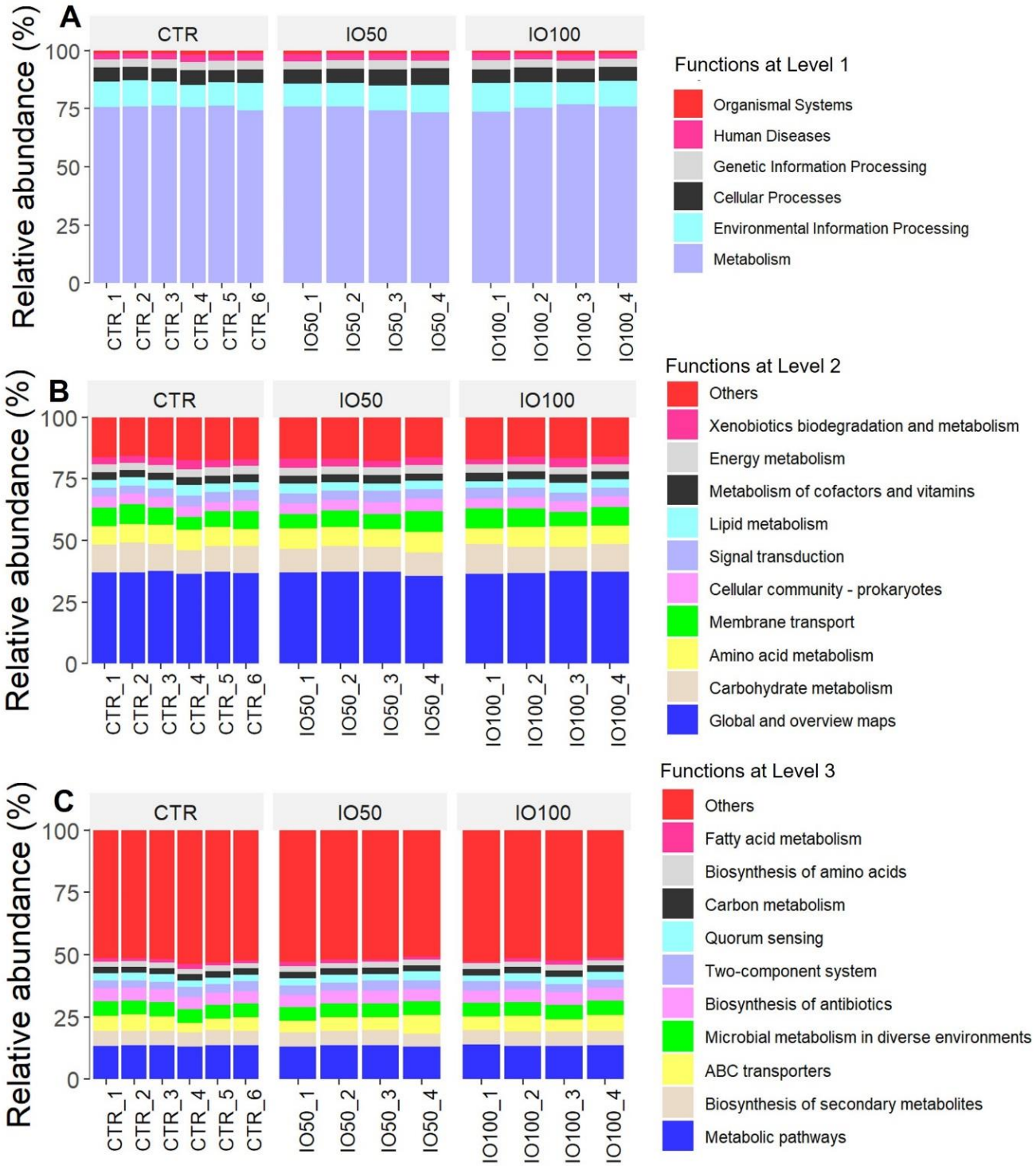

Figure 4.18. Relative abundance of bacterial pathways of European catfish juveniles fed with CTR, IO50 and IO100 diets. (**A**) At level 1, (**B**) at level 2 and (**C**) at level 3.

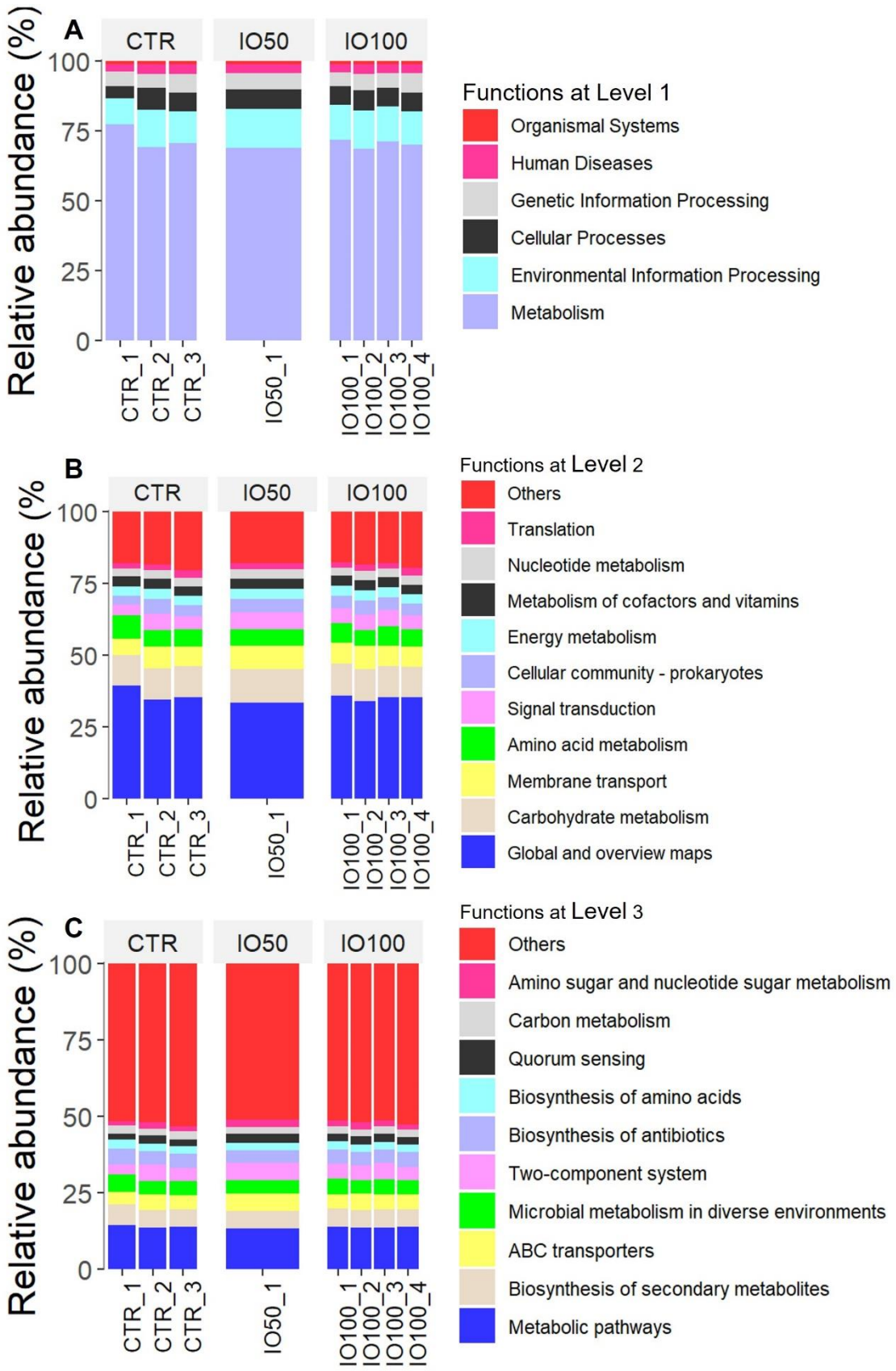

Figure 4.19. Relative abundance of bacterial pathways of African catfish hybrid juveniles fed with CTR, IO50 and IO100 diets. (**A**) At level 1, (**B**) at level 2 and (**C**) at level 3.

4.7.3. Associations between intestinal microbiota and liver antioxidation capacity

To investigate whether the effects of insect oil on antioxidation are reflected in the gut microbiota composition, Spearman's correlation analysis between the microbiota relative abundance and the levels of antioxidant indicators (TAOC, MDA, GSH, and SOD activity) in the liver was performed. Figures 4.20 and 4.21 present results of the correlation analysis in European catfish and African catfish hybrid respectively. At the phylum level, there was a positive correlation between the Firmicutes/Bacteroidota ratio and the level of liver GSH in European catfish (Figure 4.20C). The Firmicutes/Bacteroidota ratio negatively correlated with MDA content, but the results were only significant in African catfish hybrid (Figure 21A). The abundance of Proteobacteria was positively correlated with MDA content, being significant in African catfish hybrid (Figure 21C). Negative correlations between the relative abundance of Proteobacteria and liver SOD activity, as well as GSH content were observed but the results were not significant in both species. The relative abundance of Spirochaetota was negatively correlated with SOD activity (Figure 20G). Negative correlation between relative abundance of Spirochaetota and GSH was also observed in European catfish but the results were not significant (Figure 20H). On the other hand, the relative abundance of Fusobacteriota was positively correlated with SOD activity (Figure 20I) and negatively correlated with TAOC level (Figure 20K). In addition, the relative abundance of Spirochaetota was positively correlated with TAOC level (Figure 20L).

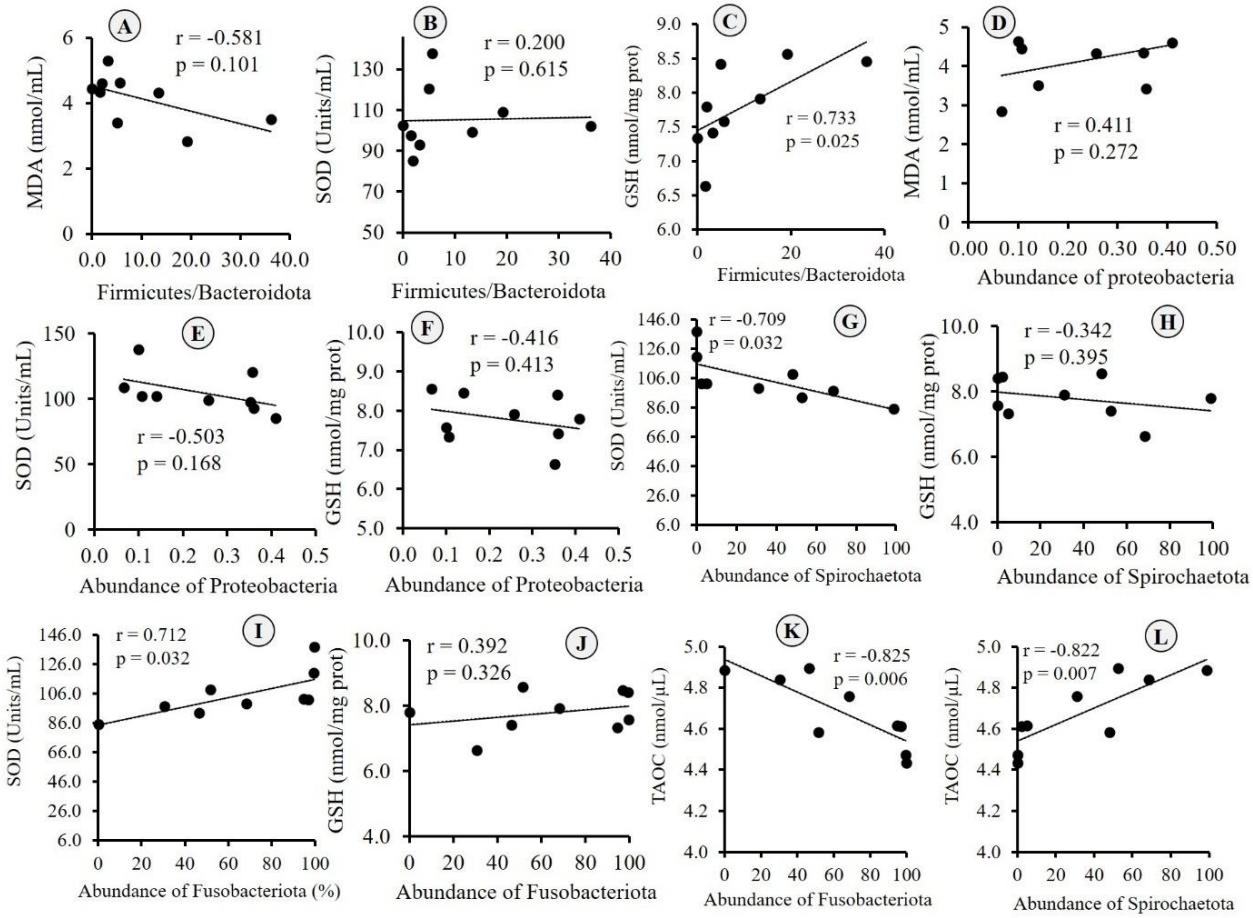

Figure 4.20. Pearson correlations between relative abundance (%) of intestinal microbiota of European catfish and the liver antioxidant indicators (TAOC, SOD, GSH, and MDA). (A) to (L) refer to correlations in the different phyla and/or their ratios.

The correlations between gut microbiota at the genus level and the liver antioxidant parameters (TAOC, SOD, GSH, and MDA) in European catfish and African catfish hybrid are presented in Tables S5 and S6, respectively. In the European catfish, relative abundance of *Cetobacterium*, the most abundant genus in the phylum Fusobacteriota, was negatively correlated with the levels of TAOC and positively correlated with SOD activity. On the contrary, relative abundances of *Brevinema* (phylum Spirochaetota) and *Diaphorobacter* (phylum Proteobacteria), positively correlated with the level of TAOC but negatively correlated with SOD activity. Similarly, the relative abundance of the genus *Pseudomonas* (phylum Proteobacteria) was negatively correlated with SOD activity. In African catfish hybrid, the relative abundance of *Bacteroides* (phylum Bacteroidota) was negatively correlated with TAOC, while *Flavobacterium* (phylum Bacteroidota) was positively correlated with MDA level. The Firmicutes *Epulopiscium* and *Turicibacter* were negatively correlated with TAOC and GSH, respectively.

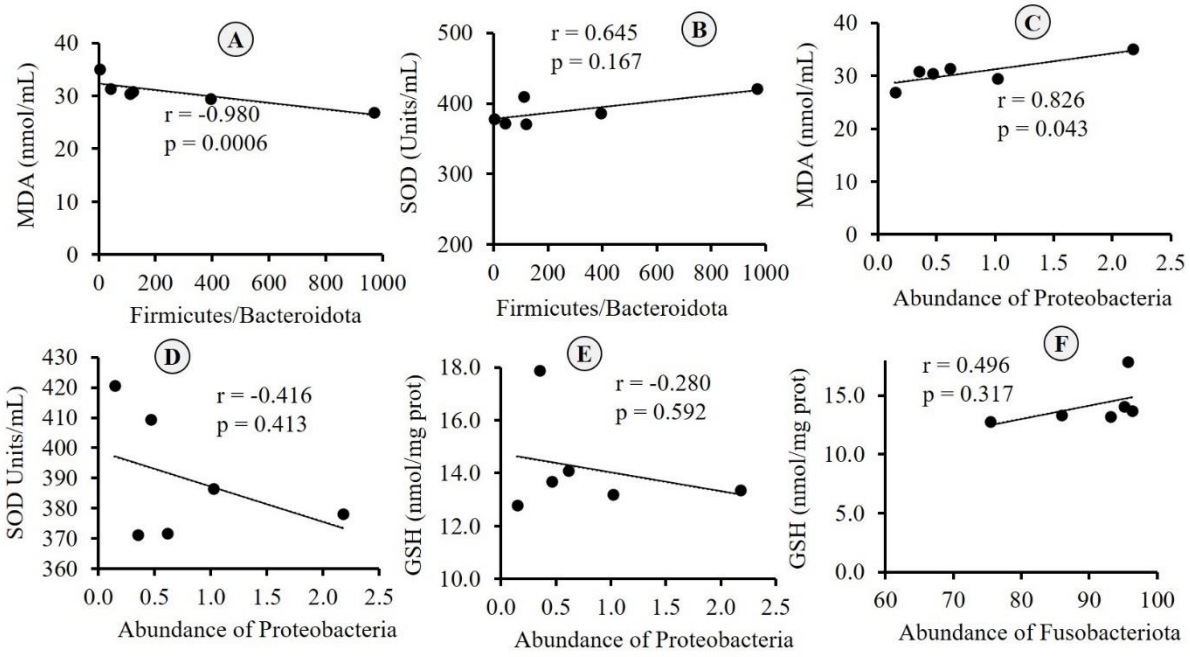

Figure 4.21. Pearson correlation analyses between the relative abundance (%) of intestinal microbiota of African catfish hybrid and the liver antioxidant indicators. (A) Correlation between Firmicutes/Bacteroidota ratio and MDA level, (B) Correlation between Firmicutes/Bacteroidota ratio and SOD activity, (C) Correlation between relative abundance of Proteobacteria and MDA level, (D) Correlation between relative abundance of Proteobacteria and SOD activity, (E) Correlation between relative abundance of Proteobacteria and GSH level, and (F) Correlation between relative abundance of Fusobacteriota and GSH level.

