

Insect meal inclusion as a novel feed ingredient in soy-based diets improves performance of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The additive effects of insect meal supplementation to diets containing a high soy protein inclusion were evaluated for growth performance, antioxidant potential and fillet nutritional quality of juvenile rainbow trout (*Oncorhynchus mykiss*). A basal diet (Soy) with a high-level soy protein (21% soybean meal, SBM plus 4–5.5% soy protein concentrate, SPC) was compared to similarly formulated soy-based diets supplemented with low (8%) and high levels (16%) of insect meal for diet groups Soy + IM_{Low} and Soy + IM_{High}, respectively. A high-level fishmeal (24%) based positive control diet (Fish meal) was also produced. All diets were iso-nitrogenous (44%) and isolipidic (20%) and were fed to triplicate groups of rainbow trout juveniles (initial weight 32 ± 0.0 g) over the course of a 10-week trial. Fish fed the positive control diet showed significantly higher final weight, % weight gain (%WG) and specific growth rate (SGR) compared to the Soy + IM_{High} and Soy diet groups, but these parameters were not significantly different from the Soy + IM_{Low} diet group. The Soy and Soy + IM_{High} diet groups showed significantly lower final weight, % WG and SGR. Feed efficiency and protein efficiency ratio was numerically higher in fish fed the Fish meal positive control diet followed by the Soy + IM_{Low} and Soy and Soy + IM_{High} diet groups, respectively. Among somatic indices, condition factor (CF) was significantly influenced by IM supplementation and the Soy + IM_{High} diet group showed significantly lower CF compared to other dietary groups. Addition of insect meal significantly increased whole body amino acids viz., arginine, histidine, valine and threonine content. Insect meal supplementation significantly increased muscle α -linolenic acid content. However, α -linolenic acid content in fish fed the Soy + IM_{High} diet was not significantly different from fish fed the Fishmeal positive control diet. Insect meal supplementation did not significantly influence EPA + DHA, total n-3 PUFA and monounsaturated fatty acid content in the muscle, whereas the significantly highest EPA/DHA and total monounsaturated fatty acids were detected in the fishmeal based positive control diet compared to other dietary treatment. The liver gene expression pattern for $\Delta 5$ desaturase was significantly higher in the Soy + IM_{Low} diet fish, while $\Delta 6$ desaturase and fatty acid synthase (FAS) were significantly higher in Soy (basal) and Soy + IM_{Low} diet fed fish. Elongase and fatty acid binding protein (FABP) were not significantly influenced by insect meal supplementation. Regarding the antioxidant potential, Soy diet group showed significantly higher superoxide dismutase (SOD) activity compared to insect meal included groups and positive control diet group. Glutathione peroxidase (GPX) activity was significantly reduced in Soy + IM_{High} diet compared to other dietary groups. Finally, we conclude that insect meal inclusion at a low level (8%) is beneficial for increased growth and select fatty acid restoration for rainbow trout fed soy-based feeds.

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1. Introduction

Historically fishmeal (FM) has been the major protein source in aquaculture diets. However, increasing world wide demand and a reducing supply of this finite protein sources makes it a limiting factor in the aquafeed industry. To overcome this limitation, several cost-effective alternative protein sources have been tested in the diets of different fish species with varying degrees of success. Due to several positive characteristics, such as availability, cost effectiveness and relatively better balanced amino acids profile, soybean meal (SBM) has been considered as the most suitable alternative protein for replacing FM. It is also well documented that SBM inclusion as a FM replacer in aquafeeds above a certain percentage (20–40%) causes amino acid imbalances and antinutrients accumulation resulting in reduced feed intake and weight gain, morphological changes of distal intestinal epithelium and abnormal health conditions of fish (Baevefjord and Kroghdahl, 1996; Burrells et al., 1999; Kroghdahl et al., 2003; Deng et al., 2006; Chen et al., 2011; Li et al., 2012; Hossain and Koshio, 2017; Hossain et al., 2018). However, supplementation strategies, i.e., supplementation of novel feed ingredients or functional additives, could improve the palatability and nutritional quality of soy-based and plant protein-based diets, which would help to recover feed intake, as well as improve overall fish performance (Hossain and Koshio, 2017; Hossain et al., 2018). Recently, protein meal derived from insects has received attention as a novel feed ingredient for aquafeeds due to having potential beneficial growth and health properties (Belghit et al., 2019; Nogales-Mérida et al., 2019; Fisher et al., 2020). Inclusion of insect meal in alternative plant protein based aquafeeds might help to increase the efficiency of utilizing alternative plant proteins which is very important for low FM based functional aquafeed development.

Insect meals from fly larvae, especially black soldier fly larvae (BSFL) have been identified as high protein (~60%) meals providing bioavailable proteins, as well as a balanced essential amino acid profile, and lipids, minerals, and vitamins suitable for fish under most rearing conditions (Barroso et al., 2014; Henry et al., 2015; Wang and Shelomi, 2017). BSFL meal has a more appropriate amino acid profile for fish than commonly used plant ingredients, such as SBM (Makkar et al., 2014; Tran et al., 2015). The oil content of BSFL is often reduced after mechanical or chemical extraction processes to produce commercial BSFL meal. Even after extraction, commercially available BSFL meal contains ~15% crude lipid. BSFL oil is rich in medium-chain fatty acids such as lauric acid (C12:0), approximately 21%–49%, and this fatty acid is advantageous in abdominal fat reduction and has been shown to have beneficial antimicrobial effects on gut bacteria (Li et al., 2016, 2017). In addition, the concentration of polyunsaturated fatty acids (linoleic, 18:2n-6, and linolenic, 18:3n-3) in BSFL oil is closely related to fatty acid ratios found in many plant oils such as soybean and sunflower oil and this could be effectively utilized by freshwater fish to biosynthesize n-3 long-chain polyunsaturated fatty acid (LC-PUFA) through a series of elongation and desaturation reactions (Oliva-Teles, 2012). The inclusion of insect meal in Nile tilapia diets and their subsequent effects on LC-PUFA biosynthesis has been reported in previous studies (Agbohessou et al., 2021; Sánchez-Muros et al., 2016). However, insect meal inclusion in fish meal replaced with SBM based diet and their subsequent effects on LC-PUFA bioconversion has not been reported in rainbow trout. So, it is of great importance to evaluate the impact of BSFL meal dietary inclusion on the bioconversion of LC-PUFA in rainbow trout. The fatty acid desaturation and elongation pathway have been extensively studied in fish at both the molecular and enzymatic levels (reviewed by Turchini et al., 2009), and several of the fatty acid desaturases ($\Delta 6$ and $\Delta 5$ desaturases) and elongases, that are critical enzymes and genes involved in this bioconversion of fatty acids have been shown to be produced in fish. However, the dietary inclusion effects of BSFL meal on those genes in rainbow trout (*Oncorhynchus mykiss*) is scarce. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are antioxidant enzymes which play indispensable role in the antioxidant protective

capacity of biological systems against free radical attack (Ighodaro and Akinloye, 2018). BSFL meal protein derivatives contain low molecular weight peptides that are known for their antioxidant and immunomodulatory effects (Nogales-Mérida et al., 2019). So, it is of great importance to evaluate the effects of BSFL meal inclusion in a soy-based diet on antioxidant activity and in rainbow trout it is yet to be reported.