5. DISCUSSION

5.1 Fish growth performance, nutrient utilisation, and fatty acid profiles

The current study showed that total replacement of FO and RO blend with BSFLO in practical diets of European catfish and African catfish hybrid does not significantly affect fish growth performance and nutrient utilisation. These results suggest 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. The final weight of all experimental fish was significantly improved when compared to the weight at the beginning of the trial, with no major differences between the dietary groups. The measurement of digestive enzyme activities provides a more accurate assessment of carbohydrate, protein, and lipid digestibility (Bakke et al., 2010), and thus, enzyme activities are considered predictors of potential feed utilisation and growth differences in fish (Lemieux et al., 1999; Sunde et al., 2001; Lin & Luo, 2011). The observed similarities in the activities of trypsin, lipase, and amylase between the different dietary fish groups indicated that the digestive processes were not significantly affected when insect oil was used as a source of lipids in the diets of the two catfish species. This corroborates the observed lack of differences in growth between the treatments.

Similar observations on fish growth performance were reported for juvenile striped catfish, *Pangasianodon hypophthalmus*, where differences in growth and body parameters including FBW, WG, DWG, SGR, and FCR between groups fed FO and BSFLO for 10 weeks were not observed (Sudha et al., 2022). In addition, our results are also similar to those reported for Jian carp (*Cyprinus carpio* var. Jian) fed BSFLO and vegetable oils for 8 weeks (Li et al., 2016); juvenile barramundi (*Lates calcarifer*) fed diets in which 30% FM and FO were replaced with *H. illucens* protein and oil (Hender et al., 2021); and rainbow trout (*Oncorhynchus mykiss*) fed diets containing 25, 50, and 100% BSFLO replacing FO (Dumas et al., 2018). In addition, there were no major differences in the growth, nutrient utilisation, and body indices of juvenile *Onychostoma macrolepis* when fed on diets with up to 50% replacement of FO with BSFLO, but significant differences in WG, SGR, and feed intake were obtained with 100% replacement, attributed to reduced feed intake (Gou et al., 2023). CTR, IO50, and IO100 diets mainly differed in FA percentage. The type and relative content rather than the total quantity of dietary lipids play a major role in promoting fish growth. In species such as channel catfish, the n-3 fatty acids were reported to be essential for growth, and a 1.0-2.0% level of 18:3n-3 or a 0.5-0.75% level of n-3 HUFA (n-3 highly unsaturated fatty acid) was essential for this species (Satoh et al., 1989b). The absence of statistical differences in the growth and nutrient utilisation between the dietary fish groups of the two species (European catfish and African catfish hybrid) was an indication that the dietary fatty acid profiles met the basic requirements for essential fatty acids (EFAs) needed by these species.

Generally, the lipid composition of fish tissues largely reflected that of their diets, with the two species exhibiting similarities in the trends of tissue FAs across the dietary groups. In the liver, increased lipid deposition can have an impact on the lipid metabolism, thereby affecting metabolic processes including the synthesis, oxidation, and FA transport (Riera-Heredia et al., 2020). In our study on European catfish and African catfish hybrid, the substitution of a mixture of FO and RO with BSFLO did not increase liver lipid deposition. 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 diets containing black soldier fly larvae (Belghit et al., 2019). The reduced lipid deposition in the liver can be attributed to the high level of MCFAs in the insect oil-based diets. The MCFA, such as lauric acid, accounted for over 50%

of the total FAs in the insect oil used for the feed and 11.46 to 23.67% of the total FA in the insect oil-based diets. However, a comparison of the lauric acid content of the liver with the levels in the corresponding diets for both European and African catfish hybrid showed a much lower content in the livers than in the diets, indicating that the lauric acid was oxidised to provide energy rather than stored in the tissues (Schönfeld and Wojtczak, 2016). In both the European catfish and African catfish hybrid, the liver DHA/EPA ratio increased with an increase in insect oil 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 observed in the diets. The increased liver DHA/EPA ratios were due to the decrease in EPA and increase in DHA content with insect oil inclusion, which could be a consequence of desaturation of EPA and selective retention of DHA or retention of DHA and selective catabolism of EPA.

In order to understand the possible health effects associated with fatty acids present in fish, the effects of C12:0, C14:0 and C16:0 were taken into account since they have been reported to increase the total serum cholesterol (Ulbricht and Southgate, 1991). The atherogenic index is an indicator of the ability of saturated fatty acids to cause atherogenic effects where the lipids adhere onto cells of the immune and circulatory systems. However, the non-saturated fatty acids are considered to be anti-atherogenic because they inhibit the formation of plaques and reduce the levels of esterified fatty acids, cholesterol, and phospholipids, thereby preventing micro- and macro-coronary events (Ulbritch and Southgate, 1991). The thrombogenic index shows the tendency towards blood clotting, while the polyene index indicates the susceptibility to PUFA damage (Küçükgülmez et al., 2018). All the lipid quality indices determined in this study were less than or equal to 1.23, indicating that the fish tissues were safe for human consumption. In addition, the whole-body PUFA/SFA ratio was higher than 0.4, which is recommended so as to reduce the risk of cardiovascular, autoimmune, and other chronic diseases (Simopoulos, 2002).

5.2 Plasma biochemistry

Plasma biochemical parameters are considered an important indicator of the health and nutritional status of fish, as well as the ability of fish to adapt to the external environment, especially when alternatives to fish meal and fish oil are used in aquafeed (Fazio et al., 2013; Faggio et al., 2014). In the present study, differences in the content of albumin, globulin and the activities of alkaline phosphatase and lipase between the dietary groups were observed in European catfish. On the other hand, glucose content significantly differed between dietary groups of African catfish hybrid, which was not the case for European catfish. In a study on stripped catfish (Sudha et al., 2022), major differences were not observed in the contents of total protein, albumin, cholesterol, and glucose, as well as the albumin-to-glucose ratio, between fish fed fish oil and those fed BSFLO. 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 partially defatted BSF larvae protein and oil as fish meal protein and oil replacement showed no significant differences between the dietary groups (Hender et al., 2021). In a study on rainbow trout (*Oncorhynchus mykiss*) fed diets in which fish oil was replaced with BSFLO, significant results were obtained for glucose and phosphorus content between the dietary groups (Dumas et al., 2018).

Plasma parameters such as total protein, immunoglobulin, lysozyme, and myeloperoxidase are related to fish health and immune function. The total protein level of plasma is the sum of albumin and globulin, with immunological and transport roles and the roles in osmoregulation (Ghelichpour et al., 2017). While globulins such as immunoglobulins are formed in the

lymphatic system, albumin is synthesised in the liver (El-Moghazy et al., 2014). Thus, changes in albumin level reflect changes in liver function, and the higher albumin level in European catfish fed the IO100 diet may indicate upregulated nutritional or metabolic activities in this species, since albumins are involved in the transport functions (Andreeva, 2010; Manna et al., 2021). The albumin to globulin ratio (A/G) is an index used to track relative changes in the composition of plasma proteins. The significantly higher A/G ratio in European catfish fed the IO100 diet was due to the increase in albumin and decrease in globulin levels. In the African catfish, plasma albumin levels were below the detection limit of the equipment, and the globulin content did not significantly differ between the dietary groups.

The non-specific immune system plays an important role in maintaining the health status of fish. The activities of plasma lysozyme and myeloperoxidase were measured as indicators of humoral innate immunity. Lysozyme activity can be used as a marker of macrophage activation (Catap et al., 2018). Levels of myeloperoxidase, which is possibly released via the degranulation process by neutrophils during oxidative respiratory burst activity, can be used as a marker for the assessment of fish health (Zhang et al., 2014). Myeloperoxidase catalyses the reaction of hydrogen peroxide with chlorine to produce hypochlorous acid, which has a strong antimicrobial effect (Bu et al., 2017). In the African catfish hybrid, a linear increase in myeloperoxidase activity with increasing insect oil fraction in the diets was observed, highlighting its influence on the non-specific immune function.

Increased plasma ALT activity is generally used as a marker of liver damage (Li et al., 2016). On the other hand, ALP hydrolyses organophosphate molecules, releasing phosphates for cellular biochemical processes, and thus, high ALP activity indicates high metabolic activity (Manna et al., 2021). Additionally, increased ALP levels in blood could be linked to haemolysis or liver damage (Gaudet et al., 1975; Banaee, 2020). However, unlike ALT activities which were similar among the dietary groups of fish, 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 relatively higher metabolic activity and not liver damage. The IO50 diet was associated with increased plasma lipase activity. As reported (Babalola et al., 2016), high plasma lipase activity may indicate accelerated clearance of plasma triglycerides, resulting in reduction of plasma triglycerides. The increase in plasma amylase activity may be attributed to damage of the pancreas (Sastry and Sharma, 1981). Thus, results from the two species (African catfish and European catfish) fed different insect oil-based diets indicated no negative influence of the diets on pancreatic health. Creatinine, a nitrogenous end product of metabolism, which is used to estimate kidney function (Ajeniyi and Solomon, 2014), was not affected by the dietary inclusion of insect oil for both species of fish under this study.

5.3 Liver antioxidant capacity

In this study, only the content of MDA differed significantly between the dietary groups. The lower content of MDA in insect oil-based dietary fish groups relative to the CTR group suggested relatively lower levels of lipid peroxidation in the insect oil-based groups. The trend toward lower SOD activities in fish fed insect oil-based diets relative to the CTR indicated relatively lower oxidative stress by ROS in fish fed insect oil-based diets. In the gilthead seabream juveniles, BSFLO increased liver antioxidant enzyme activity without affecting lipid peroxidation levels, suggesting that the use of BSFLO increased oxidative stress in the liver, thereby necessitating increased activity of antioxidant enzymes to eliminate ROS (Moutinho et al., 2025). In *Onychostoma macrolepis*, the BSFLO-based diets significantly reduced the content of MDA and increased the activity of SOD in the liver (Gou et al., 2023).

On the other hand, GSH plays a significant role in detoxification through chemical and enzymatic reactions and its content tended to be higher in the insect oil fed fish compared to the control in both species (Ross, 1988). The total non-enzymatic antioxidant capacity (TAOC), an indicator of the ability of cells to counter damage caused by oxidative stress, was insignificant between dietary groups but overall was highest in fish fed insect oil-based diets, indicating improved antioxidation. Thus, the activity of SOD and contents of MDA, GSH, and TAOC across groups of fish fed experimental diets indicated that the use of BSFLO-based diets in both the European catfish and African catfish hybrid was associated with improved antioxidation status.

5.4 Expression of lipid metabolism and immune-related genes

Irrespective of the primary mechanism behind the increased liver DHA/EPA observed in this study, the lack of significant differences in the expression of *fads2* and *elovl2* between the dietary fish groups indicated that selective retention of DHA and catabolism of EPA could have been the mechanism in European catfish, since the expression of *cpt1a* was significantly upregulated in fish fed IO100. In a related study, significantly higher expression of *cpt1a* was also reported in juvenile *Onychostoma macrolepis* fed a diet containing 100% black soldier fly larvae oil (Gou et al., 2023). The *cpt1a* gene encodes a protein that catalyses the conversion of fatty acid-CoAs into fatty acid-carnitines for entry into the mitochondrial matrix from the cytoplasm, thus facilitating the β-oxidation of long-chain fatty acids (LCFAs) (Zheng et al., 2013). Thus, increased expression of *cpt1a* as a response to dietary insect oil indicated increased oxidation of LCFAs such as EPA, unlike DHA, which is considered a poor substrate for mitochondrial β-oxidation and is thus retained in tissues (Tocher, 2003). In addition, unlike the LCFAs which require cpt1a enzyme, MCFAs such as lauric acid can pass directly through the mitochondrial membrane without the need for the carnitine shuttle (Schönfeld and Wojtczak, 2016).

In the African catfish hybrid in this study, the expression of *cpt1a* was still highest in fish fed IO100 but with no statistical difference between the dietary fish groups. Both the juveniles of African catfish hybrid and European catfish fed the IO100 diet presented a significant upregulation of the *lpl* gene encoding lipoprotein lipase. Lipoprotein lipase hydrolyses triacylglycerols present in plasma lipoproteins (e.g., very low-density lipoproteins – VLDL, and chylomicrons), thereby providing free fatty acids (FFA) for either storage or oxidation in tissues (Lu et al., 2013). It thus appears that both the LCFAs and MCFAs were highly utilised as substrates in the energy-yielding reactions in the case of European catfish. The absence of significant differences in *cpt1a* expression in African catfish hybrid showed that the diets did not significantly influence the ability of this species to utilise LCFAs in β-oxidation, highlighting species-specific differences. Thus, the highly reduced content of lauric acid in the livers of African catfish hybrid fed insect oil-based diets suggested that MCFAs served as the primary energy source, especially in fish fed insect oil-based diets.

The expression of $LC_{(C20-24)}$-PUFA biosynthesis genes such as *fads2*, *elovl2*, and *elovl5* in both African catfish hybrid and European catfish indicated no major differences between the dietary fish groups. Similar results were reported on the hepatic expression of lipid biosynthesis genes in juveniles of *Onychostoma macrolepis* and rainbow trout (*Oncorhynchus mykiss*) fed a fish oil-based control diet and experimental diets containing black soldier fly larvae oil (Fawole et al., 2021; Gou et al., 2023). This indicates that dietary insect oils rich in the precursor molecules for $LC_{(C20-24)}$-PUFA biosynthesis can be used to replace fish and vegetable oils in the practical diets of catfish. The significant differences in liver n-6 PUFAs, including γ-linolenic acid

(C18:3n-6), dihomo-γ-linolenic acid (C20:3n-6), and arachidonic acid (C20:4n-6), between the fish groups despite similarity in relative expression of *fads2*, *elovl2* and *elovl5* suggest post-transcriptional differences in the expression of these genes, creating differences in $LC_{(C20-24)}$-PUFA biosynthesis. Similar results in the liver content of arachidonic acid were observed in juvenile black seabream (Jin et al., 2017).

Fish can also biosynthesise C16:0 and C18:0 *de novo* through the action of fatty acid synthase (*fas*) (Tocher, 2003). Thus, the higher relative contents of both C16:0 and C18:0 in the fish livers than observed in the corresponding diets were an indication of increased *de novo* synthetic activity by fatty acid synthase (*fas*). The similarity in the contents of C16:0 and C18:0 between dietary fish groups corresponded with the similarity in the relative expression of *fas*, glucose 6-phosphate dehydrogenase (*g6pd*) and 6-phosphogluconate dehydrogenase (*6pgd*). However, a linear increase in the expression of *g6pd* and *6pgd* with an increase in insect oil inclusion was only observed in European catfish. The genes *g6pd* and *6pgd* are key regulatory enzymes involved in NADPH production, which is essential for fatty acid biosynthesis. Thus, higher expression of *g6pd* and *6pgd* in European catfish juveniles fed insect oil-based diets indicated that replacing fish oil with BSFLO has a positive effect on the supply of NADPH, which is needed for the synthesis of lipids, thereby suggesting that dietary BSFLO could promote lipid synthesis and metabolism in this species.

The expression of genes of enzymes involved in lipid metabolism also relies on the expression of genes of transcription factors, including *pparα, pparγ, srebp1*, and *hnf4α*. *Srebp1* encodes a membrane-bound transcriptional factor that regulates the expression of genes encoding enzymes catalysing the synthesis of cholesterol, fatty acids, TAG, and phospholipids (Jin et al., 2017), and its expression was similar between the dietary groups in both species of this study. On the other hand, *pparα*, *pparγ*, and *hnf4α* encode nuclear receptors playing pivotal roles in dietary fatty acid-mediated effects on expression of lipid metabolism genes (Khan and Heuvel, 2003; Yin et al., 2011). Apart from *hnf4α*, which was significantly upregulated in European catfish fed the IO100 diet compared to the control, no significant differences were observed in the expression of *srebp1*, *pparα*, and *pparγ* in this species. The non-significant results in *pparα* and *pparγ* expression between juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets containing soybean oil and BSFLO (50:50) and those fed 100% BSFLO are also reported (Li et al., 2016). However, in the African catfish hybrid, the expression of *hnf4α* and *pparα* was significantly different between the dietary groups of this species, the major differences being between fish fed IO50 and those fed IO100. In the spleen, expression of immune-related genes was only significant for *irf-1*, being higher in the group fed IO50 and lower in the control, while in the head kidney, all the immune-related genes showed no statistical difference between the dietary fish groups. In rainbow trout, supplementation of BSFLO in the diets had an upregulatory effect on the expression of *irf-1* and *il-8* in the fish head kidneys but not in the intestine (Kumar et al., 2021), illustrating tissue-specific differences in the expression levels of immune-related genes

5.5. Intestinal histology and microbiota composition of the species

The effects of dietary BSFLO on histological changes of fish intestine were also investigated. The intestinal structural integrity of both species under this study indicated no major differences between groups of fish fed the CTR and those fed the insect oil-based diets (IO50 and IO100 diets). In addition, the intestinal mucosa is a barrier for pathogen invasion, and the intestinal bacteria are important in maintaining the barrier function of the intestinal mucosal system. Thus, functions of the host, such as immune response, nutrient absorption, and internal environment stability, can be kept in balance by the beneficial bacteria in the intestine.

However, the presence of harmful bacteria can increase the permeability of the mucosal layer, thereby allowing bacteria in food to pass through the mucosal barrier (Hooper et al., 2002; Hooper and Macpherson, 2010; Ma et al., 2021). In addition, several studies have indicated a direct correlation between oxidative stress and gut microbiota (Qiao et al., 2013; Nie et al., 2019; Wang et al., 2019). Therefore, the intestinal microbiota composition of African catfish hybrid and European catfish were studied in order to examine the influence of dietary incorporation of BSFLO on gut microbiota. This is because of the importance of microbiota in host health and the influence of diet on the diversity, and composition of the bacterial community (Arias-Jayo et al., 2018; Larios-Soriano et al., 2021). In both the African catfish hybrid and the European catfish, there were no major differences in bacterial diversity between groups fed the different diets (CTR, IO50, and IO100). While the bacterial community in individual samples from European catfish was dominated by either Fusobacteriota or Spirochaetota, all the samples of African catfish hybrid were dominated by Fusobacteriota. Other major bacterial phyla included Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota, and Verrucomicrobiota. These bacterial phyla were also detected in channel catfish and blue catfish reared indoors in flow-through tanks, the dominant phyla being Fusobacteriota and Firmicutes, depending on the source environment of the strains (Bledsoe et al., 2018). Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota and Fusobacteriota were also reported in channel catfish fed the control diet and groups fed a diet containing *Saccharomyces cerevisiae* (Xia et al., 2022).

The inclusion of insect oil in the diets of African catfish hybrid and European catfish was associated with an increased Firmicutes to Bacteroidota ratio. A higher Firmicutes to Bacteroidota ratio is associated with enhanced oxidative response and more resistance to pathogens. This is because Firmicutes are considered to be involved in maintaining intestinal barrier integrity and play a key role in modulating host inflammation (Mariat et al., 2009; Chae et al., 2016; Louis and Flint, 2017), while Bacteroidota constitutes members with the ability to release lipopolysaccharide molecules, consequently leading to higher inflammatory responses (Ortega-Hernández et al., 2020). The observed trends in the Firmicutes/Bacteroidota ratio across the dietary groups in both species of fish examined, indicated that BSFLO exhibits antioxidant and anti-inflammatory effects closely associated with the increased Firmicutes/Bacteroidota ratio. The phylum Proteobacteria as well as Bacteroidota contain several genera known to be pathogenic to fish, with a high number of pathogens belonging to the phylum Proteobacteria. The pathogenic Proteobacteria genera such as *Aeromonas*, *Pseudomon*as, *Escherichia-shigella*, and *Legionella* identified among the gut microbiota, can generate toxins, and cause gut microbiota disturbance and oxidative stress (Mekasha and Linke, 2021). Indeed, the relative abundance of Proteobacteria was positively correlated with liver MDA levels. The levels of MDA and the relative abundance of Proteobacteria were lower in fish fed insect oil containing diets compared to the control group, indicating that the insect oil provided some level of protection in fish against these pathogens.

The phylum Fusobacteriota was dominated by the genus *Cetobacterium*, consistent with results reported on blue catfish (Bledsoe et al., 2018). In related studies on African catfish, *Cetobacterium* was reported as a common member especially in the young catfish (Skvortsova et al., 2023). In this study, *Cetobacterium* was negatively correlated with TAOC and positively correlated with liver SOD activity, indicating that it plays a role in health protection and that the administration of BSFLO in fish diet can ameliorate oxidative stress and reduce the relative abundance of *Cetobacterium*. The genus *Brevinema* was the most dominant in the phylum Spirochaetota and was identified in all digesta 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), and was also reported in the mucosa samples of European seabass (*Dicentrarchus labrax*) (Serra et al., 2021). In red hybrid tilapia (*Oreochromis* spp.), the elevation of *Brevinema* population was associated with a higher mortality rate (Paimeeka et al., 2024). Our study revealed that the relative abundance of *Brevinema* was positively correlated with liver TAOC and negatively correlated with liver SOD activity, demonstrating that *Brevinema* in European catfish flourishes in conditions of low oxidative stress. It is thus very unlikely that the *Brevinema* recorded in this study could be an opportunistic pathogen in European catfish cultured at a mean temperature of 24.5 °C. However, further studies are required to fully understand the effects of such variations in *Brevinema* abundance on the health of European catfish in aquaculture. Among the Firmicutes, the genus *Exiguobacterium* was the most dominant in European catfish while *Romboutsia* was the dominant Firmicute in African catfish hybrid. The probiotic potential of species such as *Exiguobacterium acetylicum* within the genus *Exiguobacterium* has been reported to effectively inhibit the growth of fish pathogens including *Vibrio* spp. and *Aeromonas hydrophila* (Xie et al., 2024). In our study, only one ASV belonging to the genus *Aeromonas* was encountered in each species while *Vibrio* spp. was not encountered in any sample for both species. The genus *Romboutsia* (phylum Firmicutes) in addition to *Cetobacterium* (phylum Fusobacteriota) and *Plesiomonas* (phylum Proteobacteria) have been characterised as common members in the guts of healthy yellow tail fish, with their abundances decreasing in the diseased fish (Yang et al., 2023). Differences were observed in the top 10 most abundant genera of intestinal microbiota in the two species under this study. For instance, in the European catfish, other notable genera ranked among the top 10 included the Proteobacteria genera: *Escherichia-Shigella* and *Achromobacter*; the Actinobacteriota genera: *Leifsonia*, *Corynebacterium*, and *Bifidobacterium*; and the Firmicutes: *Bacillus* and *Aerosphaera*.

In the African catfish, a majority of the genera contributing to the top 10 abundant genera were members of the phylum Firmicutes, and included *Turicibacter*, *Clostridium sensu stricto 1*, *Candidatus Arthromitus*, *Terrisporobacter*, *Epulopiscium* and *Staphylococcus*. Other genera were members of the phylum Proteobacteria (e.g., *Plesiomonas*), and phylum Actinobacteriota (e.g., *Aurantimicrobium*). The high lipid content of SFAs such as lauric acid has been reported to be associated with increased abundance of lactic acid bacteria, LAB (Rimodi et al., 2018; Huyben et al., 2020). In our study such enrichment was not observed similar to the observations reported on Atlantic salmon (Weththasinghe et al., 2022). In addition, the relative abundances of bacterial functional pathways in gut microbiota of European catfish and African catfish hybrid were not significantly different between fish fed the CTR, IO50, and IO100 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, *Rachycentron canadum* (Reinoso et al., 2023).

6. CONCLUSIONS

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.

7. NEW SCIENTIFIC RESULTS

7.1 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 Cº rearing temperature.

7.2 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.

7.3 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.

7.4 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.

7.5 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.

8. SUMMARY

Dietary intake of long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is associated with health benefits in humans. Fish is regarded as the primary source of n-3 LC-PUFA and thus, dietary intake of fish has been recommended as a way of promoting human health. However, many commonly consumed fish from aquaculture have relatively low levels of n-3 LC-PUFA and a higher n-6 LC-PUFA, caused by the increasing usage of n-6 PUFA-rich vegetable oils (VO) in aquafeeds—a strategy to avoid over-reliance on the scarce fish oil. This consequently results in a low intake of n-3 LC-PUFA and a concurrent high intake of n-6 PUFA. In search for new alternatives to fish oil, insects might be part of the solution, because they form part of the natural diets of many fish species in the wild.

Among the insects with potential as alternative source of lipids for aquafeeds, is the black soldier fly (*Hermetia illucens*). Black soldier fly larvae (BSFL) are rich in saturated- and monounsaturated-fatty acids (SFA and MUFA, respectively) which have been implicated in aiding LC-PUFA retention, thereby enabling the fish to meet its physiological demands. It also contains sufficient quantities of α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) required for LC- PUFA biosynthesis in freshwater fish. Although a number of investigations have been conducted utilizing black soldier fly larvae meal, limited studies have investigated the nutritional value of black soldier fly larvae oil especially in catfish. Thus, the influence of dietary oil derived from black soldier fly on fish growth performance, lipid metabolism, immunity, antioxidation and intestinal microbiota remain to be fully explored. The overall objective of this study was to increase the production of farmed catfish while maintaining lipid quality and decreasing the use of fish- and vegetable-oils in the feeds. Therefore, two feeding trials with a similar experimental set up were designed to examine the influence of black soldier fly larvae oil (BSFLO) on the growth performance of African catfish hybrid (*Clarias gariepinus* × *Heterobranchus longifilis*) and European catfish (*Silurus glanis*, L).

Three diets (a control and two experimental) were formulated, the main differences being the oil source (Fish oil, rapeseed oil and BSFLO) and physical properties of the pellets (sinking feed for European catfish, floating feed for African catfish). The control diet (CTR) contained a mixture of fish oil and rapeseed oil (50:50), while the two experimental diets contained either BSFLO replacing 50% of the fish oil and rapeseed oil mixture (diet IO50) or 100% BSFLO (diet IO100). Thus, during the two feeding trials lasting for 7 weeks (African catfish hybrid trial) or 8 weeks (European catfish trial), fish were either fed a control diet (CTR) or insect oil-based diets (IO50 and IO100). Results from the two trials indicated that all the diets promoted adequate growth and nutrient utilization with no major differences observed between the dietary treatments. The activities of digestive enzymes which are considered predictors of potential feed utilization and growth differences in fish were similar between the dietary fish groups for both species. However, the apparent digestibility coefficients (ADCs) of dry matter and crude protein were significantly different between the dietary groups of European catfish but similar between dietary groups of African catfish. Apart from moisture content which significantly varied between dietary groups of African catfish hybrid, the whole-body crude protein, crude lipid, and ash were similar between dietary groups in both species.

The lipid composition of fish tissues largely reflected that of their diets, with the two species exhibiting similarities in the trends of tissue fatty acids across the dietary groups. While the whole-body saturated fatty acid profile of European catfish indicated a higher content in fish fed insect oil-based diets (IO50 and IO100) than in fish fed the CTR diet, such statistical differences were not observed in African catfish hybrid. However, statistical differences were

observed in the individual fatty acids between dietary groups in each species. In addition, the whole-body content of MUFA, EPA, DHA, and total n-3 PUFA significantly decreased with increase in dietary insect oil fraction in both species. The whole-body total PUFA as well as n-6 PUFA indicated no statistical difference between the dietary groups in both species. Although the total lipid content of the liver was not affected in both species, the individual fatty acid profiles were significantly influenced by the diets. The liver DHA content showed no statistical difference between the dietary groups, but there was a general tendency of DHA content to increase with increase in insect oil fraction in the diets for both species. The nutritional quality of the lipids in fish fed the experimental diets was assessed by calculating the Atherogenic Index, Thrombogenic Index, and Polyene Index. In the liver of European catfish, these indexes were statistically similar between the dietary groups while in the whole body, significant results were obtained, with the Atherogenic and Thrombogenic Indices linearly increasing with increasing BSFLO fraction in the diets and a linear decrease in Polyene index with increase in BSFLO fraction in the diets. In African catfish hybrid, all the lipid quality indices of the liver and whole body were similar between the different dietary groups. The levels of all lipid quality indices determined were within the range considered low and safe for humans.

Differences and similarities in the hepatic expression of genes involved in LC-PUFA biosynthesis (*elovl2*, *elovl5*, and *fads2*), de novo fatty acid synthesis (*fas*, *g6pd* and *6gpd*), triacylglycerol metabolism and β-oxidation (*cpt1a*, *lpl* and *hadh*), and regulation of lipids (*pparα*, *pparγ*, *srebp1c* and *hnf4α*) were observed. In the European catfish, the expression of *cpt1a*, *lpl*, and *hnf4α* was significantly highest in fish fed IO100 diet, while in the African catfish hybrid, only *hadh*, *lpl*, *pparα* and *hnf4α* significantly differed between fish fed IO50 and fish fed IO100 diets. Thus, the low content of medium chain fatty acids (MCFAs) in the liver compared to the diets and the significant expression of *cpt1a*, indicated that European catfish fed IO100 highly utilised both the long chain fatty acids (LCFAs) and MCFAs in the energy yielding reactions. The highly reduced content of lauric acid in the livers of African catfish hybrid fed insect oil-based diets suggested that MCFAs served as the primary energy source in fish fed insect oil-based diets. The expression of liver *sod-1* gene involved in antioxidation was similar between the dietary groups in both species. Moreover, the activities of liver SOD enzyme were similar between the dietary groups of fish. Based on the content of MDA, TAOC and reduced GSH, there was a general tendency for liver antioxidant capacity to increase with increase in insect oil inclusion in the diets in both species of this study. The expression of immune-related genes in the spleen of European catfish was only significant for *irf-1* and not *tlr-5*, *il-8*, *rag-1* and *tgf-β1*, while in the head kidney, all the immune related genes showed no statistical difference between the dietary fish groups, illustrating tissue specific differences.

The histological examination showed normal histological structures of the intestine in both species. In addition, alpha diversity and community composition of intestinal microbiota of fish fed different diets were similar between the dietary groups in both species. The bacterial community in individual samples from European catfish was dominated by either Fusobacteriota or Spirochaetota, while all the samples of African catfish hybrid were dominated by Fusobacteriota. Other bacterial phyla included Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota, and Verrucomicrobiota, considered common members of fish microbiota. An increase in the Firmicutes/Bacteroidota ratio with dietary insect oil fraction indicated that BSFLO exhibits antioxidant and anti-inflammatory effects closely associated with the increased Firmicutes/Bacteroidota ratio. The relative abundances of bacterial functional pathways were similar between fish fed the CTR, IO50, and IO100 diets. The most abundant pathways were associated with metabolism, environmental information processing, cellular processes, and genetic information processing.

9. ÖSSZEFOGLALÁS

A hosszú szénláncú, többszörösen telítetlen n-3 zsírsavak (n-3 LC-PUFA), például az eikozapentaénsav (EPA) és a dokozahexaénsav (DHA) étrendi bevitele előnyökkel jár az emberi egészségre nézve. A halakat az n-3 LC-PUFA elsődleges forrásának tekintik, ezért a halak étrendi fogyasztása az emberi egészség támogatásának egyik eszköze. Sok, gyakran fogyasztott, akvakultúrából származó hal azonban viszonylag alacsony n-3 LC-PUFA-szintet és magasabb n-6 LC-PUFA-szintet tartalmaz. Ennek oka az n-6 PUFA-ban gazdag növényi olajok (VO) növekvő használata az akvakultúrákban és a hal takarmányiparban, melyet a tengeri eredetű források szűkülése váltott ki. Ez a tendencia azonban alacsonyabb n-3 LC-PUFA és magasabb n-6 PUFA bevitelét eredményez a halak esetében. A halolaj új alternatíváinak keresése során a rovarok ígéretes megoldásként szolgálhatnak, mivel a vadon élő halak étrendjében természetes módon szerepelnek.

A rovarok közül a fekete katonalégy (*Hermetia illucens*) az akvakultúrákban használt takarmányok alternatív lipidforrása lehet. A fekete katonalégy lárvái (BSFL) gazdagok telített és egyszeresen telítetlen zsírsavakban (SFA és MUFA), amelyekről kimutatták, hogy elősegítik az LC-PUFA-k megtartását, lehetővé téve ezzel a halak számára, hogy kielégítsék élettani igényeiket. Emellett megfelelő mennyiségben tartalmaznak az édesvízi halak LC-PUFA-bioszintéziséhez szükséges α-linolénsavat (18:3n-3) és linolsavat (18:2n-6).

Bár a fekete katonalégy lárvájából készült liszt táplálóértékét számos tanulmány vizsgálta, a lárvából származó zsír táplálóértékére, különösen harcsák esetében, csak korlátozott számú kutatás irányult. Így a fekete katonalégyből származó takarmány olajnak a halak növekedési teljesítményére, lipidanyagcseréjére, immunrendszerére, antioxidáns kapacitására és bélmikrobiótájára gyakorolt hatását még nem tárták fel teljes mértékben. A vizsgálataink általános célja az volt, hogy növeljük a tenyésztett harcsák termelési teljesítményét, megőrizve a lipidminőséget és csökkentve a takarmányok hal- és növényi eredetű olaj tartalmát. Ennek érdekében két, hasonló kísérleti elrendezésű takarmányozási vizsgálatot végeztünk el, amelyek célja annak értékelése volt, hogy a fekete katonalégy lárvaolajának (BSFLO) milyen hatása van az afrikai harcsa hibrid (*Clarias gariepinus* × *Heterobranchus longifilis*) és az európai harcsa (*Silurus glanis* L.) ivadékok növekedési teljesítményére.