There are some limitations of insect meal incorporation in aquafeed including its nutritional composition (fatty acid, amino acids profile) and presence of chitin in insect meal (Ng et al., 2001; Sanchez-Muros et al., 2014). In comparison to FM, insect meals are generally poor in n-3 LC-PUFA (Ekpo and Onigbinde, 2007; Katayama et al., 2008; Barroso et al., 2014). Despite these limitations, using insect meal as partial FM substitutes in aquafeeds obtained interesting results on different physiological aspects in several fish species without any negative effects on growth performance like, in rainbow trout (Renna et al., 2017; Dumas et al., 2018; Elia et al., 2018), Atlantic salmon, *Salmo salar* (Lock et al., 2016; Belghit et al., 2018), European seabass, *Dicentrarchus labrax* (Magalhães et al., 2017), Pacific white shrimp, *Litopenaeus vannamei* (Cummins et al., 2017) channel catfish, *Ictalurus punctatus* (Yildirim-Aksoy et al., 2020) and juvenile dusky kob (Madibana et al., 2020). In contrast, reduced growth and feed utilization have been reported in juvenile turbot and rainbow trout when FM was replaced with BSF meal at 65–756 g kg⁻¹ diet (St-Hilaire et al., 2007; Kroeckel et al., 2012). On the other hand, the partial substitution of FM with the larval meal of BSF and poultry by-product meal did not affect growth, utilization and feeding efficiency, but affected the whole-body fatty acid composition of Nile tilapia (Devic et al., 2018). Recently, Agbohessou et al. (2021) also reported that, total replacement of FM by enriched-fatty acid BSFL meal did not substantially affect growth parameters or innate immune status but improved whole body biochemical quality of Nile tilapia. However, so far, no investigations have been performed on rainbow trout using insect meal as a novel feed ingredient in a soybean-based diet. In the current study, the supplementation effects of BSFL meal in soy-based diets fed to rainbow trout on growth, body amino acid and fatty acid compositions, and antioxidant defense has been evaluated.

2. Materials and methods

2.1. Experimental design and test diets

All ingredients used in the experimental feeds were analyzed for protein and amino acid content at the Hagerman Fish Culture Experiment Station (HFCES), and analyzed values were used to refine feed formulations to achieve target protein lipid and key amino acid levels. Four experimental feeds for rainbow trout were produced as shown in Table 1. Briefly, a high level FM (24%) based positive control diet (Fish meal) was produced, then 40% of FM protein was replaced with SBM protein, and this was the high soy protein based (21% of SBM plus 4–5.5% soy protein concentrate, SPC) basal diet (Soy), which was then supplemented with low (8%) and high levels (16%) of BSFL meal for diet groups Soy + IM_{Low}, Soy + IM_{High}, respectively. All dry ingredients used in the formulation of experimental diets were ground to a particle size of <500 µm using a pulverizer. All ingredients were mixed thoroughly in a Hobart mixer, water was added (20–25%) and then cold pelleted at the University of Idaho's HFCES using a laboratory-scale California pellet mill (California Pellet Mill Company, San Francisco, CA, USA) fitted with 3–4 mm die. After 36 h drying in a forced-air dryer at 37 °C, the feeds were stored at ambient temperature until fed.

2.2. Experimental fish and feeding trial

Rainbow trout fingerlings, hatched from eggs obtained from Trout Lodge (Sumner, WA), were used in the study. Rainbow trout juveniles with an average initial weight of 32 ± 0.01 g were randomly stocked into 12,145-L tanks at 25 fish per tank. Each tank was supplied with 8 L/min of constant temperature (15 °C) spring water supplied by gravity to

Table 1

Formulation of the experimental diets.

Ingredients (%)	Diet groups			
	Fishmeal	Soy	Soy + IM Low	Soy + IM High
Fish meal	24	9.5	9.5	9.5
Soybean meal	0	21	21	21
Insect meal	0	0	8	16
Poultry meal	10.2	11.8	7.8	5.5
Soy protein concentrate	6	5.5	5	4
Wheat gluten	5	5	5	5
Corn Protein Concentrate	6	6.5	6	5
Blood meal	5	5	5	4.5
Wheat flour	24.9	15.59	13.26	11.03
Fish oil	16	17	16.4	15.5
Dicalcium phosphate	1.2	1.2	1.2	1.2
Choline chloride (60%)	0.6	0.6	0.6	0.6
Vitamin premix†	0.8	0.8	0.8	0.8
Trace Mineral mixture, Trouw nutrition‡	0.1	0.1	0.1	0.1
Vitamin C, Stay C-35)	0.2	0.2	0.2	0.2
Lysine	0.0	0.11	0.06	0.02
Methionine	0.0	0.1	0.08	0.05
Total	100	100	100	100

† Vitamin premix supply the following to the diet (mgkg⁻¹ diet): D calcium pantothenate, 46.47; pyridoxine (pyridoxine HCl), 13.68; riboflavin, 9.58; niacinamide, 21.78; folic acid, 2.49; thiamine (thiamine mononitrate), 9.1; inositol, 599; biotin, 0.33; vitamin B₁₂, 0.03; menadione sodium bisulfite complex, 1.1; vitamin E (DL α -tocopherol acetate), 131.9 IU; vitamin D₃ (stabilized), 6594 IU; vitamin A (vitamin A palmitate, stabilized), 9641 IU; ethoxyquin, 198.

‡ Trace mineral premix supply the following to the diet (mgkg⁻¹ diet): Zn (as ZnSO₄ 7H₂O), 50; Mn (as MnSO₄), 7.5; Cu (as CuSO₄ 5H₂O), 2.5; I (as KIO₃), 1; selenium (as Na₂SeO₃), 0.05.

the fish rearing laboratory. Each diet was fed twice daily by hand to triplicate tanks of fish to apparent satiation, six-day per week for 10 weeks. Tanks were assigned diets using a completely randomized design to account for any tank position effects. Photoperiod was held constant at 14 h light: 10 h dark with electric timers. Tanks were cleaned daily, and any dead fish were removed and recorded. All fish handling and experimental protocols were approved by and conducted in accordance with the guidelines of the University of Idaho's Animal Use and Care Committee (IACUC-2019-76).

2.3. Evaluation of performance parameters

At the end of the feeding trial, the following parameters were calculated using the live-weight and feed consumption data as per Hardy and Barrows (2002) to evaluate the growth and feed utilization performances of the fish.