A kísérletekhez háromféle takarmányt állítottunk össze hasonló beltartalmi paraméterekkel, amelyek közötti fő különbség az olajforrásokban (halolaj, repceolaj és BSFLO) és a pelletek fizikai tulajdonságaiban rejlettek: süllyedő takarmányt használtunk az európai harcsák számára, míg lebegőt az afrikai harcsák esetében. A kontrolltáp (CTR) halolaj és repceolaj keverékét tartalmazta (50:50 arányban), míg a két kísérleti tápban a halolaj és repceolaj keverékének 50%-át (IO50 táp) vagy 100%-át (IO100 táp) fekete katonalégy lárvaolajjal (BSFLO) helyettesítettük. Az etetési kísérletet nyolc héten keresztül folytattuk európai harcsa ivadékokkal és hét héten keresztül az afrikai harcsa hibrid állománnyal a Halászati Kutatóközpont halnevelő rendszerében. A vizsgálatok eredményei azt mutatták, hogy mindhárom táp elősegítette a megfelelő növekedést és tápanyag-hasznosítást, és a takarmányozási kezelések között nem mutatkoztak jelentős különbségek.

Az emésztőenzimek aktivitása, amelyet a halak takarmányhasznosítási képességének és növekedési potenciáljának indikátoraként használnak, mindkét faj esetében hasonló volt a különböző diétás csoportok között. A szárazanyag és a nyersfehérje látszólagos emészthetőségi együtthatók (ADC) ugyanakkor eltéréseket mutattak az európai harcsa egyes csoportjai között, míg az afrikai harcsák esetében ezek hasonlóak voltak.

A haltest lipidjének zsírsavösszetétele nagymértékben tükrözte a táplálék összetételét, és mindkét faj esetében hasonló zsírsav-összetételi trendek voltak megfigyelhetők a táplálékcsoportok között. Az európai harcsa egész testre kiterjedő telített zsírsavprofilja a rovarolaj-alapú étrenddel (IO50 és IO100) táplált halaknál magasabb volt, mint a kontroll (CTR) étrenddel etetett halaknál. Ezzel szemben az afrikai harcsa hibrideknél ilyen statisztikai különbségeket nem figyeltünk meg. Az egyes zsírsavak szintjén azonban mindkét faj esetében statisztikai eltérések mutatkoztak az egyes táplálékcsoportok között. A MUFA, az EPA, a DHA és az összes n-3 PUFA teljes testtartalma szignifikánsan csökkent a táplálékban lévő rovarolaj-frakció növekedésével mindkét faj esetében. Ugyanakkor az egész test összes PUFA-tartalma, valamint az n-6 PUFA szintje nem mutatott statisztikai különbséget a táplálékcsoportok között egyik fajnál sem. A máj összes lipidtartalmát egyik faj esetében sem befolyásolta a különböző étrend, azonban jelentősen módosította a táplálék összetétel az egyes zsírsavprofilokat. A máj DHA-tartalma nem mutatott statisztikai különbséget a táplálékcsoportok között, de a DHA-tartalom általános tendenciája mindkét faj esetében a rovarolaj-frakció növekedésével nőtt.

A kísérleti táppal etetett halak lipidjeinek minőségét az aterogén index, a trombogén index és a polién index segítségével értékeltük. Az európai harcsa esetében a májban ezek az indexek statisztikailag hasonlóak voltak a különböző takarmányozási csoportok között. Az egész test lipidjei esetében azonban szignifikáns különbségek mutatkoztak: az aterogén és trombogén indexek lineárisan növekedtek a takarmányok BSFLO-frakciójának növekedésével, míg a polién index lineárisan csökkent. Az afrikai harcsa hibridnél csak a máj trombogén indexe mutatott szignifikáns eltérést a táplálékcsoportok között. Mindazonáltal az összes meghatározott lipidminőségi index értéke az emberi táplálkozás szempontjából alacsonynak és biztonságosnak tekintett tartományon belül maradt, így a halak lipidjei továbbra is egészséges táplálékforrásnak számítanak.

Az LC-PUFA bioszintézisben (*elovl2, elovl5, fads2*), a *de novo* zsírsavszintézisben (*fas, g6pd, 6gpd*), a triacilglicerin-anyagcserében és β-oxidációban (*cpt1a, lpl, hadh*), valamint a lipidek szabályozásában (*pparα, pparγ, srebp1c, hnf4α*) részt vevő gének májban történő expressziójában egyaránt hasonlóságokat és különbségeket figyeltünk meg a két faj esetében. Az európai harcsában a *cpt1a*, az *lpl* és a *hnf4α* expressziója szignifikánsan magasabb volt az IO100 táppal etetett halakban. Az afrikai harcsa hibrid esetében viszont csak a *hadh,* az *lpl*, a *pparα* és a *hnf4α* expressziója mutatott szignifikáns eltérést az IO50 és az IO100 táppal etetett csoportok között. Az európai harcsák esetében a májban az MCFA-k alacsony tartalma, valamint a *cpt1a* jelentős expressziója arra utalt, hogy az IO100 táppal etetett halak mind az LCFA-kat, mind az MCFA-kat intenzíven használták energiatermelő reakciókban. Szintén hasonló megfigyelést tettünk az afrikai harcsa hibridek májában, hogy a rovarolaj-alapú táplálék hatására jelentősen csökkent a laurinsavtartalom, mely arra engedett következtetni, hogy az MCFA-k az elsődleges energiaforrásként szolgáltak ezeknél a halaknál. Az antioxidáns rendszer működésében szerepet játszó májban vizsgált *sod-1* gén expressziója mindkét faj esetében hasonló volt a táplálkozási csoportok között, ahogy a máj SOD enzim aktivitása is. A máj antioxidáns kapacitását vizsgálva az MDA, a TAOC és a redukált GSH tartalma alapján mind az afrikai harcsa hibrideknél, mind az európai harcsáknál általában növekedés volt megfigyelhető a rovarolaj-tartalom étrendbeli emelkedésével. Az immunrendszerrel kapcsolatos gének expressziója az európai harcsa lépében csak az *irf-1* esetében volt szignifikáns, a *tlr-5, il-8, rag-1* és *tgf β-1* esetében nem, míg a fejvese esetében az egyik immunrendszerrel kapcsolatos gén sem mutatott statisztikai különbséget a csoportok között, ami szövetspecifikus különbségeket mutat. Hasonlóan nem találtunk eltérést az afrikai harcsa hibrid esetében a megvizsgált két immungén, a *tlr-5* és *tgf β-1*esetében sem.

A szövettani vizsgálat mindkét fajnál normális szövettani struktúrákat mutatott a bélben. Ezenkívül a különböző táplálékkal etetett halak bélmikrobiótájának alfa-diverzitása és közösségi eloszlása mindkét fajnál hasonló volt a táplálékcsoportok között. Az európai harcsa egyedi mintáiban a baktériumközösséget vagy a Fusobacteriota vagy a Spirochaetota dominálta, míg az afrikai harcsa hibrid összes mintájában a Fusobacteriota dominált. Az egyéb baktériumtársulások közé tartoztak a Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota és Verrucomicrobiota, amelyek a halak mikrobiótájának gyakori tagjai. A Firmicutes/Bacteroidota arány növekedése a táplálékkal bevitt rovarolaj-frakcióval azt jelezte, hogy a BSFLO antioxidáns és gyulladáscsökkentő hatásai szorosan kapcsolódnak a megnövekedett Firmicutes/Bacteroidota arányhoz. A bakteriális funkcionális útvonalak relatív abundanciája hasonló volt a CTR, IO50 és IO100 táplálékkal etetett halak között. A legnagyobb mennyiségben előforduló útvonalak az anyagcseréhez, a környezeti információk feldolgozásához, a sejtfolyamatokhoz és a genetikai információk feldolgozásához kapcsolódtak.

10. ACKNOWLEDGEMENTS

The work of Robert Egessa was supported by the Stipendium Hungaricum Scholarship Program of the Hungarian Government. The research work was also supported by the Hungarian National Research, Development, and Innovation Office, Serbian-Hungarian Joint Research project (contract number 2023-1.2.4-TÉT-2023-00018). The development of feed was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant number 451–03-66/2024–03/200222).

First and foremost, I would like to thank the Almighty God for His kindness, mercy, and love. He has protected and kept me and my family healthy throughout my studies.

I would like to express my sincere appreciation and thanks to my supervisor, Dr Zsuzsanna J. Sandor, for the continuous and overwhelming support towards my PhD research and related studies, for the patience, motivation, and guidance. You inspired me and provoked my thinking towards successful research work. Thank you once again for initiating this research and making me to come here in Hungary to explore the field of lipid nutrition research on catfish.

I would like to acknowledge the staff at Research Centre for Aquaculture and Fisheries (HAKI), for their help and guidance during this work, right from the experimental set up and feeding trials to the sampling and sample analyses. I am also grateful for the good companionship and support rendered to me by fellow PhD students at HAKI.

I would like to thank my family especially my wife, Nakirijja Harriet for the support and for taking care of our last-born child (Wejuli Ephraim Egessa), who I left when he was only two months old, and to the rest of the children (Esther Nekesa, Ethan Egessa and Were Elvis) for their endurance during my absence.

REFERENCES

Adeoye, A.A., Akegbejo-Samsons, Y., Fawole, F.J., Davies, S.J., 2020. Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*Clarias gariepinus*): Impact on growth, body index, and hematological parameters. J. World Aquac. Soc. 51, 1024-1033.

Agbaga, M., 2016. Different mutations in ELOVL4 affect very long chain fatty acid biosynthesis to cause variable neurological disorders in humans, in: Bowes Rickman, C., LaVail, M.M., Anderson, R.E., Grimm, C., Hollyfield, J., Ash, J. (Eds.), Retinal Degenerative Diseases. Adv. Exp. Med. Biol. 854, 129–135.

Ajeniyi, S.A., Solomon, R.J., 2014. Urea and creatinine of *Clarias gariepinus* in three different commercial ponds. Nat. Sci. 12, 124–138.

Alfiko, Y., Xie, D., Astuti, R. T., Wong, J., Wang, L., 2021. Insects as a feed ingredient for fish culture: Status and trends. Aquac. Fish. 7, 166-178.

Alp, A., Kara, C., Büyükçapar, H. M., 2004. Reproductive biology in a native European catfish, *Silurus glanis* L., 1758, population in Menzelet Reservoir. Turkish J. Vet. Anim. Sci. 28, 613-622.

Andreeva, A. M., 2010. Structure of fish serum albumins. J. of Evol. Biochem. and Phys. 46, 135-144.

AOAC., 2000. Methods of analysis of AOAC International. AOAC INTERNATIONAL, Maryland, USA

Arias-Jayo, N., Abecia, L., Alonso-Sáez, L., Ramirez-Garcia, A., Rodriguez, A., Pardo, M.A., 2018. High-Fat Diet Consumption Induces Microbiota Dysbiosis and Intestinal Inflammation in Zebrafish. Microb. Ecol. 76, 1089–1101.

Arts, M.T., Kohler, C.C., 2009. Health and Condition in Fish: The Influence of lipids on Membrane Competency and Immune Response, in: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds), Lipids in Aquatic Ecosystems. Springer, New York, 237-256.

Ayisi, C.L., Yamei, C., Zhao, J.L., 2018. Genes, transcription factors and enzymes involved in lipid metabolism in fin fish. Agri Gene 7, 7-14.

Azadmanesh, J., Lutz, W. E., Coates, L., Weiss, K. L., Borgstahl, G. E., 2021. Direct detection of coupled proton and electron transfers in human manganese superoxide dismutase. Nat. Commun. 12, 2079.

Babalola, T. O., Oyawale, F. E., Adejumo, I. O., Bolu, S. A., 2016. Effects of dietary fish oil replacement by vegetable oil on the serum biochemical and haematological parameters of African catfish (*Heterobranchus longifilis*) fingerlings. Iran. J. Fish. Sci. 15, 775-788.

Bakke, A. M., Glover, C., Krogdahl, Å., 2011. Feeding, Digestion and Absorption of Nutrients, in: Grossel, M., Farell A. P., Brauner, C.J. (Eds.), The Multifunctional Gut of Fish. Academic Press, San Diego, 57-110.

Balcázar, J. L., De Blas, I., Ruiz-Zarzuela, I., Vendrell, D., Gironés, O., Muzquiz, J. L., 2007. Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (*Oncorhynchus mykiss*). FEMS Immunol. Med. Microbiol. 51, 185-193.

Banaee, M., 2020. Alkaline phosphatase activity as a biochemical biomarker in aqua-toxicological studies. Int. J. Aquat. Biol. 8, 143-147.

Banerjee, G., Ray, A.K., 2017. Bacterial symbiosis in the fish gut and its role in health and metabolism. Symbiosis 72, 1-11.

Barroso, F.G., de Haro, C., Sánchez-Muros, M.J., Venegas, E., Martínez-Sánchez, A., Pérez-Bañón, C., 2014. The potential of various insect species for use as food for fish. Aquaculture 422-423, 193–201.

Bartoli, M.L., Novelli, F., Costa, F., Malagrinò, L., Melosini, L., Bacci, E., Cianchetti, S.,

Dente, F.L., Di Franco, A., Vagaggini, B., Paggiaro, P.L., 2011. Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. Mediators Inflamm. 891752.

Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E., Guillemin, K., 2006. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev. Biol. 297, 374–86.

Belghit, I., Waagbø, R., Lock, E.-J., Liland, N.S., 2019. Insect-based diets high in Lauric acid reduce liver lipids in freshwater Atlantic Salmon. Aquac. Nutr. 25, 343–357

Bell, J. G., McGhee, F., Campbell, P. J., Sargent, J. R., 2003. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". Aquaculture 218, 515-528.

Bledsoe, J.W., Waldbieser, G.C., Swanson, K.S., Peterson, B.C. Small, B.C., 2018. Comparison of channel catfish and blue catfish gut microbiota assemblages shows minimal effects of host genetics on microbial structure and inferred function. Front. Microbiol. 9, 1073.

Boukouvala, E., Antonopoulou, E., Favre-Krey, L., Diez, A., Bautista, J. M., Leaver, M. J., Tocher, D.R., Krey, G., 2004. Molecular characterization of three peroxisome proliferator-activated receptors from the sea bass (*Dicentrarchus labrax*). Lipids 39, 1085-1092.

Boyd, C. E., 2015. Overview of aquaculture feeds: Global impacts of ingredient use, in: Allen Davis, D. (Eds.), Feed and feeding practices in aquaculture. Woodhead Publishing, 3-25.

Bruton M., 1979. The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. Trans. Zool. Soc. London 35, 47–114.

Bu, X., Chen, A., Lian, X., Chen, F., Zhang, Y., Muhammad, I., Ge, X., Yang, Y., 2017. An evaluation of replacing fish meal with cottonseed meal in the diet of juvenile Ussuri catfish *Pseudobagrus ussuriensis*: Growth, antioxidant capacity, nonspecific immunity and resistance to *Aeromonas hydrophila*. Aquaculture 479, 829-837.

Burgos, F. A., Ray, C. L., Arias, C. R., 2018. Bacterial diversity and community structure of the intestinal microbiome of Channel Catfish (*Ictalurus punctatus*) during ontogenesis. Syst. Appl. Microbiol. 41, 494-505.

Butts, I. A. E., Baeza, R., Støttrup, J. G., Krüger-Johnsen, M., Jacobsen, C., Pérez, L., ..., Tomkiewicz, J., 2015. Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in European eel. Comp. Biochem. and Phys. Part A: Mol. Integr. Physiol. 183, 87-96.

Cabano, M. O., 2017. Impact of fish meal replacement by alternative and sustainable ingredients in diets for gilthead seabream (*Sparus aurata* Linnaeus, 1758) juveniles (Doctoral dissertation, Universidade do Algarve (Portugal).

Calder, P.C., 2005. Polyunsaturated fatty acids and inflammation. Biochem. Soc. Trans. 33, 423-427.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A. W., Johnson, A. J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. methods 13, 581-583.

Cani, P.D., Neyrinck, A.M., Fava, F., Knauf, C., Burcelin, R.G., Tuohy, K.M., Gibson, G.R., Delzenne, N.M., 2007. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50, 2374–2383.

Carmona-Antoñanzas, G., Tocher, D. R., Martinez-Rubio, L., Leaver, M. J., 2014. Conservation of lipid metabolic gene transcriptional regulatory networks in fish and mammals. Gene 534, 1-9.

Carol, J., Benejam, L., Benito, J., García-Berthou, E., 2009. Growth and diet of European

catfish (*Silurus glanis*) in early and late invasion stages. Fundam. Appl. Limnol. 174, 317-328.
Castro, L.F.C., Monroig, Ó., Leaver, M.J., Wilson, J., Cunha, I., Tocher, D.R., 2012. Functional desaturase Fads1 (Δ5) and Fads2 (Δ6) orthologues evolved before the origin of jawed vertebrates. PLoS One 7, e31950
Castro, L.F.C., Tocher, D.R., Monroig, O., 2016. Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. Prog. in Lipid Res. 62, 25-40.
Catap, E. S., Kho, M. J. L., Jimenez, M. R. R., 2018. In vivo nonspecific immunomodulatory and antispasmodic effects of common purslane (*Portulaca oleracea* Linn.) leaf extracts in ICR mice. J. of Ethnopharmac. 215, 191-198.
Chae, J.P., Pajarillo, E.A., Oh, J.K., Kim, H., Kang, D.K., 2016. Revealing the combined effects of lactulose and probiotic enterococci on the swine faecal microbiota using 454 pyrosequencing. Microb. Biotechnol. 9,486– 95.
Cherian, D. A., Peter, T., Narayanan, A., Madhavan, S. S., Achammada, S., Vynat, G. P., 2019. Malondialdehyde as a marker of oxidative stress in periodontitis patients. J. Pharm. Bioallied Sci. 11(Suppl 2), S297.
Cho, J. H., Kim, I. H., 2011. Fish meal–nutritive value. J. of Anim. Physiol. Anim. Nutr. 95, 685-692.
Clarke, S. D., Jump, D. B., 1996. Polyunsaturated fatty acid regulation of hepatic gene transcription. Lipids 31, S7-S11.
Codabaccus, B.M., Carter, C.G., Bridle, A.R., Nichols, P.D., 2012. The "n-3 LC-PUFA sparing effect" of modified dietary n-3 LC-PUFA content and DHA to EPA ratio in Atlantic salmon smolt. Aquaculture 356, 135–140.
Cook, H.W., McMaster, C.R., 2002. Fatty acid desaturation and chain elongation in eukaryotes, in: Vance, D.E., Vance, J.E. (Eds.), Lipids, Lipoproteins and Membranes (4th Ed.). Elsevier, 181–204.
Corrêa, C. F., Nobrega, R. O., Mattioni, B., Turchini, G. M., Fracalossi, D. M., 2023. LC-PUFA endogenous biosynthesis is improved in Nile tilapia fed plant-oil sources at cold suboptimal temperature. Aquaculture 562, 738832.
Costantini, L., Molinari, R., Farinon, B., Merendino, N., 2017. Impact of omega-3 fatty acids on the gut microbiota. Int. J. Mol. Sci. 18, 2645.
Craig, S., Helfrich, L., Kuhn, D.D., Schwarz, M. H., 2017. Understanding fish nutrition, feeds, and feeding. Virginia Cooperative extension, publication 420-256
Cucherousset, J., Horky, P., Slavík, O., Ovidio, M., Arlinghaus, R., Boulêtreau, S., Britton, R., Garcia-Berthou, E., Santoul, F., 2018. Ecology, behaviour and management of the European catfish. Rev. Fish Biol. Fish. 28, 177-190.
Daniel, N., 2018. A review on replacing fish meal in aqua feeds using plant protein sources. Int. J. Fish. Aquat. Stud. 6, 164-179.
Das, U.N., 2008. Can essential fatty acids reduce the burden of disease (s)? Lipids Health Dis. 7, 1-5.
David, J. A., 2006. Water quality and accelerated winter growth of European catfish using an enclosed recirculating system. Water Environ. J. 20, 233-239.
Davis, S., Ramm, K., Ju, Z.Y., Soller, F., 2017. Insects as a Feed Ingredient in Aquafeeds. by World Aquaculture Magazine. 2017, 61-63
Dayrit, F. M., 2015. The properties of lauric acid and their significance in coconut oil. J. Am. Oil Chem. Soc. 92, 1–15.
Deng, J., Mai, K., Chen, L., Mi, H., Zhang, L., 2015. Effects of replacing soybean meal with rubber seed meal on growth, antioxidant capacity, non-specific immune response, and resistance to *Aeromonas hydrophila* in tilapia (*Oreochromis niloticus* × *O. aureus*). Fish

Shellfish Immunol. 44, 436–444.
Dumas, A., Raggi, T., Barkhouse, J., Lewis, E., Weltzien, E., 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 492, 24-34.
Eichmann, T. O., Kumari, M., Haas, J. T., Farese, R. V., Zimmermann, R., Lass, A., Zechner, R., 2012. Studies on the substrate and stereo/regioselectivity of adipose triglyceride lipase, hormone-sensitive lipase, and diacylglycerol-O-acyltransferases. J. of Biol. Chem. 287, 41446-41457.
Eichmiller, J.J., Hamilton, M.J., Staley, C., Sadowsky, M.J., Sorensen, P.W., 2016. Environment shapes the fecal microbiome of invasive carp species. Microbiome 4, 44.
Ellis, A. I., 1990. Lysozyme assays, in: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., Van Muiswinkel, W.B. (Eds), Techniques in fish immunology, vol 1. SOS Publications, Fair Haven, USA, 101-103.
El-Moghazy, M., Zedan, N. S., El-Atrsh, A. M., El-Gogary, M., Tousson, E., 2014. The possible effect of diets containing fish oil (omega-3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney. Biomed. Prev. Nutr. 4, 371-377.
Erlanger, B.F., Kokowsky, N., Cohen, W., 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophy. 95, 271–278.
Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*)–possibilities and limitations for modification through diet. Waste Manag. 102, 40–47
Faggio, C., Fedele, G., Arfuso, F., Panzera, M., Fazio, F., 2014. Haematological and biochemical response of *Mugil cephalus* after acclimation to captivity. Cah. Biol. Mar. 55, 31-36.
Fawole, F.J., Adeoye, A.A., Tiamiyu, L.O., Ajala, K.I., Obadara, S.O., Ganiyu, I.O., 2020. Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias gariepinus*): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. Aquaculture 518, p.734849.
Fawole, F.J., Labh, S.N., Hossain, M.S., Overturf, K., Small, B.C., Welker, T.L., Hardy, R. W., Kumar, V., 2021. Insect (black soldier fly larvae) oil as a potential substitute for fish or soy oil in the fish meal-based diet of juvenile rainbow trout (*Oncorhynchus mykiss*). Anim. Nutr. 7, 1360-1370.
Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M., Faggio, C., 2013. Haematological and serum protein profiles of *Mugil cephalus*: effect of two different habitats. Ichthyol. Res. 60, 36–42.
Figueiredo-Silva, A. C., Kaushik, S., Terrier, F., Schrama, J. W., Médale, F., Geurden, I., 2012. Link between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in medium-chain TAG. Br. J. Nutr. 107, 1714–1725.
Filho, W.D., Giulivi, C., Boveris, A., 1993. Antioxidant defences in marine fish—I. Teleosts. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 106, 409-413.
Folch, J., Lees, M., Sloane-Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 496-509.
Fonseca-Madrigal, J., Navarro, J.C., Hontoria, F., Tocher, D.R., Martínez-Palacios, C.A., Monroig, Ó., 2014. Diversification of substrate specificities in teleostei Fads2: Characterization of Δ4 and Δ6Δ5 desaturases of *Chirostoma estor*. J. Lipid Res. 55, 1408–1419.
Gaudet, M., Racicot, J. G., Leray, C., 1975. Enzyme activities of plasma and selected tissues in rainbow trout *Salmo gairdneri* Richardson. J. of Fish Biol. 7, 505-512.
Gebremichael, A., Kucska, B., Ardó, L., Biró, J., Berki, M., Lengyel-Kónya, É., Tömösközi-

Farkas, R., Egessa, R., Müller, T., Gyalog, G., Sáandor, Z.J., 2023. Physiological response of grower African catfish to dietary black soldier Fly and mealworm meal. Animals 13, 968.

Ghanbari, M., Kneifel, W., Domig, K. J., 2015. A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture 448, 464-475.

Ghelichpour, M., Taheri Mirghaed, A., Mirzargar, S. S., Joshaghani, H., Ebrahimzadeh Mousavi, H., 2017. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquac. Res. 48, 5666-5676.

Glencross, B. D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1, 71-124.

Goh, P. T., Kuah, M. K., Chew, Y. S., Teh, H. Y., Shu-Chien, A. C., 2020. The requirements for sterol regulatory element-binding protein (Srebp) and stimulatory protein 1 (Sp1)-binding elements in the transcriptional activation of two freshwater fish Channa striata and Danio rerio elovl5 elongase. Fish Phys. Biochem. 46, 1349-1359.

Goodrich, J. K., Di Rienzi, S. C., Poole, A. C., Koren, O., Walters, W. A., Caporaso, J. G., ..., Ley, R. E., 2014. Conducting a microbiome study. Cell 158, 250-262.

Gou, N., Wang, K., Jin, T., Yang, B., 2023. First Insights on the Administration of Insect Oil (Black Soldier Fly Larvae) in the Diet of Juvenile *Onychostoma macrolepis*. Animals 13, 518.

Gregory, M.K., James, M.J., 2014. Rainbow trout (*Oncorhynchus mykiss*) Elovl5 and Elovl2 differ in selectivity for elongation of omega-3 docosapentaenoic acid. Biochim. Biophys. Acta 1841, 1656–1660.

Guillou, H., Zadravec, D., Martin, P.G.P., Jacobsson, A., 2010. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog. Lipid Res. 49, 186–199.

Hecht, T., 2013. A review of on-farm feed management practices for North African catfish (*Clarias gariepinus*) in sub-Saharan Africa, in: Hasan, M.R., and New, M.B. (Eds). On-farm feeding and feed management in aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO. 463–479.

Hender, A., Siddik, M. A., Howieson, J., Fotedar, R., 2021. Black soldier fly, *Hermetia illucens* as an alternative to fishmeal protein and fish oil: impact on growth, immune response, mucosal barrier status, and flesh quality of juvenile barramundi, *lates calcarifer* (Bloch, 1790). Biology 10, 505.

Henderson, R. J., 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5-22.

Henry, M.A., Gasco, L., Chatzifotis, S., Piccolo, G., 2018. Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus labrax*. Dev. Comp. Immunol. 81, 204–209.

Hilge, V., 1985. The influence of temperature on the growth of the European catfish (*Silurus glanis* L.). J. Appl. Ichthyol. 1, 27-31.

Hoffman, L.C., Prinsloo, J.F., 1995. Genetic and nutritional influence on the total lipid fatty acid profile of *Clarias gariepinus* muscle. Aquat. Living Resour. 8, 415-421.

Hooper, L.V., Macpherson, A.J., 2010. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat. Rev. Immunol. 10, 159–169.

Hooper, L.V., Midtvedt, T., Gordon, J.I., 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu. Rev. Nutr. 22, 283–307.

Hoseinifar, S. H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., Carnevali, O., 2020. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev. Fish. Sci. Aquac. 29, 198-217.

Howe, E.R., Simenstad, C.A., Toft, J.D., Cordell, J.R., Bollens, S.M., 2014**.** Macroinvertebrate prey availability and fish diet selectivity in relation to environmental variables in natural and restoring north San Francisco Bay tidal marsh channels. San Franc. Estuary Watershed Sci. 12, 1–46.

Huyben, D., Roehe, B.K., Bekaert, M., Ruyter, B., Glencross, B., 2020. Dietary lipid: protein ratio and n-3 long-chain polyunsaturated fatty acids alters the gut microbiome of Atlantic salmon under hypoxic and normoxic conditions. Front. Microbiol. 11, 589898.

Izquierdo, M. S., Fernandez-Palacios, H., Tacon, A. G. J., 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197, 25-42.

Jakobsson, A., Westerberg, R., Jacobsson, A., 2006. Fatty acid elongases in mammals: Their regulation and roles in metabolism. Prog. Lipid Res. 45, 237–249.

Jin, M., Lu, Y., Yuan, Y.E., Li, Y.I., Qiu, H., Sun, P., Zhou, Q.C., 2017. Regulation of growth, tissue fatty acid composition, biochemical parameters and lipid related genes expression by different dietary lipid sources in juvenile black seabream, *Acanthopagrus schlegelii*. Aquaculture 479, 25-37.

Kamala, B. S., Panserat, S., Aguirre, P., Geurden, I., Fontagné-Dicharry, S., Médale, F., 2013. Selection for high muscle fat in rainbow trout induces potentially higher chylomicron synthesis and PUFA biosynthesis in the intestine. Comp. Biochem. Physiol., Mol. amp; Integr. Physiol. 164, 417-427.

Kanazawa, A., 1985. Essential fatty acid and lipid requirement of fish, Nutrition and Feeding in Fish. Academic Press, 281-298.

Khan, S. A., Heuvel, J. P. V., 2003. Reviews: current topics role of nuclear receptors in the regulation of gene expression by dietary fatty acids. The J. Nutr. Biochem. 14, 554-567.

Khedkar, G.D., Jadhao, B.V., Chavan, N.V., Khedkar, C.D., 2003. FISH | Demersal Species of Tropical Climates. Encyclopedia of Food Sciences and Nutrition (2nd ed.). 2438-2442

Knudsen, B. E., Bergmark, L., Munk, P., Lukjancenko, O., Priemé, A., Aarestrup, F. M., Pamp, S. J., 2016. Impact of sample type and DNA isolation procedure on genomic inference of microbiome composition. MSystems 1, e00095-16.

Kokou, F., Rigos, G., Henry, M., Kentouri, M., Alexis, M., 2012. Growth performance, feed utilization and non-specific immune response of gilthead sea bream (*Sparus aurata* L.) fed graded levels of a bioprocessed soybean meal. Aquaculture 364, 74–81.

Küçükgülmez, A., Yanar, Y., Çelik, M., Ersor, B., 2018. Fatty acids profile, atherogenic, thrombogenic, and polyene lipid indices in golden grey mullet (*Liza aurata*) and gold band goatfish (*Upeneus moluccensis*) from Mediterranean Sea. J. Aquat. Food Prod. Technol, 27, 912-918.

Kumar, V., Fawole, F. J., Romano, N., Hossain, M. S., Labh, S. N., Overturf, K., Small, B. C., 2021. Insect (black soldier fly, *Hermetia illucens*) meal supplementation prevents the soybean meal-induced intestinal enteritis in rainbow trout and health benefits of using insect oil. Fish Shellfish Immunol. 109, 116-124.

Lal, K.K., Singh, R.K., Mohindra, V., Singh, B., Ponniah, A.G., 2003. Genetic makeup of exotic catfish, *Clarias gariepinus* in India. Asian Fish. Sci. 16, 229–234.

Lall, S.P., Dumas, A., 2015. Nutritional requirements of cultured fish: Formulating nutritionally adequate feeds, in: Allen Davis, A.D. (Ed.), Feed and feeding practices in aquaculture. Woodhead publishing, 53-109.

Larios-Soriano, E., Re-Araujo, A.D., Gómez-Gil, B., Tovar-Ramírez, D., Trejo-Escamilla, I., Galaviz, M.A., 2021. Reciprocal Effect of Temperature and Dietary Lipids on Metabolic Performance and Gut Microbiota of Yellowtail Kingfish (*Seriola lalandi*) Juveniles. Aquac. Res. 52, 6189–6204.