Weight gain (g/fish) = (g mean final weight – g mean initial weight)

$$\text{Mean weight gain (\%)} = \left[\frac{(\text{g mean final weight} - \text{g mean initial weight})}{\text{g mean initial weight}} \right] \times 100$$

$$\text{Specific growth rate (SGR; \% / d)} = \left[\frac{\text{Ln (g final weight)} - \text{Ln (g initial weight)}}{\text{duration}} \right] \times 100$$

Feed consumed (g/fish) = g total feed consumed/number of surviving fish

Feed efficiency (FE) = g wet weight gain per fish/g feed consumed per fish

$$\text{Survival} = \left(\frac{\text{number of fish at the end of the trial}}{\text{number of fish at the beginning}} \right) \times 100$$

Protein efficiency ratio (PER) = g weight gain/g protein consumed

$$\text{Nutrient retention (\%)} = \left(\frac{\text{g nutrient gain}}{\text{g nutrient consumed}} \right) \times 100$$

2.4. Sample collection and biochemical analysis

As an initial sample, 15 fish from the experimental population were sacrificed and frozen for whole-body analysis. At the end of the feeding trial, all experimental fish were fasted for 24 h prior to sampling. All the fish were anesthetized with tricaine methanesulfonate (MS-222, 100 mg/L). Then, individual body weight and length of fish were measured, and the growth parameters were calculated accordingly. Three randomly selected anesthetized fish from each dietary replicate tank were used for the collection of blood by puncture of the caudal vasculature using non-heparinized disposable syringes, and serum was obtained by centrifugation at 7000 xg for 10 min at 4 °C. Liver and viscera were dissected from the fish identified above following euthanasia (MS-222, 300 mg/L) and were weighed individually to calculate the hepatosomatic index (HSI) and viscerasomatic index (VSI). A small section of liver and muscle samples was collected from each of the fish identified above, snap-frozen in liquid nitrogen, and stored at -80 °C until analyses.

Experimental diets used in the trial were analyzed for proximate composition, amino acids, fatty acid, and gross energy at the Hagerman lab using standard protocols (as described below) prior to the start of the feeding trial to ensure that calculated values were achieved (Table 2 and Table 3). Proximate composition (moisture, protein, fat and ash) were determined using Association of Official Analytical Chemists procedures (AOAC, 1995). Samples were dried in a convection oven at 105 °C for 12 h to determine moisture level. Dried samples were finely ground by mortar and pestle and analyzed for crude protein (total nitrogen x 6.25) using a combustion method with a nitrogen determinator (rapid N exceed, Elementar, Langensfeld, Germany). Crude lipid was analyzed by subjecting samples to acid hydrolysis using an ANKOM HCL hydrolysis system (ANKOM Technology, Macedon, NY) and extracting samples with petroleum ether using an ANKOM XT15 extractor. Ash was analyzed by incineration at 550 °C in a muffle furnace for 5 h. Energy content of samples was determined using an isoperibol bomb calorimeter (Parr 6300, Parr Instrument Company Inc., Moline, IL). Analyses of amino acids in fish whole body and feeds were conducted using a Bio-Chrom 30+ amino acid analyzer. Briefly, sample containing approximately 100 mg protein was taken into hydrolysis tube and 1 mL hydrochloric acid was added. Afterward, using vacuum pump and N₂

Table 2

Analyzed proximate and amino acid composition of experimental diets (% as-fed basis).

Parameters	Diet groups			
	Fishmeal	Soy	Soy + IM Low	Soy + IM High
Moisture	5.97	5.83	6.02	5.93
Crude protein	44.80	44.47	43.90	43.76
Crude lipid	20.97	21.01	21.1	20.84
Ash	7.57	7.08	7.01	7.23
Energy (Cal/g)	5352.5	5383.0	5421.5	5389.0
<i>Amino acid profiles</i>				
Arginine (Arg)	2.29	2.39	2.20	2.17
Cysteine (Cys)	4.07	5.17	4.88	4.78
Histidine (His)	1.71	2.82	2.66	2.70
Isoleucine (Ile)	2.27	2.29	2.19	2.27
Leucine (Leu)	4.34	4.43	4.22	4.43
Lysine (Lys)	6.55	7.20	7.15	6.93
Methionine (Met)	1.29	1.23	1.27	1.25
Phenylalanine (Phe)	0.64	0.59	0.76	0.69
Threonine (Thr)	1.70	1.68	1.59	1.70
Tryptophan (Trp)	3.02	3.84	3.77	4.02
Tyrosine (Tyr)	4.25	3.73	3.35	3.75
Valine (Val)	4.72	4.86	4.44	4.56
Alanine (Ala)	2.78	2.98	2.82	2.95
Aspartate (Asp)	3.69	3.84	3.72	3.74
Glutamate (Glu)	12.40	12.99	11.98	12.48
Glycine (Gly)	2.66	2.77	2.70	2.79
Proline (Pro)	2.66	2.50	2.40	2.77
Serine (Ser)	2.17	2.14	2.07	2.10

Table 3

Analyzed fatty acids composition (% of total fatty acids) of experimental diets (% as-fed basis).

Variables	Name	Diet groups			
		Fishmeal	Soy	Soy + IM Low	Soy + IM High
C14:0	Myristic acid	5.20 ± 0.12	5.26 ± 0.23	5.31 ± 0.11	5.76 ± 0.16
C16:0	Palmitic acid	16.57 ± 0.49	16.70 ± 0.96	15.89 ± 0.30	16.49 ± 0.22
C16:1	Palmitoleic acid	5.49 ± 0.12	5.79 ± 0.25	5.49 ± 0.00	5.38 ± 0.05
C18:0	Stearic acid	3.63 ± 0.06	3.63 ± 0.18	3.53 ± 0.05	3.42 ± 0.00
C18:1n-9	Oleic acid	21.38 ± 0.17	22.99 ± 1.16	21.38 ± 0.04	21.25 ± 0.02
C18:2n-6	linoleic acid	8.24 ± 0.13	9.46 ± 0.45	9.49 ± 0.02	10.07 ± 0.06
C18:3n-3	α-Linolenic acid	1.03 ± 0.03	1.30 ± 0.05	1.30 ± 0.01	1.30 ± 0.01
C20:1n-9	11-Eicosenoic acid or Gondoic acid	12.00 ± 0.07	12.41 ± 0.00	12.54 ± 0.17	11.84 ± 0.11
C20:5n-3	Eicosapentaenoic acid	9.33 ± 0.50	9.14 ± 0.01	8.95 ± 0.06	8.58 ± 0.02
C22:1n-9	Erucic acid	7.58 ± 0.07	8.13 ± 0.28	7.95 ± 0.08	7.57 ± 0.04
C22:5n-3	Docosapentaenoic acid	0.83 ± 0.03	0.84 ± 0.02	0.81 ± 0.03	0.79 ± 0.02
C22:6n-3	Docosahexaenoic acid	8.38 ± 0.34	7.72 ± 0.49	7.35 ± 0.14	7.09 ± 0.00