Legendre, M., Kerdchuen, N., Corraze, G., Bergot, P., 1995. Larval rearing of an African catfish *Heterobranchus longifilis* (Teleostei, Clariidae): effect of dietary lipids on growth,

survival and fatty acid composition of fry. Aquat. Living Resour. 8, 355-363.
Lemieux, H., Blier, P., Dutil, J. D., 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (Gadus morhua)? Fish Phys. Biochem. 20, 293-303.
Leray, C., 2014. Lipids: nutrition and health. CRC Press.
Lewis, M. J., Francis, D. S., Blyth, D., Moyano, F. J., Smullen, R. P., Turchini, G. M., Booth, M. A., 2019. A comparison of in-vivo and in-vitro methods for assessing the digestibility of poultry by-product meals using barramundi (*lates calcarifer*); impacts of cooking temperature and raw material freshness. Aquaculture 498, 187-200.
Li, M., Xu, C., Ma, Y., Ye, R., Chen, H., Xie, D., Zhang, G., Zhang, M., Whang, M., You, C., Wang, S., Ning, L., Lou, M., Li, Y., 2020. Effects of dietary n-3 highly unsaturated fatty acids levels on growth, lipid metabolism and innate immunity in juvenile golden pompano (*Trachinotus ovatus*). Fish Shellfish Immunol. 105, 177-185.
Li, S., Ji, H., Zhang, B., Tian, J., Zhou, J., Yu, H., 2016. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 465, 43-52.
Li, S., Monroig, Ó., Wang, T., Yuan, Y., Navarro, J.C., Hontoria, F., Liao, K., Tocher, D.R., Mai, K., Xu, W., Ai, Q., 2017. Functional characterization and differential nutritional regulation of putative Elovl5 and Elovl4 elongases in large yellow croaker (*Larimichthys crocea*). Sci. Rep. 7, 2303.
Li, Y., Bruni, L., Jaramillo-Torres, A., Gajardo, K., Kortner, T.M., Krogdahl, Å., 2021. Differential response of digesta-and mucosa-associated intestinal microbiota to dietary insect meal during the seawater phase of Atlantic salmon. Anim. Microbiome 3, 1-18.
Li, Y., Yin, Z., Dong, Y., Wang, S., Monroig, Ó., Tocher, D. R., You, C., 2019. Pparγ is involved in the transcriptional regulation of liver LC-PUFA biosynthesis by targeting the Δ6Δ5 fatty acyl desaturase gene in the marine teleost *Siganus canaliculatus*. Mar. Biotechnol. 21, 19-29.
Linhart, O., Gela, D., Rodina, M., Kocour, M., 2004. Optimization of artificial propagation in European catfish, *Silurus glanis* L. Aquaculture 235, 619-632.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.
Louis, P., Flint, H.J., 2017. Formation of propionate and butyrate by the human colonic microbiota. Environ. Microbiol. 19:29–41.
Lu, K. L., Xu, W. N., Li, X. F., Liu, W. B., Wang, L. N., Zhang, C. N., 2013. Hepatic triacylglycerol secretion, lipid transport and tissue lipid uptake in blunt snout bream (*Megalobrama amblycephala*) fed high-fat diet. Aquaculture 408, 160-168.
Lubis, Z., Buckle, K. A., 1990. Rancidity and lipid oxidation of dried-salted sardines. Int. J. Food Sci. Technol. 25, 295–303.
Ma, Y.N., Yin, Z.H., Li, L., Chen, B.N., Dai, H.Y., Wu, D.D., Cong, J.X., Ye, L., Liao, C.H., Li, L.Y., Ye, Z.Z., Huang, Z., 2021. Food antigens exacerbate intestinal damage and inflammation following the disruption of the mucosal barrier. Int. Immunopharmacol. 96, 107670.
Manna, S. K., Das, N., Bera, A. K., Baitha, R., Maity, S., Debnath, D., ..., Patil, P. K., 2021. Reference haematology and blood biochemistry profiles of striped catfish (*Pangasianodon hypophthalmus*) in summer and winter seasons. Aquac. Rep. 21, 100836.
Mariat, D., Firmesse, O., Levenez, F., Guimaraes, V., Sokol, H., Doré, J., et al., 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol. 9,123.
Mekasha, S., Linke, D., 2021. Secretion systems in gram-negative bacterial fish

pathogens. Front. Microbiol. 12, 782673.
Merrifield, D. L., Rodiles, A., 2015. The fish microbiome and its interactions with mucosal tissues, in: Beck, B. H., Peatman, E. (Eds). Mucosal Health in Aquaculture. Academic Press, San Diego, 273-295.
Miles, R. D., Chapman, F. A., 2006. The benefits of fish meal in aquaculture diets: FA122/FA122, 5/2006. Edis, 2006(12).
Mokkala, K., Houttu, N., Cansev, T., Laitinen, K., 2020. Interactions of dietary fat with the gut microbiota: Evaluation of mechanisms and metabolic consequences. Clin. Nutr. 39, 994-1018.
Monroig, O., Tocher, D. R., Castro, L. F. C., 2018. Polyunsaturated fatty acid biosynthesis and metabolism in fish, in: Burdge, G. C. (Ed.), Polyunsaturated Fatty Acid Metabolism. AOCS Press, 31-60
Monroig, Oh., Kabeya, N., 2018. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. Fish. Sci. 84, 911-928.
Moutinho, S., Oliva-Teles, A., Pulido-Rodríguez, L., Magalhaes, R., Monroig, ´O., Parisi, G., Peres, H., 2023. Black soldier fly larvae oil as an alternative lipid source in diets for gilthead seabream (*Sparus aurata*) juveniles. Aquaculture 574, 739705.
Moutinho, S., Peres, H., Fontinha, F., Estevão-Rodrigues, T., Monroig, Ó., Magalhães, R., ... Oliva-Teles, A., 2025. *Hermetia illucens* larvae oil as an alternative lipid source: Effects on immune function, antioxidant activity, and inflammatory response in gilthead seabream juveniles. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 276, 111059.
Mulligan, B.L., Trushenski, J.T., 2013. Use of standard or modified plant derived lipids as alternatives to fish oil in feeds for juvenile Nile tilapia. J. Aquat. Food Prod. Technol. 22, 47–57.
Nakamura, M. T., Nara, T. Y., 2004. Structure, function, and dietary regulation of (delta) 6, (delta) 5, and (delta) 9 desaturases. Annu. Rev. Nutr. 24, 345.
Nakamura, M. T., Cheon, Y., Li, Y., Nara, T. Y., 2004. Mechanisms of regulation of gene expression by fatty acids. Lipids 39, 1077-1083.
Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., Tomberlin, J.K. and Thelen, E.E., 2005. The Black Soldier Fly, *Hermetia illucens*, as a manure management/resource recovery tool. Proceedings of the Symposium State of the Science, Animal Manure and Waste Management, San Antonio, TX, 5-7 January 2005.
Nie, Y., Hu, J., Hou, Q., Zheng, W., Zhang, X., Yang, T., et al., 2019. Lactobacillus frumenti improves antioxidant capacity via nitric oxide synthase 1 in intestinal epithelial cells. Faseb J. 33:10705–16.
Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., Józefiak, A., 2019. Insect meals in fish nutrition. Rev. Aquac. 11, 1080–1103
Nordrum, S., Olli, J. j., Rosjo, C., Holm, H., Krogdahl, A., 2003. Effects of graded levels of medium chain triglycerides and cys- teine on growth, digestive processes and nutrient utilization in sea water reared Atlantic salmon (*Salmo salar*, L.) under *ad libitum* feeding regime. Aquac. Nutr. 9, 263–274.
Oboh, A., Betancor, M. B., Tocher, D. R., Monroig, O., 2016. Biosynthesis of long-chain polyunsaturated fatty acids in the African catfish *Clarias gariepinus*: Molecular cloning and functional characterisation of fatty acyl desaturase (fads2) and elongase (elovl2) cDNAs7. Aquaculture 462, 70-79.
Ogunji, J. O., Awoke, J., 2017. Effect of environmental regulated water temperature variations on survival, growth performance and haematology of African catfish, *Clarias gariepinus*. Our Nature 15.
Oliva-Teles, A., Enes, P., Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish, in: Davis, A.D. (Ed.), Feed and feeding practices in

aquaculture. Woodhead Publishing, 203-233.
Ortega-Hernández, A., Martínez-Martínez, E., Gómez-Gordo, R., López-Andrés, N., Fernández-Celis, A., Gutiérrrez-Miranda, B., et al., 2020. The interaction between mitochondrial oxidative stress and gut microbiota in the cardiometabolic consequences in diet-induced obese rats. Antioxidants (Basel) 9, 640.
Paimeeka, S., Tangsongcharoen, C., Lertwanakarn, T., Setthawong, P., Bunkhean, A., Tangwattanachuleeporn, M., Surachetpong, W., 2024. Tilapia lake virus infection disrupts the gut microbiota of red hybrid tilapia (*Oreochromis* spp.). Aquaculture 586, 740752.
Polakof, S., Médale, F., Larroquet, L., Vachot, C., Corraze, G., Panserat, S., 2011. Regulation of de novo hepatic lipogenesis by insulin infusion in rainbow trout fed a high-carbohydrate diet. J. Anim. Sci. 89, 3079-3088.
Qiao, Y., Sun, J., Ding, Y., Le, G., Shi, Y., 2013. Alterations of the gut microbiota in high fat diet mice is strongly linked to oxidative stress. Appl. Microbiol. Biotechnol. 97:1689–97. doi: 10.1007/s00253-012-4323-6
Qin, Y., Dalen, K. T., Gustafsson, J. Å., Nebb, H. I., 2009. Regulation of hepatic fatty acid elongase 5 by LXRα–SREBP-1c. Biochim. Biophys. Acta (BBA)-Molecular and Cell Biology of Lipids, 1791(2), 140-147.
Qiu, X., 2003. Biosynthesis of docosahexaenoic acid (DHA, 22:6-4,7,10,13,16,19): Two distinct pathways. Prostaglandins Leukot. Essent. Fat. Acids 68, 181–186.
Quast, C., Pruesse. E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Opens external link in new window. Nucl. Acids Res. 41 (D1): D590-D596.
R Core Team., 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
Reinoso, S., Gutiérrez, M.S., Reyes-Jara, A., Toro, M., García, K., Reyes, G., Argüello-Guevara, W., Bohórquez-Cruz, M., Sonnenholzner, S., Navarrete, P., 2023. Feed regime slightly modifies the bacterial but not the fungal communities in the intestinal mucosal microbiota of cobia fish (*Rachycentron canadum*). Microorganisms 11, 2315.
Richter, C.K., Skulas-Ray, A.C. and Kris-Etherton, P.M., 2016. Recommended intake of fish and fish oils worldwide, in: Raatz, S.K. and Bibus, D.M. (Eds.), Fish and Fish Oil in Health and Disease Prevention. Academic Press, San Diego, 27-48.
Rick, W., Stegbauer, H. P., 1974. α-Amylase measurement of reducing groups. In Bergmeyer, H.U. (Eds) Methods of Enzymatic Analysis, Academic Press, 885-890.
Ridha, M.T., Cruz, E.M., 2001. Effect of biofilter media on water quality and biological performance of the Nile tilapia *Oreochromis niloticus* L. reared in a simple recirculating system, Aquac. Eng. 24,157-166
Riera-Heredia, N., Lutfi, E., Sánchez-Moya, A., Gutiérrez, J., Capilla, E., Navarro, I., 2020. Short term responses to fatty acids on lipid metabolism and adipogenesis in rainbow trout (*Oncorhynchus mykiss).* Int. J. Mol. Sci. 21(5).
Rimoldi, S., Terova, G., Ascione, C., Giannico, R., Brambilla, F., 2018. Next generation sequencing for gut microbiome characterization in rainbow trout (*Oncorhynchus mykiss*) fed animal by-product meals as an alternative to fishmeal protein sources. PLoS One 13(3), e0193652.
Ringø, E., Hoseinifar, S. H., Ghosh, K., Doan, H. V., Beck, B. R., Song, S. K., 2018. Lactic acid bacteria in finfish—An update. Front. Microbiol. 1818.
Rombenso, A.N., Turchini, G.M., Trushenski, J.T., 2021. The omega-3 sparing effect of saturated fatty acids: a reason to reconsider common knowledge of fish oil replacement. Rev. Aquac. 14, 213–217.
Ross, D., 1988. Glutathione, free radicals and chemotherapeutic agents: mechanisms of free-

radical induced toxicity and glutathione-dependent protection. Pharmacol. Therapeut. 37,231–249.
Rowley, A. F., Knight, J., Lloyd-Evans, P., Holland, J. W., Vickers, P. J., 1995. Eicosanoids and their role in immune modulation in fish—a brief overview. Fish Shellfish Immunol. 5, 549-567.
Rumpold, B. A., Schlüter, O. K., 2013. Nutritional composition and safety aspects of edible insects. Mol. Nutr. Food Res. 57, 802-823.
Sado-Kamdem, S. L., Vannini, L., Guerzoni, M. E., 2009. Effect of α- linolenic, capric and lauric acid on the fatty acid biosynthesis in *Staphylococcus aureus*. Int. J. Food Microbiol. 129, 288–294.
Sampath, H., Ntambi, J. M., 2005. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu. Rev. Nutr. 25, 317-40.
Sándor, Z.J., Banjac, V., Vidosavljević, S., Káldy, J., Egessa, R., Lengyel-Kónya, É., Tömösközi-Farkas, R., Zalán, Z., Adányi, N., Libisch, B. and Biró, J., 2022. Apparent digestibility coefficients of black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), and blue bottle fly (*Calliphora vicina*) insects for juvenile African catfish hybrids (*Clarias gariepinus*× *Heterobranchus longifilis*). Aquac. Nutr. 2022, 1, 4717014.
Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids, in: Halver, J.E., Hardy, R.W. (Eds.), Fish nutrition. Academic Press, San Diego, 181–257.
Sastry, K. V., Sharma, K., 1981. Diazinon-induced histopathological and hematological alterations in a freshwater teleost, *Ophiocephalus punctatus*. Ecotoxicol. Environ. Saf. 5, 329-340.
Satoh, S., Poe, W. E., Wilson, R. P., 1989a. Effect of dietary n-3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish. J. Nutr. 119, 23-28.
Satoh, S., Poe, W.E., Wilson, R.P., 1989b. Studies on the essential fatty acid requirement of channel catfish, *Ictalurus punctatus*. Aquaculture 79, 121-128.
Schmitz, G., Ecker, J., 2008. The opposing effects of n− 3 and n− 6 fatty acids. Prog. lipid Res. 47, 147-155.
Schoeler, M., Caesar, R., 2019. Dietary lipids, gut microbiota and lipid metabolism. Rev. Endocr. Metab. Disord. 20, 461-472.
Schönfeld, P., Wojtczak, L., 2016. Short-and medium-chain fatty acids in energy metabolism: the cellular perspective. J. Lipid Res. 57, 943–954.
Serra, C.R., Oliva-Teles, A., Enes, P., Tavares, F., 2021. Gut microbiota dynamics in carnivorous European seabass (Dicentrarchus labrax) fed plant-based diets. Sci. Rep. 11, 1–13.
Sharma, A., Deo, A. D., Riteshkumar, S. T., Chanu, T. I., Das, A., 2010. Effect of *Withania somnifera* (L. Dunal) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in *Labeo rohita* (Hamilton) fingerlings. Fish Shellfish Immunol. 29, 508-512.
Simopoulos, A.P., 2002: The importance of the ratio of omega-6/omega-3 essential fatty acids, Biomed. Pharmacother. 56, 365-379.
Skelton, P., 2001. A complete guide to the freshwater fishes of Southern Africa. Struik Publishers, Cape Town.
Skvortsova, E., Filinskaya, O., Postrash, I., Bushkareva, A. and Mostofina, A., 2023. Biodiversity of gut microorganisms in aquacultured African catfish. In E3S Web of Conferences, 463, 01039. EDP Sciences.
Sorbera, L.A, Francisco Asturiano, J., Carrillo, M., Zanuy, S., 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biol. Reprod. 64, 382-389.

Sperling, P., Ternes, P., Zank, T.K., Heinz, E., 2003. The evolution of desaturases. Prostaglandins Leukot. Essent. Fat. Acids 68, 73–95.

Standen, B. T., Rodiles, A., Peggs, D. L., Davies, S. J., Santos, G. A., Merrifield, D. L., 2015. Modulation of the intestinal microbiota and morphology of tilapia, Oreochromis niloticus, following the application of a multi-species probiotic. Appl. Microbiol. Biotechnol. 99, 8403-8417.

Steffens, W., 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture 151, 97–119

St-Onge, M.-P., Bosarge, A., Goree, L. L. T., Darnell, B., 2008. Medium chain triglyceride oil consumption as part of a weight loss diet does not lead to an adverse metabolic profile when compared to olive oil. J. Am. Coll. Nutr. 27, 547–552.

Sudha, C., Ahilan, B., Felix, N., Uma, A., Chidambaram, P., Prabu, E., 2022. Replacement of fish oil with black soldier fly larvae oil and vegetable oils: Effects of growth, whole-body fatty acid profile, digestive enzyme activity, haemato-biochemical responses and muscle growth-related gene expression of juvenile striped catfish, *Pangasianodon hypophthalmus*. Aquac. Res. 53, 3097-3111.

Sunde, J., Taranger, G. L., Rungruangsak-Torrissen, K., 2001. Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (Salmo salar L.). Fish Phys. Biochem. 25, 335-345.

Talwar, C., Nagar, S., Lal, R., Negi, R. K., 2018. Fish gut microbiome: current approaches and future perspectives. Indian J. Microbiol. 58, 397-414.

Tarnecki, A. M., Burgos, F. A., Ray, C. L., Arias, C. R., 2017. Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. J. Appl. Microbiol. 123, 2-17.

Terova, G., Gini, E., Gasco, L., Moroni, F., Antonini, M., Rimoldi, S., 2021. Effects of full replacement of dietary fishmeal with insect meal from *Tenebrio molitor* on rainbow trout gut and skin microbiota. J. Anim. Sci. Biotechnol. 12, 1-14.

Thejaswini, S., Jojy, S., Vijayan, A., Martin, P.A., 2022. Isolation of Gut Actinobacteria from Fishes, in: Dharumadurai, D. (Ed.), Methods in Actinobacteriology. Springer Protocols Handbooks. Humana, New York, NY.

Tian, J., Du, Y., Yu, E., Lei, C., Xia, Y., Jiang, P.,.., Wang, G., 2022. Prostaglandin 2α promotes autophagy and mitochondrial energy production in fish hepatocytes. Cells 11, 1870.

Tocher, D. R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107-184.

Tocher, D. R., Glencross, B. D., 2015. Lipids and fatty acids, in: Lee, C.S, Lim, C., Gatlin, D.M., Webster, C.D. (Eds.), Dietary Nutrients, Additives, and Fish Health. Wiley-Blackwell, 47-94.

Tocher, D.R., 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449, 94–107.