gas, all the atmosphere inside the hydrolysis tube was evacuated. After de-gassing the hydrolysis tubes with samples were placed in a heating block pre-heated to 110 °C exactly for 24 h. Afterwards, using a 3 mL syringe and stainless-steel needle, the hydrolyzed sample was transferred into test tube with 9 mL of BioChrom Loading Buffer. Then, 2 mL diluted sample was taken out and using NaOH, pH was adjusted to between 2 and 2.2. Using the 3 mL syringe again pH adjusted 2 mL sample was filtered (25 mm syringe filter) into a labeled 1.5 mL sample vial. The pH adjusted samples were prepared for analysis by using 100 µL of filtered sample, 100µL filtered 2500 µmol/L norleucine standard, 800 µL filtered Biochrom Loading Buffer. Finally, after vortexing the sample was run on the BioChrom 30+ for amino acid analysis. The fatty acid composition of the liver and fillet samples was determined in line with the modified AOAC method (AOAC, 1995). Briefly, samples were dried for 5–6 h under an N₂ stream at 50 °C (OA-SYS heating system, Organomation Associates, Inc., Berlin, MA, USA). Thereafter, 2 mL of 0.5 N NaOH was added for sample saponification at 70 °C for 60 min. Following sample cooling, the free fatty acid was methylated by the addition of 2 mL 14% BF₃ (Boron trifluoride) in methanol and incubated at 70 °C for 60 min. After the samples were allowed to cool, 2 mL of hexane was added, inverted repeatedly for 60 s, and 1 mL of saturated NaCl was added. The samples were again inverted repeatedly for 60 s and then centrifuged at 2000 ×g for 5 min. An aliquot (100 µL) of the clarified hexane extract was diluted in hexane (1:10) and put into autosampler vials for gas chromatography/mass spectroscopy (GC/MS) analysis. The injection mode with a helium flow rate and the column temperature was as described by Overturf et al. (2013). All the analyses were done in duplicate.

2.5. Antioxidant enzymes, glucose and total serum protein assay

The superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzyme activities in the serum were measured using Cayman chemical assay kits (#706002, #707002, and #703102, respectively). Total serum protein content was quantified using the Pierce™ BCA protein assay kit (Thermo Scientific). The serum glucose concentration was quantified using a commercial kit (Cayman chemical, #10009582).

2.6. Hepatic gene expression

The total RNA was isolated individually from the collected liver samples ($n = 9$ fish per diet) using the TRIzol reagent (Invitrogen, USA) extraction method following the manufacturer's protocol. The RNA purity and quantity of the RNA samples were examined by measuring absorbance at 260 and 280 nm (NanoDrop 2000 spectrophotometer, Thermo Scientific, USA). To ascertain the level of lipid metabolism-related gene expression, quantitative real-time RT-PCR was performed with an ABI Prism7900HT Sequence Detection System using the TaqMan One-Step RT-PCR Master Mix Reagents kit in line with the procedure provided by ABI (Foster City, CA, USA). The concentration of each reaction was: Master Mix, 1× (contains AmpliTaq Gold enzyme, dNTPs including dUTP, a passive reference, and buffer components); Multi-Scribe reverse transcriptase, 0.25 U µL⁻¹; RNase inhibitor mix, 0.4 U µL⁻¹; forward primer 600 nM; reverse primer 600 nM; probe, 250 nM; total RNA, 75 ng. The primer sequences of each gene, accession numbers, and probes are depicted in Table 4. The β-actin gene was used as the reference gene. The thermal cycling conditions used for the tested genes consisted of 30 min at 48 °C, 10 min at 95 °C, then 40 PCR cycles of 95 °C for 15 s followed by 60 °C for 1 min. After carrying out the melting curve analysis to confirm a single PCR product in each reaction, the comparative CT method was used to determine the relative expression of the target genes viz., fatty acid-binding protein (FABP), fatty acid synthase (FAS), fatty acid elongase, Δ5 and Δ6 desaturases, and the values were normalized by the 2-ΔΔCT method (Livak and Schmittgen, 2001) with β-actin as the reference gene. Each assay sample was run in duplicate.

2.7. Statistical analysis of data

Tank mean values were used as units of observation for statistical analysis. All performance data were tested for normality and homogeneity of variance prior to one-way Analysis of Variance (ANOVA). If significant differences were found, data were subjected to Tukey's HSD test to separate the means at a significance level of $P < 0.05$ using the IBM SPSS version 22 package.

3. Results

3.1. Growth feed utilization performances and somatic indices

Growth performance, feed utilization and somatic indices of rainbow trout fed experimental diets are presented in Table 5. Significantly higher final weight, % weight gain (%WG) and specific growth rate (SGR) was observed in fish fed the positive control diet to the Soy and Soy + IM_{High}, but it was not significantly different from the low insect meal supplemented group (Soy + IM_{Low}). Both the Soy and Soy + IM_{High} group showed significantly lower final weight, % WG and SGR. Feed efficiency and protein efficiency ratio were numerically higher in the fish meal based positive control diet group followed by Soy + IM_{Low}, Soy and Soy + IM_{High} diet groups, respectively. However, feed utilization parameters and protein retention were not significantly influenced by insect meal supplementation. Survival rate ranged between 97.3 and 100% and were not significantly different among diet groups. Among the measured somatic indices only condition factor (CF) was significantly influenced by insect meal supplementation and Soy + IM_{High} diet group showed significantly lower CF compared to other dietary groups.

3.2. Whole-body composition of major nutrients and amino acids

The whole-body major nutrients (moisture, protein, lipid, ash) were not significantly influenced by dietary treatment groups (Table 6). Several whole-body amino acids (arginine, histidine, threonine, valine, alanine, aspartic acid and glycine) were significantly influenced by

Table 4

Primers and probes used for one-step quantitative real-time (RT-PCR).

Genes	Primers (5'-3')	Probes	Accession numbers
Fatty acid synthase, <i>FAS</i>	Fw: 5'-GCCAGCGTGCCATGTTTC-3' Rv: 5'-GGGCGGTTGAAGTCAAAGAA-3'	5'-CCAACCGACTCTCC-3'	XM_021576228
Fatty acid binding protein, <i>FABP</i>	Fw: 5'-AGAGAAAGTTCTCCACATCAGAGA-3' Rv: 5'-CTTGCTTATCCTGGTGAGGGTT-3'	5'-TCATGCGGCGCTCAAC-3'	XM_020500137.2
Elongase, <i>Elovl</i>	Fw: 5'-CTGATGTACTCTTACTATGGGCTCTCT-3' Rv: 5'-AACTGAATCAGCTGTACTTGTGTGA-3'	5'-CCTTGGCGGCTATCT-3'	AY605100
Delta -6- desaturase, $\Delta 6$ desaturase	Fw: 5'-GAC CAT GTT TTC ACA ACG GAA CTG-3' Rv: 5'-CAG AAG AAG CGA AGG TAG AAA GTC A-3'	5'-CATGACGGCGACTACC-3'	AY458652
Delta -5- desaturase, $\Delta 5$ desaturase	Fw: 5'-CTGGGCCACATCC-3' Rv: 5'-CCAAAGCCAGGGCCTCTAG-3'	5'-GCCTCCTGTTCTTCAGCCTCTA-3'	XM_014170388
Beta-actin, β act	Fw: 5'-CCC TCT TCC AGC CCT CCTT-3' Rv: 5'-AGT TGT AGG TGG TCT CGT GGA TA-3'	5'-CCGCAA GACTCC ATACCGA-3'	AF254414

Table 5

Growth performance, feed utilization and somatic parameters of rainbow trout fed with experimental diets for 70 days*

Variables	Diet groups			
	Fishmeal	Soy	Soy + IM Low	Soy + IM High
Initial weight	32.0 ± 0.01	32.0 ± 0.01	32.0 ± 0.01	32.0 ± 0.01
Final body weight	186.61 ± 1.89 ^b	174.02 ± 0.44 ^a	184.71 ± 2.78 ^b	170.04 ± 0.55 ^a
Weight gain (%)	483.15 ± 5.89 ^b	443.82 ± 1.37 ^a	477.22 ± 8.69 ^b	431.37 ± 1.71 ^a
Specific growth rate (%/day)	2.52 ± 0.02 ^b	2.42 ± 0.00 ^a	2.51 ± 0.03 ^b	2.39 ± 0.07 ^a
Feed intake	151.18 ± 1.15	147.42 ± 6.55	154.88 ± 0.94	144.30 ± 1.32
Feed efficiency	1.03 ± 0.01	0.97 ± 0.05	0.99 ± 0.02	0.96 ± 0.01
Protein efficiency ratio	2.38 ± 0.01	2.25 ± 0.11	2.29 ± 0.03	2.23 ± 0.02
Protein retention (%)	40.32 ± 0.35	37.71 ± 0.66	39.83 ± 0.40	37.94 ± 1.39
Survival (%)	100 ± 0.00	98 ± 2.00	100 ± 0.00	97.33 ± 1.33
CF ^a	1.48 ± 0.02 ^a	1.45 ± 0.02 ^a	1.49 ± 0.01 ^a	1.39 ± 0.02 ^b
HSI ^b	1.24 ± 0.06	1.19 ± 0.03	1.22 ± 0.04	1.15 ± 0.02
VSI ^c	10.34 ± 0.56	10.63 ± 0.25	10.78 ± 0.35	10.94 ± 0.48

* Means ($n = 3$) in the same row that share the same superscript are not statistically different ($P > 0.05$; Completely Randomized Design, One-factor ANOVA; Tukey's HSD test).

^a Condition factor (CF, %) = weight of fish / (length of fish)³ × 100.

^b Hepatosomatic index (HSI, %) = weight of liver / weight of fish × 100.

^c Viscerosomatic index (VSI, %) = viscera weight/fish weight × 100.

insect meal supplementation (Fig. 1). These amino acid contents were significantly higher in insect meal supplemented groups compared to the fish fed the fishmeal control and Soy diets.

3.3. Fatty acid composition of muscle (fillet) and hepatic tissue

Tables 7 and 8 and Figs. 2 and 3 show the fatty acids composition of the rainbow trout fillet and hepatic tissue. The fatty composition of the muscle and liver were influenced by the insect meal as well as the inclusion of alternative protein. In comparison to the Soy diet, insect meal supplemented groups showed significantly higher fillet α -linolenic acid content and fish fed Soy + IM_{high} diet group showed similar fillet α -linolenic acid content to fish fed the fishmeal control diet. Total SFA, PUFA, DHA and EPA + DHA content in fillets were not significantly influenced by insect meal inclusion, but fish fed the soy-based basal diet and insect meal supplemented diet showed significantly higher content of these fatty acids in comparison to high FM-based positive control diet (Fig. 2a, b). In contrast, the positive control diet group showed significantly higher total MUFA and EPA/DHA ratio compared to fish fed other

Table 6

Analyzed whole body proximate (% wet basis) and amino acids composition (AVG, % DM) of rainbow trout fed test diets for 70 days.

Parameters	Initial	Diet groups			
		Fishmeal	Soy	Soy + IM Low	Soy + IM High
Moisture (%)	75.52	66.98 ± 0.39	67.70 ± 0.93	66.61 ± 0.22	67.96 ± 0.72
Protein (%)	13.40	16.89 ± 0.12	16.72 ± 0.53	17.23 ± 0.25	16.61 ± 0.37
Lipid (%)	7.94	13.81 ± 0.35	13.58 ± 0.53	14.64 ± 0.11	13.41 ± 0.62
Ash (%)	2.19	2.17 ± 0.02	2.07 ± 0.01	2.17 ± 0.08	2.19 ± 0.02
<i>Amino acids</i>					
Cysteine (Cys)	4.93	4.72 ± 0.14	4.61 ± 0.06	4.73 ± 0.12	4.82 ± 0.06
Isoleucine (Ile)	1.88	2.34 ± 0.49	2.15 ± 0.27	2.46 ± 0.11	2.45 ± 0.09
Leucine (Leu)	4.05	4.03 ± 0.15	3.90 ± 0.16	4.17 ± 0.05	4.08 ± 0.16
Lysine (Lys)	4.75	4.30 ± 0.97	3.47 ± 0.64	4.81 ± 0.06	4.48 ± 0.13
Methionine (Met)	1.13	0.83 ± 0.19	0.92 ± 0.17	1.16 ± 0.02	1.14 ± 0.03
Tyrosine (Tyr)	3.47	4.01 ± 0.34	3.59 ± 0.27	4.18 ± 0.31	3.87 ± 0.42
Alanine (Ala)	2.84	2.80 ± 0.04 ^a	3.09 ± 0.30 ^{ab}	3.63 ± 0.06 ^b	3.51 ± 0.09 ^b
Aspartate (Asp)	3.66	3.45 ± 0.15 ^a	4.13 ± 0.61 ^{ab}	5.45 ± 0.11 ^b	5.28 ± 0.18 ^b
Glutamate (Glu)	12.01	11.79 ± 0.34	11.05 ± 0.08	11.41 ± 0.21	11.0 ± 0.34
Glycine (Gly)	2.65	2.78 ± 0.07 ^a	3.59 ± 0.53 ^{ab}	4.64 ± 0.05 ^b	4.50 ± 0.11 ^b
Proline (Pro)	2.33	2.54 ± 0.11	2.44 ± 0.32	2.08 ± 0.02	2.10 ± 0.13
Serine (Ser)	2.10	1.99 ± 0.06	2.02 ± 0.13	2.33 ± 0.05	2.27 ± 0.04
Σ Amino acid	60.12	57.90 ± 2.59	56.48 ± 3.77	65.02 ± 1.02	63.16 ± 1.82

dietary groups (Fig. 2b). Total EPA and ARA content showed no significant difference among dietary groups. Liver total SFA, PUFA, EPA + DHA, ARA, DHA were not significantly influenced by dietary treatment. Soy + IM_{low} diet group showed significantly higher liver total MUFA but did not differ significantly from the positive control diet group (Fig. 3a). Insect meal inclusion at both high and low levels significantly reduced EPA content in liver (Fig. 3b); whereas, the ratio of EPA to DHA was significantly lower only when high insect meal was included (Soy + IM_{high}).