Tocher, D.R., Agaba, M., Hastings, N., Teale, A.J., 2003. Biochemical and molecular studies of the polyunsaturated fatty acid desaturation pathway in fish, in: Browman, H., Skiftesvik, A.B. (Eds.), The Big Fish Bang - Proceedings of the 26th Annual Larval Fish Conference. Institute of Marine Research, Bergen, Norway, 211–227

Trushenski, J.T., Rombenso, A.N., 2020. Trophic levels predict the nutritional essentiality of polyunsaturated fatty acids in fish—introduction to a special section and a brief synthesis. N. Am. J. Aquac. 82, 241–250.

Turchini, G.M., Hermon, K., Cleveland, B.J., Emery, J.A., Rankin, T., Francis, D.S., 2013. Seven fish oil substitutes over a rainbow trout grow-out cycle: I) effects on performance and fatty acid metabolism. Aquacult. Nutr. 19, 82–94.

Turchini, G.M., Ng, W.K., Tocher, D.R., 2010. Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press.

Ulbricht, T.L.V., Southgate, D.A.T., 1991. Coronary heart disease: seven dietary factors. Lancet 338, 985–992.
Vagner, M. Santigosa, E., 2011. Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review. Aquaculture 315, 131-143.
Van der Waal, B.C.W., 1998. Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. Koedoe - African Protected Area Conservation and Science 41, 131-138.
Wang, H., Hu, C., Cheng, C., Cui, J., Ji, Y., Hao, X., et al., 2019. Unraveling the association of fecal microbiota and oxidative stress with stillbirth rate of sows. Theriogenology 136, 131-7.
Wang, S., Chen, J., Jiang, D., Zhang, Q., You, C., Tocher, D. R., ..., Li, Y., 2018. Hnf4α is involved in the regulation of vertebrate LC-PUFA biosynthesis: insights into the regulatory role of Hnf4α on expression of liver fatty acyl desaturases in the marine teleost *Siganus canaliculatus*. Fish Phys. Biochem. 44, 805-815.
Wang, X., Ma, Q., Chen, L., Wu, H., Chen, L. Q., Qiao, F., ..., Du, Z. Y., 2022. Peroxisome proliferator-activated receptor gamma is essential for stress adaptation by maintaining lipid homeostasis in female fish. Biochim. Biophys. Acta (BBA)-Mol. Cell Biol. Lipids, 1867, 159162.
Wemheuer, F., Taylor, J.A., Daniel, R., Johnston, E., Meinicke, P., Thomas, T. and Wemheuer, B., 2020. Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environ. Microbiome 15, 1-12.
Weththasinghe, P., Rocha, S.D., Øyås, O., Lagos, L., Hansen, J.Ø., Mydland, L.T., Øverland, M., 2022. Modulation of Atlantic salmon (*Salmo salar*) gut microbiota composition and predicted metabolic capacity by feeding diets with processed black soldier fly (*Hermetia illucens*) larvae meals and fractions. Anim. Microbiome 4,9.
Williams, I., Williams, K. C., Smith, D. M., Jones, M. (2006). Polka- dot grouper, *Cromileptes altivelis*, can utilize dietary fat efficiently. Aquaculture Nutrition, 12, 5, 379–387.
Wilson, R.P., Moreau, Y., 1996. Nutrient requirements of catfishes (*Siluroidei*). Aquat. Living Resour. 9, 103-111.
Winkler, U.K., Stuckman, M., 1979. Glycogen, hyluronate and some other polysaccharides greatly enhance the formation of exolipase by *Serratiz marcescens*. J. Bacteriol. 138, 663–670.
Wirth, M., Steffens, W., Meinelt, T., Steinberg, C., 1997. Significance of docosahexaenoic acid for rainbow trout (*Oncorhynchus mykiss*) larvae. Lipid/Fett. 99, 251-253.
Xia, R., Hao, Q., Xie, Y., Zhang, Q., Ran, C., Yang, Y., Zhou, W., Chu, F., Zhang, X., Wang, Y. and Zhang, Z., 2022. Effects of dietary *Saccharomyces cerevisiae* on growth, intestinal and liver health, intestinal microbiota and disease resistance of channel catfish (*Ictalurus punctatus*). Aquac. Rep. 24, 101157.
Xie, D., Chen, C., Dong, Y., You, C., Wang, S., Monroig, Oh., ..., Li, Y., 2021. Regulation of long-chain polyunsaturated fatty acid biosynthesis in teleost fish. Prog. Lipid Res. 82, 101095.
Xie, G.; Zhang, M.; Zhong, Z.; Zhu, Y.; Fan, X.; Li, M.; Xing, K.; Shu, H., 2024. Functional Genomic Characterization Reveals the Probiotic Tendency and Safety Assessment of Exiguobacterium acetylicum G1–33 Isolated from the Gut of the Hybrid Grouper (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂). Aquac. Rep. 36, 102127.
Xu J., Gordon J. I., 2003. Honor thy symbionts. Proc. Natl. Acad. Sci. 100, 18, 10452–10459.
Xu, H., Turchini, G.M., Francis, D.S., Liang, M., Mock, T.S., Rombenso, A., Ai, Q., 2020. Are fish what they eat? A fatty acid's perspective. Prog. Lipid Res. 80, 101064
Xu, Y. H., Tan, X. Y., Xu, Y. C., Zhao, T., Zhang, L. H., Luo, Z., 2019. Novel insights for SREBP-1 as a key transcription factor in regulating lipogenesis in a freshwater teleost,

grass carp *Ctenopharyngodon idella*. Br. J. Nutr. 122, 1201-1211.
Yalçın, S., Akyurt, I., Solak, K., 2001. Stomach contents of the catfish *Clarias gariepinus* (Burchell, 1822) in the river Asi (Turkey). Turk. J. Zool. 25: 461–468.
Yanes-Roca, C., Rhody, N., Nystrom, M., Main, K.L., 2009. Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). Aquaculture 287, 335-340.
Yang, J., Lin, Y., Wei, Z., Wu, Z., Zhang, Q., Hao, J., Wang, S., Li, A., 2023. *Edwardsiella ictaluri* almost completely occupies the gut microbiota of fish suffering from Enteric Septicemia of catfish (Esc). Fishes 8, 30.
Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Opens external link in new window. Nucl. Acids Res. 42: D643-D648
Yin, L., Ma, H., Ge, X., Edwards, P. A., Zhang, Y., 2011. Hepatic hepatocyte nuclear factor 4α is essential for maintaining triglyceride and cholesterol homeostasis. Arterioscler. Thromb. Vasc. Biol. 31, 328-336.
Yoshikawa, T., Ide, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Matsuzaka, T., ..., Yamada, N., 2003. Cross-talk between peroxisome proliferator-activated receptor (PPAR) α and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling. Mol. Endocrinol. 17, 1240-1254.
Yukgehnaish, K., Kumar, P., Sivachandran, P., Marimuthu, K., Arshad, A., Paray, B. A., Arockiaraj, J., 2020. Gut microbiota metagenomics in aquaculture: Factors influencing gut microbiome and its physiological role in fish. Rev. Aquac. 12, 1903-1927.
Zhang, C., Derrien, M., Levenez, F., Brazeilles, R., Ballal, S.A., Kim, J., et al., 2016. Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. ISME J. 10, 2235–2245.
Zhang, J. F., Wang, X. R., Guo, H. Y., Wu, J. C., Xue, Y. Q., 2004. Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, *Carassius auratus*. Ecotoxicol. Environ. Saf. 58, 110-116.
Zhang, Y. Y., Liu, B., Ge, X. P., Liu, W. B., Xie, J., Ren, M., ..., Yu, Y., 2014. The influence of various feeding patterns of emodin on growth, non-specific immune responses, and disease resistance to *Aeromonas hydrophila* in juvenile Wuchang bream (*Megalobrama amblycephala*). Fish Shellfish Immunol. 36, 187-193.
Zheng, J. L., Luo, Z., Zhu, Q. L., Tan, X. Y., Chen, Q. L., Sun, L. D., Hu, W., 2013. Molecular cloning and expression pattern of 11 genes involved in lipid metabolism in yellow catfish *Pelteobagrus fulvidraco*. Gene 531, 53-63.
Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G., Teale, A.J., 2005. Highly unsaturated fatty acid synthesis in vertebrates: New insights with the cloning and characterization of a Δ6 desaturase of Atlantic salmon. Lipids 40, 13–24.

SUPPLEMENTARY MATERIALS

Table S1. Amino acid composition (g/kg DM) of experimental diets

	CTR	IO50	IO100
Essential amino acids			
Arginine	28.45	26.74	27.64
Histidine	9.91	9.44	9.66
Isoleucine	17.40	17.11	18.07
Leucine	31.10	30.49	32.04
Lysine	23.75	23.54	27.24
Methionine	10.57	11.92	10.94
Phenylalanine	19.95	19.08	19.46
Threonine	16.75	16.12	16.94
Tryptophan	0.93	0.87	0.94
Valine	20.14	19.91	20.96
Nonessential amino acids			
Alanine	21.90	21.91	23.39
Aspartic acid	27.23	25.56	29.29
Cysteine	5.80	2.80	5.11
Glutamic acid	59.36	59.29	63.69
Glycine	26.59	27.05	26.93
Proline	27.84	28.50	29.21
Serine	22.05	21.30	22.52
Tyrosine	13.96	13.03	13.19

Values are presented based on duplicate analyses

Table S2. Bacterial counts and relative abundance (%) of phyla and genera (with at least 0.01% detection threshold) detected in intestinal digesta samples of European catfish fed the CTR and insect oil-based diets (IO50 and IO100).

	Samples (CTR group)								Samples (IO50 group)						Samples (IO100 group)					
Taxa	**CTR1**	**CTR2**	**CTR3**	**CTR4**	**CTR5**	**CTR6**	**Counts**	**Rel. abund (%)**	**IO501**	**IO502**	**IO503**	**IO504**	**Counts**	**Rel. abund. (%)**	**IO100**	**IO1002**	**IO1003**	**IO1004**	**Counts**	**Rel. abund (%)**
Phylum																				
Fusobacteriota	114368	70476	37878	4	135757	132667	491150	**83.51**	72046	101604	667	71	174388	**45.30**	71788	102307	2238	87019	263352	**71.23**
Spirochaetota	93	9645	206	84559	1	111	94615	**16.09**	32865	53	95287	80705	208910	**54.26**	10	2432	90452	10731	103625	**28.03**
Proteobacteria	148	61	421	16	70	198	914	**0.16**	271	72	60	334	737	**0.19**	258	148	668	23	1097	**0.30**
Firmicutes	123	181	290	16	32	64	706	**0.12**	241	90	6	200	537	**0.14**	60	398	394	86	938	**0.25**
Actinobacteriota	50	60	385	0	33	17	545	**0.09**	54	24	9	201	288	**0.07**	32	121	271	41	465	**0.13**
Bacteroidota	0	0	189	2	15	2	208	**0.04**	18	0	5	101	124	**0.03**	12	11	143	7	173	**0.05**
Chloroflexi	0	0	0	0	3	0	3	**0.00**	0	0	0	0	0	**0.00**	0	0	0	0	0	**0.00**
Patescibacteria	0	0	0	0	1	0	1	**0.00**	0	0	0	6	6	**0.00**	0	34	2	0	36	**0.01**
Bdellovibrionota	0	0	0	0	0	0	0	**0.00**	0	0	0	0	0	**0.00**	0	4	0	0	4	**0.00**
Campilobacterota	0	0	0	0	0	0	0	**0.00**	0	8	0	0	8	**0.00**	0	0	0	0	0	**0.00**
Cyanobacteria	0	0	0	0	0	0	0	**0.00**	0	0	0	0	0	**0.00**	0	0	15	0	15	**0.00**
Verrucomicrobiota	0	0	0	0	0	0	0	**0.00**	0	0	0	6	6	**0.00**	0	0	0	0	0	**0.00**
Total	114782	80423	39369	84597	135912	133059	588142	**100**	105495	101851	96034	81624	385004	**100**	72160	105455	94183	97907	369705	**100**
Genus																				
Cetobacterium	114368	70476	37878	4	135757	132667	491150	**83.51**	72046	101604	667	71	174388	**45.30**	71788	102307	2236	87019	263350	**71.23**
Brevinema	93	9645	206	84559	1	111	94615	**16.09**	32865	53	95287	80705	208910	**54.26**	10	2432	90452	10731	103625	**28.03**
Escherichia-Shigella	84	13	178	0	32	149	456	**0.08**	49	12	1	29	91	**0.02**	236	9	19	4	268	**0.07**
Exiguobacterium	71	75	92	7	7	56	308	**0.05**	66	36	6	80	188	**0.05**	60	230	98	41	429	**0.12**
Leifsonia	39	48	82	0	0	1	170	**0.04**	5	13	7	11	36	**0.01**	5	56	78	29	168	**0.05**
Corynebacterium	0	0	112	0	29	0	141	**0.02**	7	0	0	51	58	**0.02**	18	16	68	3	105	**0.03**
Aerosphaera	45	24	55	1	0	0	125	**0.02**	16	0	0	8	24	**0.01**	0	7	0	10	17	**0.01**
Bifidobacterium	0	0	111	0	0	0	111	**0.02**	8	1	0	83	92	**0.02**	0	1	31	0	32	**0.01**
Enterococcus	0	57	52	1	0	0	110	**0.02**	1	4	0	15	20	**0.01**	0	0	0	0	0	**0**
Bacillus	0	16	19	3	7	4	49	**0.01**	62	5	0	38	105	**0.03**	0	30	153	14	197	**0.05**
Others	82	69	584	22	79	71	907	**0.15**	370	123	66	533	1092	**0.28**	43	367	1048	56	1514	**0.41**
Total	114782	80423	39369	84597	135912	133059	588142	**100**	105495	101851	96034	81624	385004	**100**	72160	105455	94183	97907	369705	**100**

Table S3. Bacterial counts and relative abundance (%) of phyla and genera (with at least 0.01% detection threshold) detected in intestinal digesta samples of African catfish hybrid fed the CTR and insect oil-based diets (IO50 and IO100).

	Samples (CTR group)				Rel. Abund	Sample	Rel. Abund	Samples (IO100 group)					Rel. Abund
Taxa	**CTR1**	**CTR2**	**CTR3**	**Counts**	**(%)**	**IO501**	**(%)**	**IO1001**	**IO1002**	**IO1003**	**IO1004**	**Counts**	**(%)**
Phylum													
Fusobacteriota	64330	101779	114715	280824	**89.503**	83296	**75.441**	133886	87593	120121	118140	459740	**94.555**
Firmicutes	6259	9943	4892	21094	**6.723**	26765	**24.241**	1795	12031	4894	3845	22565	**4.641**
Proteobacteria	373	3838	740	4951	**1.578**	161	**0.146**	288	2143	439	571	3441	**0.708**
Actinobacteriota	854	70	68	992	**0.316**	154	**0.139**	70	54	100	88	312	**0.064**
Bacteroidota	29	5579	117	5725	**1.825**	28	**0.025**	13	22	41	35	111	**0.023**
Verrucomicrobiota	110	1	18	129	**0.041**	8	**0.007**	1	0	13	5	19	**0.004**
Campilobacterota	2	0	0	2	**0.001**	0	**0.000**	1	0	1	7	9	**0.002**
Desulfobacterota	0	0	1	1	**0.000**	0	**0.000**	1	7	0	1	9	**0.002**
Dadabacteria	3	0	0	3	**0.001**	0	**0.000**	0	0	3	0	3	**0.001**
Patescibacteria	13	0	6	19	**0.006**	0	**0.000**	3	0	0	0	3	**0.001**
Bdellovibrionota	0	2	0	2	**0.001**	0	**0.000**	0	0	0	0	0	**0.000**
Chloroflexi	7	0	0	7	**0.002**	0	**0.000**	0	0	0	0	0	**0.000**
Gemmatimonadota	3	0	0	3	**0.001**	0	**0.000**	0	0	0	0	0	**0.000**
Planctomycetota	6	0	0	6	**0.002**	0	**0.000**	0	0	0	0	0	**0.000**
Total	71989	121212	120557	313758	**100**	110412	**100**	136058	101850	125612	122692	486212	**100**
Genus													
Cetobacterium	64327	101779	114715	280821	**89.50**	83296	**75.44**	133886	87593	120121	118140	459740	**94.56**
Romboutsia	1333	3929	1438	6700	**2.14**	8969	**8.12**	615	3803	1843	796	7057	**1.45**
Plesiomonas	288	3827	621	4736	**1.51**	121	**0.11**	269	2129	426	545	3369	**0.69**
Turicibacter	0	410	248	658	**0.21**	2954	**2.68**	8	2756	39	216	3019	**0.62**
Clostridium sensu stricto 1	7	752	626	1385	**0.44**	1214	**1.10**	39	1138	77	375	1629	**0.34**
Candidatus Arthromitus	3	0	1722	1725	**0.55**	0	**0.00**	0	0	0	1220	1220	**0.25**
Terrisporobacter	59	512	98	669	**0.21**	182	**0.16**	178	51	129	12	370	**0.08**
Epulopiscium	0	345	156	501	**0.16**	44	**0.04**	8	196	10	2	216	**0.04**
Aurantimicrobium	675	60	45	780	**0.25**	135	**0.12**	38	27	81	61	207	**0.04**
Cutibacterium	100	6	7	113	**0.04**	16	**0.01**	7	18	13	2	40	**0.01**
Corynebacterium	31	1	2	34	**0.01**	1	**0.00**	11	3	4	10	28	**0.01**
Others	5166	9591	879	15636	**4.98**	13480	**12.21**	999	4136	2869	1313	9317	**1.92**
Total	71989	121212	120557	313758	**100**	110412	**100**	136058	101850	125612	122692	486212	**100**

Table S4. Bacterial counts and relative abundance of genera (with at least 0.01% detection threshold) detected in feed samples of African catfish hybrid and European catfish.