3.4. Hepatic lipid metabolism-related genes expression

The relative mRNA (RT-qPCR) expression of hepatic fatty acid

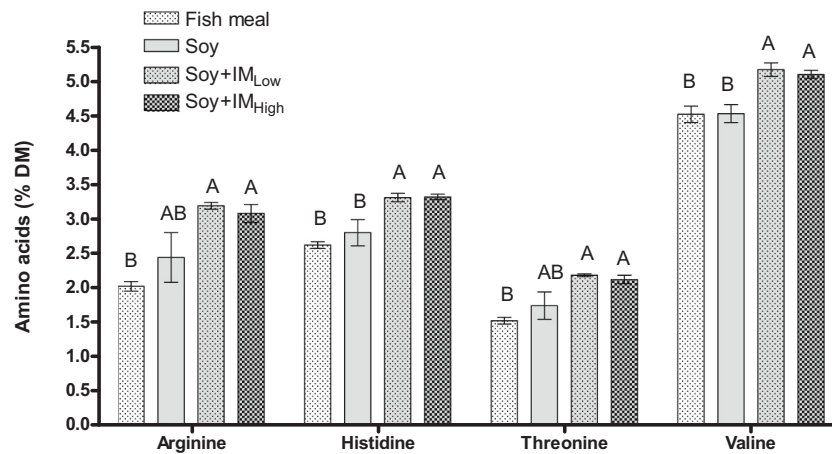


Fig. 1. Whole-body amino acid compositions in rainbow trout fed test diets for 10 weeks. Values with different letters within dietary treatment comparisons are significantly different ($P < 0.05$). Error bars denote standard error.

Table 7

Fatty acid composition of the total lipid fraction (% of total fatty acids) in rainbow trout fillet, ($n = 9$).

Fatty acids		Diet groups			
Type	Name	Fishmeal	Soy	Soy + IM _{Low}	Soy + IM _{High}
<i>Saturates</i>					
C14:0	Myristic acid	3.35 ± 0.07	3.28 ± 0.15	3.33 ± 0.14	3.24 ± 0.22
C16:0	Palmitic acid	14.64 ± 0.81 ^b	20.74 ± 0.69 ^{ab}	19.36 ± 0.76 ^a	19.56 ± 0.20 ^a
C18:0	Stearic acid	4.96 ± 0.09 ^b	4.79 ± 0.10 ^b	5.04 ± 0.13 ^b	5.71 ± 0.19 ^a
ΣSAT ^a		22.95 ± 0.80 ^b	28.81 ± 0.83 ^a	27.74 ± 0.87 ^a	28.52 ± 0.29 ^a
<i>Monoene's</i>					
C16:1	Palmitoleic acid	5.23 ± 0.08 ^a	4.09 ± 0.19 ^b	4.07 ± 0.16 ^b	3.96 ± 0.19 ^b
C18:1n-9	Oleic acid	23.38 ± 0.19 ^a	19.63 ± 0.82 ^b	19.65 ± 0.76 ^b	18.69 ± 0.63 ^b
C20:1n-9	11-Eicosenoic acid	12.56 ± 0.62 ^a	7.50 ± 0.50 ^b	8.51 ± 0.53 ^b	7.45 ± 0.26 ^b
C22:1n-9	Erucic acid	5.70 ± 0.78 ^a	3.19 ± 0.24 ^b	3.87 ± 0.29 ^b	3.68 ± 0.16 ^b
ΣMUFA ^b		46.86 ± 1.48 ^a	34.41 ± 1.66 ^b	36.10 ± 1.68 ^b	33.78 ± 1.13 ^b
<i>Polyunsaturated</i>					
C18:2n-6	linoleic acid	8.50 ± 0.19 ^a	7.04 ± 0.37 ^b	7.31 ± 0.42 ^b	7.69 ± 0.36 ^{ab}
C22:5n-3	Docosapentaenoic acid	1.98 ± 0.08 ^a	1.64 ± 0.05 ^b	1.73 ± 0.05 ^b	1.73 ± 0.09 ^b
DHA/EPA		1.81 ± 0.16 ^b	3.15 ± 0.21 ^a	2.67 ± 0.13 ^a	2.93 ± 0.12 ^a

^a SAT: Saturated fatty acid.

^b MUFA: Monounsaturated fatty acid.

metabolism related genes fatty acid elongase, fatty acid desaturases ($\Delta 5$ and $\Delta 6$ desaturase), FABP and FAS in rainbow trout fed different experimental diets are presented in Fig. 4. Fish fed Soy + IM_{Low} diet group showed significantly higher $\Delta 5$ desaturase expression compared to other dietary groups. Fatty acid elongase and FABP expression pattern were not influenced by IM supplementation, and no significant difference was observed among the diet groups. Relative expression of $\Delta 6$ desaturase was significantly higher in basal (Soy) diet group and did not differ significantly from fish fed the Soy + IM_{Low} diet followed by positive control (Fishmeal) and Soy + IM_{Low} diet. FAS expression was significantly higher in Soy (basal) and Soy + IM_{Low} diet groups compared to Soy + IM_{High} and the positive control diet groups.

3.5. Serum antioxidant enzyme activity, total serum protein and glucose content

Fish fed the Soy diet showed significantly higher SOD enzyme activity compared to insect meal supplemented groups and high FM-based positive control group (Fig. 5). Higher insect meal inclusion significantly reduced GPX activity compared to other dietary groups, whereas no significant difference was recorded in the CAT activity among the dietary groups (Fig. 5). Total serum protein content was not significantly influenced by dietary treatment groups. Significantly higher serum glucose levels were observed in fish fed the positive control diet compared to other treatment dietary groups (Fig. 6).

4. Discussion

In the current study, replacement of FM with alternative protein sources, as well as the inclusion of BSFL meal, did not make any remarkable changes in the nutritional composition (amino acids and fatty acids) of the test diets. The limiting amino acids in soy protein (lysine and methionine) were supplemented in the soy-based basal diet and IM supplemented diets during formulation so as to overcome the limitation of amino acids. Overall, the dietary formulation satisfies the recommended dietary nutrients requirements of rainbow trout (NRC, 2011).

In the present study low insect meal inclusion of the soy-based diet significantly increased the growth performance of fish and fish fed the low insect meal supplemented diet (Soy + IM_{Low}) were found to have similar final body weights and % weight gain as the fish fed the high FM-based positive control diet. The efficacy of insect meal inclusion as a FM substitute in several fish feeding trials has successfully demonstrated that fish growth performance is not negatively influenced by the inclusion of insect meal as certain levels of substitution for FM protein (Lock et al., 2016; Cummins et al., 2017; Magalhães et al., 2017; Renna et al., 2017; Belghit et al., 2018; Dumas et al., 2018; Elia et al., 2018; Fisher et al., 2020). In a recent study, Fisher et al. (2020) reported BSFL meal as a potential, sustainable and high protein feed ingredient for salmonid diets. However, high inclusion of insect meal either as a FM substitute or as a practical protein source to supplement FM failed to increase the growth performances of rainbow trout in the current study and has also been noted in other previous studies on several fish species including salmonids (St-Hilaire et al., 2007; Kroeckel et al., 2012; Gasco et al., 2016; Fisher et al., 2020). Fisher et al. (2020) reported that, Atlantic salmon fed BSFL meal included diets at a level of ≥ 300 g kg⁻¹ did not perform as well as fish fed BSFL meal included diet at a level 0 to 200 g kg⁻¹. In fresh water Atlantic salmon, up to 600 g kg⁻¹ of BSFL meal in

Table 8

Fatty acid composition of the total lipid fraction (% of total fatty acids) in rainbow trout liver ($n = 9$).