		African catfish feed						European catfish feed					
		HA_1 (CTR)		HA_2 (IO50)		HA_3 (IO100)		HE_1 (CTR)		HE_2 (IO50)		HE_3 (IO100)	
Phylum	**Genus**	Count	%	Count	%	Count	%	Count	%	Count	%	count	%
Firmicutes	*Lactococcus*	207	25.68	181	28.11	145	18.90	162	26.17	234	21.01	259	26.0
Firmicutes	*Leuconostoc*	208	25.81	148	22.98	191	24.90	129	20.84	240	21.54	244	24.5
Proteobacteria	*Pseudomonas*	57	7.07	47	7.30	77	10.04	60	9.69	72	6.46	39	3.9
Proteobacteria	*Aeromonas*	4	0.50	1	0.16	0	0.00	27	4.36	68	6.10	80	8.0
Firmicutes	*Lactobacillus*	44	5.46	22	3.42	62	8.08	22	3.55	47	4.22	36	3.6
Firmicutes	*Kurthia*	6	0.74	72	11.18	20	2.61	0	0.00	6	0.54	4	0.4
Firmicutes	*Lysinibacillus*	2	0.25	1	0.16	2	0.26	18	2.91	52	4.67	38	3.8
Proteobacteria	*Acinetobacter*	0	0.00	1	0.16	3	0.39	18	2.91	18	1.62	20	2.0
Fusobacteriota	*Fusobacterium*	11	1.36	9	1.40	6	0.78	7	1.13	11	0.99	7	0.7
Proteobacteria	*Shewanella*	1	0.12	2	0.31	3	0.39	7	1.13	24	2.15	19	1.9
Firmicutes	*unclassified*	26	3.23	18	2.80	25	3.26	19	3.07	25	2.24	41	4.1
Proteobacteria	*Providencia*	1	0.12	0	0.00	1	0.13	6	0.97	16	1.44	21	2.1
Firmicutes	*Pediococcus*	8	0.99	4	0.62	21	2.74	5	0.81	7	0.63	0	0.0
Fusobacteriota	*Psychrilyobacter*	10	1.24	9	1.40	7	0.91	5	0.81	15	1.35	4	0.4
Bacteroidota	*Flavobacterium*	0	0.00	0	0.00	0	0.00	4	0.65	16	1.44	7	0.7
Firmicutes	*Carnobacterium*	2	0.25	5	0.78	4	0.52	4	0.65	4	0.36	0	0.0
Firmicutes	*Savagea*	3	0.37	1	0.16	1	0.13	4	0.65	4	0.36	3	0.3
Firmicutes	*Streptococcus*	1	0.12	1	0.16	0	0.00	4	0.65	1	0.09	1	0.1
Fusobacteriota	*Cetobacterium*	3	0.37	1	0.16	5	0.65	4	0.65	3	0.27	2	0.2
Proteobacteria	*Vibrio*	3	0.37	0	0.00	0	0.00	4	0.65	29	2.60	18	1.8
Firmicutes	*Clostridium sensu stricto 1*	0	0.00	2	0.31	5	0.65	3	0.48	6	0.54	3	0.3
Firmicutes	*Clostridium sensu stricto 5*	1	0.12	0	0.00	2	0.26	3	0.48	2	0.18	3	0.3
Firmicutes	*Enterococcus*	10	1.24	11	1.71	3	0.39	3	0.48	3	0.27	6	0.6
Firmicutes	*Erysipelothrix*	0	0.00	0	0.00	0	0.00	3	0.48	7	0.63	7	0.7
Proteobacteria	*Alcaligenes*	0	0.00	0	0.00	0	0.00	3	0.48	3	0.27	2	0.2
Proteobacteria	*Massilia*	6	0.74	4	0.62	15	1.96	3	0.48	6	0.54	3	0.3
Proteobacteria	*Proteus*	3	0.37	1	0.16	0	0.00	3	0.48	5	0.45	9	0.9
Actinobacteriota	*Cutibacterium*	10	1.24	6	0.93	16	2.09	2	0.32	4	0.36	6	0.6
Firmicutes	*Paenibacillus*	4	0.50	0	0.00	1	0.13	2	0.32	4	0.36	0	0.0
Firmicutes	*Staphylococcus*	4	0.50	5	0.78	1	0.13	2	0.32	5	0.45	4	0.4
Myxococcota	*Pajaroellobacter*	3	0.37	0	0.00	6	0.78	2	0.32	0	0.00	1	0.1

Proteobacteria	*Serratia*	3	0.37	2	0.31	1	0.13	2	0.32	4	0.36	0	0.0
Actinobacteriota	*Corynebacterium*	19	2.36	2	0.31	2	0.26	1	0.16	2	0.18	5	0.5
Actinobacteriota	*Micrococcus*	6	0.74	1	0.16	1	0.13	1	0.16	1	0.09	2	0.2
Bacteroidota	*Empedobacter*	2	0.25	0	0.00	0	0.00	1	0.16	0	0.00	4	0.4
Bacteroidota	*Hymenobacter*	2	0.25	0	0.00	2	0.26	1	0.16	1	0.09	0	0.0
Bacteroidota	*Pedobacter*	0	0.00	0	0.00	2	0.26	1	0.16	0	0.00	0	0.0
Bacteroidota	*Sphingobacterium*	6	0.74	1	0.16	1	0.13	1	0.16	8	0.72	4	0.4
Firmicutes	*Bacillus*	3	0.37	5	0.78	2	0.26	1	0.16	10	0.90	2	0.2
Firmicutes	*Clostridium sensu stricto 15*	0	0.00	1	0.16	2	0.26	1	0.16	5	0.45	1	0.1
Firmicutes	*Proteocatella*	0	0.00	0	0.00	0	0.00	1	0.16	0	0.00	2	0.2
Proteobacteria	*Aliivibrio*	3	0.37	5	0.78	8	1.04	1	0.16	5	0.45	2	0.2
Proteobacteria	*Brevundimonas*	4	0.50	0	0.00	0	0.00	1	0.16	1	0.09	4	0.4
Proteobacteria	*Duganella*	0	0.00	0	0.00	1	0.13	1	0.16	0	0.00	0	0.0
Proteobacteria	*Moritella*	2	0.25	3	0.47	1	0.13	1	0.16	4	0.36	2	0.2
Proteobacteria	*Psychrobacter*	0	0.00	2	0.31	0	0.00	1	0.16	1	0.09	1	0.1
Proteobacteria	*Psychromonas*	6	0.74	4	0.62	2	0.26	1	0.16	4	0.36	1	0.1
Proteobacteria	*Sphingomonas*	2	0.25	1	0.16	2	0.26	1	0.16	7	0.63	2	0.2
Actinobacteriota	*Bifidobacterium*	4	0.50	0	0.00	2	0.26	0	0.00	0	0.00	1	0.1
Actinobacteriota	*Lawsonella*	5	0.62	1	0.16	2	0.26	0	0.00	1	0.09	1	0.1
Bacteroidota	*Cloacibacterium*	3	0.37	1	0.16	0	0.00	0	0.00	1	0.09	1	0.1
Bacteroidota	*Dyadobacter*	0	0.00	0	0.00	0	0.00	0	0.00	2	0.18	0	0.0
Bacteroidota	*Fluviicola*	0	0.00	0	0.00	2	0.26	0	0.00	0	0.00	0	0.0
Bacteroidota	*Moheibacter*	0	0.00	0	0.00	2	0.26	0	0.00	0	0.00	0	0.0
Bacteroidota	*Unclassified*	16	1.99	0	0.00	1	0.13	0	0.00	0	0.00	0	0.0
Bacteroidota	*Prevotella*	0	0.00	0	0.00	3	0.39	0	0.00	1	0.09	1	0.1
Campilobacterota	*Arcobacter*	1	0.12	2	0.31	0	0.00	0	0.00	1	0.09	1	0.1
Cyanobacteria	*Unclassified*	1	0.12	1	0.16	2	0.26	0	0.00	2	0.18	0	0.0
Firmicutes	*Allofustis*	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	0.3
Firmicutes	*Atopostipes*	0	0.00	0	0.00	1	0.13	0	0.00	3	0.27	0	0.0
Firmicutes	*Cerasibacillus*	2	0.25	1	0.16	1	0.13	0	0.00	1	0.09	2	0.2
Firmicutes	*Clostridium sensu stricto 7*	4	0.50	1	0.16	4	0.52	0	0.00	0	0.00	5	0.5
Firmicutes	*Rummeliibacillus*	1	0.12	3	0.47	7	0.91	0	0.00	0	0.00	0	0.0
Firmicutes	*Weissella*	4	0.50	7	1.09	6	0.78	0	0.00	0	0.00	1	0.1
Firmicutes	*ZOR0006*	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	2	0.2
Proteobacteria	*Burkholderia-Caballeronia-Paraburkholderia*	0	0.00	1	0.16	1	0.13	0	0.00	1	0.09	0	0.0
Proteobacteria	*Enhydrobacter*	3	0.37	0	0.00	0	0.00	0	0.00	1	0.09	0	0.0

Proteobacteria	*Limnobacter*	1	0.12	0	0.00	0	0.00	0	0.00	0	0.00	0	0.0
Proteobacteria	*Lysobacter*	0	0.00	2	0.31	0	0.00	0	0.00	0	0.00	0	0.0
Proteobacteria	*Marinomonas*	3	0.37	0	0.00	0	0.00	0	0.00	0	0.00	0	0.0
Proteobacteria	*Methylobacterium-Methylorubrum*	3	0.37	0	0.00	0	0.00	0	0.00	2	0.18	0	0.0
Proteobacteria	*Morganella*	0	0.00	1	0.16	3	0.39	0	0.00	2	0.18	0	0.0
Proteobacteria	*Nevskia*	10	1.24	0	0.00	18	2.35	0	0.00	3	0.27	3	0.3
Proteobacteria	*Stenotrophomonas*	7	0.87	1	0.16	3	0.39	0	0.00	5	0.45	2	0.2
Synergistota	*Jonquetella*	4	0.50	0	0.00	0	0.00	0	0.00	0	0.00	0	0.0
Actinobacteriota	*Unclassified*	14	1.74	11	1.71	26	3.39	16	2.58	8	0.72	8	0.8
Proteobacteria	*Unclassified*	18	2.23	24	3.73	20	2.61	14	2.26	19	1.71	8	0.8
Firmicutes	*Vagococcus*	0	0.00	5	0.78	2	0.26	10	1.62	21	1.89	9	0.9
Proteobacteria	*Pantoea*	5	0.62	3	0.47	12	1.56	9	1.45	15	1.35	9	0.9
Aquificota	*Sulfurihydrogenibium*	0	0.00	0	0.00	0	0.00	7	1.13	0	0.00	1	0.1
Firmicutes	*Solibacillus*	1	0.12	0	0.00	0	0.00	7	1.13	35	3.14	18	1.8

Table S5. Significant correlations between the relative abundance of gut bacterial genera and liver antioxidation parameters in European catfish. Significant values are in bold. p- values are enclosed in brackets.

Phylum	Genus	MDA	GSH	SOD	TAOC
Fusobacteriota	*Cetobacterium*	-0.201 (0.603)	0.223 (0.564)	**0.716 (0.030)**	**-0.803 (0.009)**
Proteobacteria	*Diaphorobacter*	0.243 (0.528)	-0.214 (0.581)	**-0.697 (0.037)**	**0.796 (0.010)**
Proteobacteria	*Pseudomonas*	-0.075 (0.849)	-0.181 (0.642)	**-0.698 (0.036)**	0.610 (0.081)
Spirochaetota	*Brevinema*	0.207 (0.593)	-0.216 (0.576)	**-0.714 (0.031)**	**0.800 (0.010)**

Table S6. Significant correlations between the relative abundance of gut bacterial genera and liver antioxidation parameters in African catfish hybrid. Significant values are in bold. p- values are enclosed in brackets.

Phylum	Genus	MDA	GSH	SOD	TAOC
Bacteroidota	*Bacteroides*	0.439 (0.384)	-0.587 (0.221)	-0.202 (0.701)	**-0.870 (0.024)**
Bacteroidota	*Flavobacterium*	**0.828 (0.042)**	0.150 (0.777)	-0.638 (0.173)	-0.317 (0.541)
Firmicutes	*Epulopiscium*	0.541 (0.268)	-0.430 (0.395)	-0.488 (0.326)	**-0.879 (0.021)**
Firmicutes	*Turicibacter*	-0.594 (0.214)	**-0.823 (0.044)**	0.641 (0.170)	-0.490 (0.323)

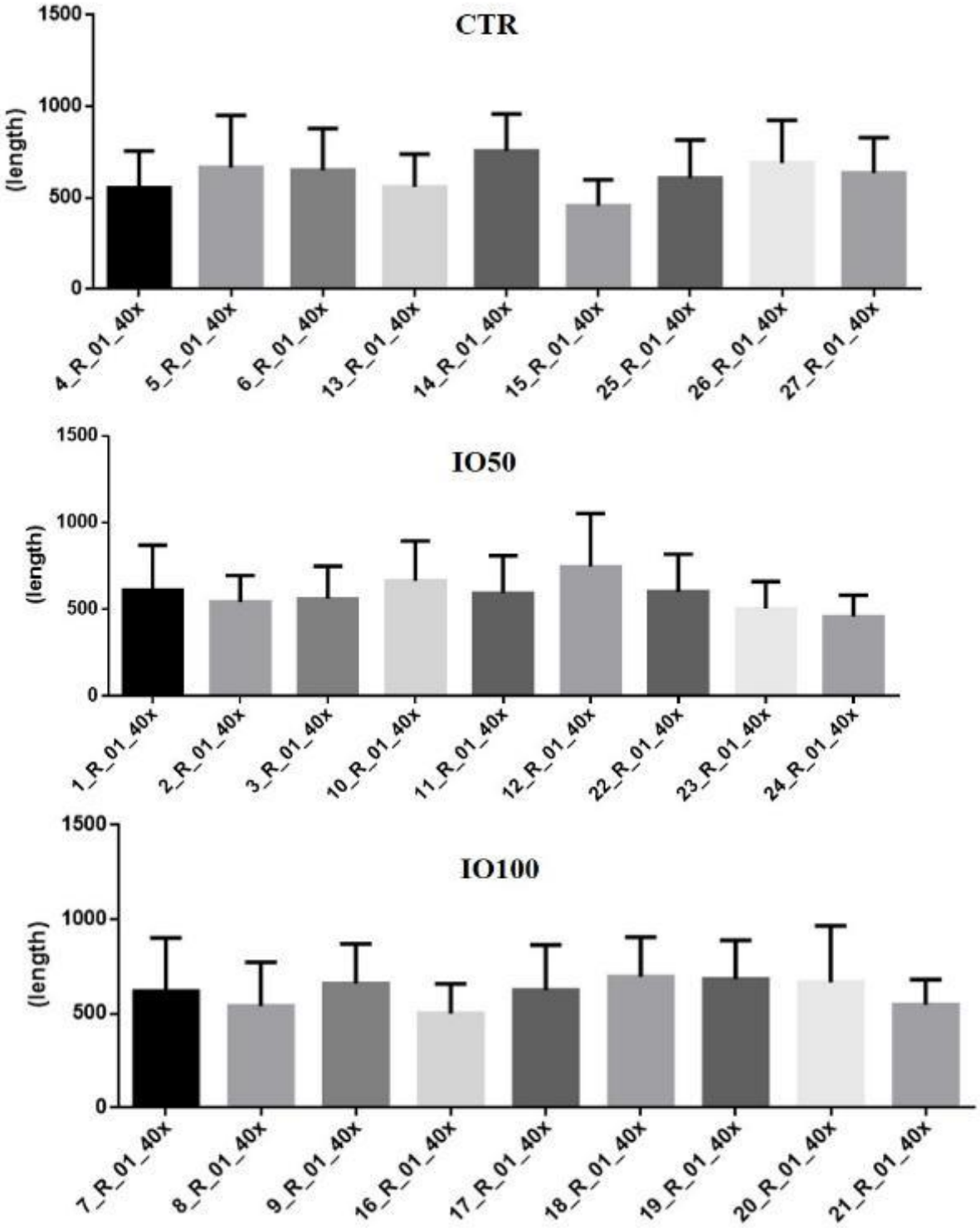

Figure S1. Intestinal villi length of individual specimens of African catfish hybrid fed a control diet (CTR, 40×) and insect oil-based diets (IO50 and IO100 both 40×).