Fatty acids		Diet groups			
Type	Name	Fishmeal	Soy	Soy + IM _{Low}	Soy + IM _{High}
Saturates					
C14:0	Myristic acid	2.04 ± 0.07 ^{ab}	1.83 ± 0.05 ^b	2.27 ± 0.12 ^a	2.07 ± 0.09 ^{ab}
C16:0	Palmitic acid	15.10 ± 0.26 ^{ab}	15.61 ± 0.40 ^{ab}	14.80 ± 0.41 ^b	15.91 ± 0.32 ^a
C18:0	Stearic acid	6.74 ± 0.14	6.98 ± 0.17	6.41 ± 0.22	7.15 ± 0.59
ΣSAT ^a		23.88 ± 0.26	24.42 ± 0.34	23.47 ± 0.43	25.13 ± 0.76
Monoenes					
C16:1	Palmitoleic acid	2.64 ± 0.23 ^{ab}	2.33 ± 0.19 ^b	3.06 ± 0.25 ^a	2.14 ± 0.12 ^b
C18:1n-9	Oleic acid	17.42 ± 1.01 ^{ab}	16.22 ± 0.85 ^b	19.77 ± 1.12 ^a	16.56 ± 0.58 ^b
C20:1n-9	11-Eicosenoic acid or Gondoic acid	6.27 ± 0.35 ^a	5.48 ± 0.29 ^{ab}	6.33 ± 0.46 ^a	4.76 ± 0.14 ^b
C22:1n-9	Erucic acid	1.56 ± 0.13 ^{ab}	1.22 ± 0.12 ^{bc}	1.73 ± 0.20 ^a	0.99 ± 0.08 ^c
ΣMUFA ^b		27.90 ± 1.69 ^{ab}	25.25 ± 1.38 ^b	30.88 ± 1.95 ^a	24.46 ± 0.75 ^b
Polyunsaturate					
C18:2n-6	linoleic acid	3.05 ± 0.11 ^b	4.05 ± 0.14 ^a	4.23 ± 0.21 ^a	4.49 ± 0.12 ^a
C18:3n-3	α-Linolenic acid	0.34 ± 0.01	0.35 ± 0.01	0.37 ± 0.02	0.35 ± 0.02
C20:2n-6	Eicosadienoic acid	0.84 ± 0.03 ^c	1.15 ± 0.06 ^{ab}	1.03 ± 0.05 ^b	1.20 ± 0.05 ^a
C20:3n-6	Dihomo-γ-linolenic acid	0.33 ± 0.01 ^b	0.45 ± 0.02 ^a	0.42 ± 0.03 ^a	0.44 ± 0.03 ^a
C22:5n-3	Docosapentaenoic acid	1.91 ± 0.04 ^a	1.86 ± 0.05 ^a	1.77 ± 0.08 ^a	1.43 ± 0.05 ^b
DHA/EPA		4.04 ± 0.15 ^b	3.93 ± 0.13 ^b	4.40 ± 0.20 ^b	5.10 ± 0.18 ^a

^a SAT: Saturated fatty acid.

^b MUFA: Monounsaturated fatty acid.

combination with black soldier fly oil has been included in the diets without affecting the growth performance or the feed utilization compared with diets containing FM and SPC (Belghit et al., 2018). A considerable reduction in growth was also observed in blue tilapia (*Oreochromis aureus*) when fed fresh larvae of whole or chopped BSF (Bondari and Sheppard, 1987). The same trend was observed by Kroeckel et al. (2012) who reported a negative effect on growth of turbot juveniles (*Psetta maxima*) when the amount of BSFL meal in the diet increased from 330 to 756 g kg⁻¹. BSFL cuticle is a product composed of chitin (1.6% to 7.3%) in a matrix with cuticular proteins, lipids and other compounds (Sheppard et al., 1994; Diener et al., 2009). High incorporation of insect meal in fish diet can result in a high level of chitin (St-Hilaire et al., 2007; Kroeckel et al., 2012; Barroso et al., 2014) which may interfere with the digestive processes and thereby decrease feed efficiency and food intake with subsequent negative impacts on growth for several species. Kroeckel et al. (2012) reported reduced growth and feed intake with the 10% inclusion of chitin in rainbow trout diet. Karlsten et al. (2017) also reported reduced growth rate and apparent protein digestibility when chitin content increased from 1% to 2%, and

5% in salmon diets. Although digestibility of nutrients were not measured in the current study, reduced growth and feed utilization parameters in fish fed the high insect meal included diet suggests high levels of chitin in this diet may have contributed to the reduced fish performance in that group. In contrast to the negative effects of a high level of chitin on aquatic species, dietary chitin has also been shown to have a beneficial effect on intestinal microbiota in Atlantic salmon (Askarian et al., 2012), which suggests that this dietary component could have a positive effect on growth at low dietary levels, and our findings with trout fed a low BSFL meal diet substantiate the above findings.

In general, the BSFL meal amino acid (indispensable and dispensable) profile is comparable to FM (Nogales-Mérida et al., 2019). Although we formulated diets considering amino acid balance and formulated the diets such that all analyzed amino acids levels would be similar among diet groups, the significantly higher whole body amino acids (arginine, histidine, threonine, valine, alanine, aspartic acid and glycine) content in the insect meal supplemented groups might be due to better digestibility, absorption, utilization and bioavailability of those amino acids in insect meal than SPC. Fisher et al. (2020) also reported BSFL meal as slightly more digestible compared to other commercially used plant-based ingredients (SBM and corn protein concentrate, CPC) and also showed higher absorption of micronutrients in Atlantic Salmon which strongly supports our hypothesis regarding the higher deposition of certain amino acids found in the muscle of rainbow trout in the present study. Higher deposition of those amino acids was also indicated by the numerically higher protein retention obtained in the current study (Table 5), especially in the low insect meal incorporated diet group.

In the current study, muscle fatty acid compositions showed a remarkable change after feeding insect meal supplemented diets as well as FM replaced with the alternative plant protein based basal diet. In comparison to the positive control diet, fish in the other dietary groups had significantly higher and lower fillet saturated and monounsaturated fatty acid content, respectively (Fig. 2a). The higher total SFA was due to the higher saturated fatty acid content in the insect meal as well as in plant ingredients. Although insect meal inclusion had no significant effect on EPA and ARA content in fish fillet, comparison with fish fed the FM positive control diet, soy-based basal diet and low and high insect meal included groups showed significantly higher EPA + DHA and total PUFA. This phenomenon was further revealed by the activity of Δ6 and Δ5 fatty acid desaturase which were invariably higher in those diet groups. Significantly decreased mRNA expression levels of Δ5 and Δ6 desaturases in the FM positive control group was likely due to feedback inhibition effects resulting from higher levels of EPA and DHA in the FM positive control diet. Previous studies have also reported that high dietary concentrations of EPA and DHA have been reported to inhibit the pathway responsible for LC-PUFA synthesis in fish (Tocher et al., 2000; Jordal et al., 2005; Teoh and Ng, 2016).

FABP are intracellular cytoplasmic proteins that are active in the transport of fatty acids and other lipid-soluble substances throughout the cytoplasm (Torstensen et al., 2009; Castro et al., 2015). In this study, the relative mRNA expression of FABP was not significantly influenced by dietary treatment. Related to this study, no significant differences were noted in the hepatic FABP gene expression of European seabass (*D. labrax*) fed vegetable oil blend and fish oil (Castro et al., 2015). Fatty acid synthase plays a crucial role in the synthesis of fatty acids and its activity could be correlated with dietary saturated fatty acid composition. In our study, FAS expression was significantly higher in Soy and Soy + IM_{Low} diet groups, and this correlated with higher SFA levels in these diets. Similar to our study, Peng et al. (2017) reported higher FAS expression in turbot when fish fed diets substituting fish oil with vegetable oils, and this was also related to higher SFA levels in those diets.

Liver fatty acid compositions were not significantly influenced by dietary treatments, except for liver EPA, total MUFA and EPA/DHA (Fig. 3a,b). Significantly lower liver EPA content in insect meal

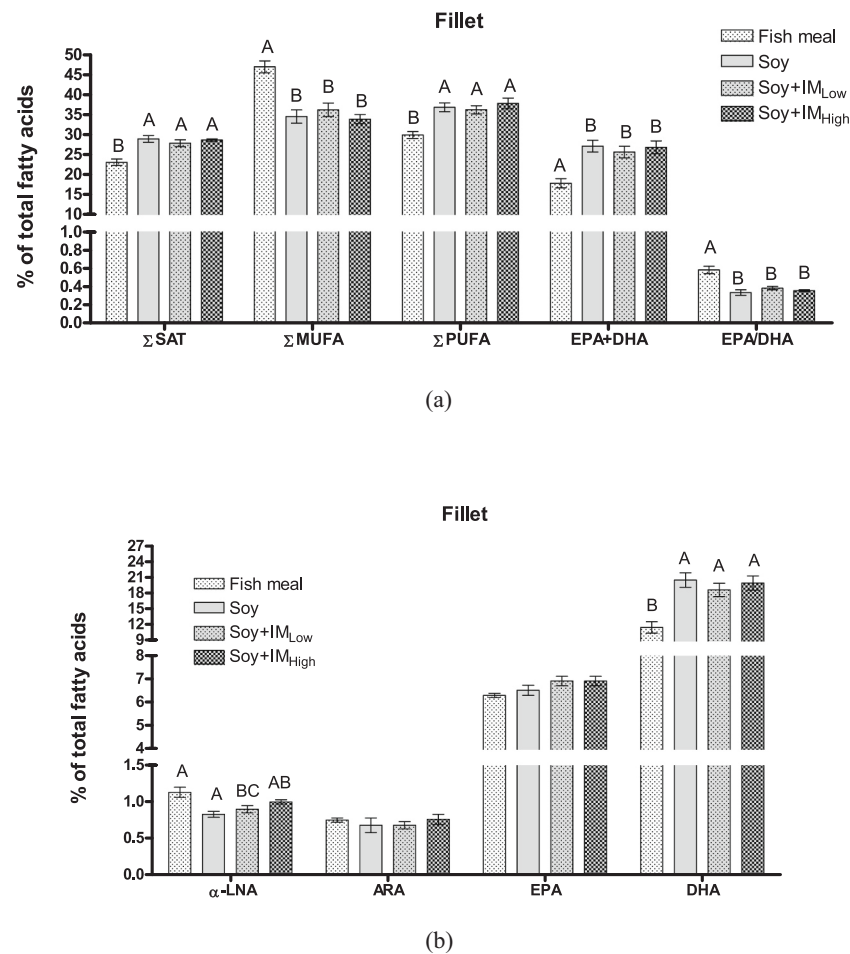


Fig. 2. a,b. Fillet fatty acids composition (% of total fatty acids) in rainbow trout fed test diets for 10 weeks. Values with different letters are significantly different ($P < 0.05$). Error bars denote standard error.

supplemented groups indicated higher conversion of liver EPA to fillet EPA which is also confirmed by higher EPA content of fish fillet fed insect meal supplemented diets. The high content of lauric acid in the insect-based diets likely led to a decreased liver lipid storage compared to when the fish fed a control diet without insects (Belghit et al., 2019). In the current study, overall liver fatty acid composition of the total lipid fraction (% of total fatty acids) showed lower values specially Σ SFA in the low insect meal included diet.

Dietary BSFL meal did not significantly influence serum glucose content of fish, but in comparison to the fishmeal based positive control diet, insect meal-included diets and the soy-based basal diets resulted in significantly lower values. Dumas et al. (2018) also reported no significant effect of BSFL meal inclusion on blood glucose content in rainbow trout. Significantly higher glucose content in the positive control diet group might be due to higher levels of wheat flour incorporated into the positive control diet compared to other dietary treatments. The total protein content, which is considered an indicator of nutritional status (McCarthy et al., 1973), was not significantly affected by insect meal inclusion in the present study. Mikołajczak et al. (2020) also reported no significant effect of hydrolyzed insect meals on serum protein content in sea trout fingerling (*Salmo trutta m. trutta*) diets. According to Panettieri et al. (2020), variations in the total protein content in the blood serum of fish can be a response to a number of physiological changes, i.e., tissue injury or destruction, differences in blood volume and plasma hydration and organism response to stress conditions. In the present study, numerically higher total serum protein in fish fed the soy-based basal

diet might be due to intestinal tissue injury or stressful physiological conditions of fish fed this particular diet. However, to confirm this hypothesis intestinal histological analysis is necessary, which also warrants further investigation.

SOD, CAT and GPx are important antioxidant enzymes which help in protecting cells against ROS-triggered damage in fish (Yu et al., 2019; Fawole et al., 2020). Superoxide dismutase generates H_2O_2 from cell generated superoxide radicals which are toxic to the body. In the present study, the activity of SOD was significantly higher in fish fed the soy-based basal diet compared to other dietary groups. The increased activity indicates some level of stress (Fawole et al., 2020) associated with the addition of the alternative soy protein. In the present study, catalase activity followed the same trend of SOD activity and non-significantly higher CAT activity observed in the soy-based basal diet group. Insect meal inclusion reduced the GPx activity, and it was significantly lower in fish fed the high insect meal supplemented diet. As GPx and CAT perform similar functions, reduced activity of these enzymes in insect meal supplemented groups indicates a lower number of precursors (H_2O_2) available for the activity of GPx and CAT in these diet groups. Simultaneously, these situations also indicate lower oxidative stress is occurring in the insect meal included diet groups.

In conclusion, dietary low inclusion of BSFL meal (8%) of a high soy-based diet significantly improved the growth performance of rainbow trout. Addition of BSFL meal significantly increased some of the whole-body amino acids (arginine, histidine, valine and threonine) and α -linolenic fatty acid. A significantly higher hepatic gene expression

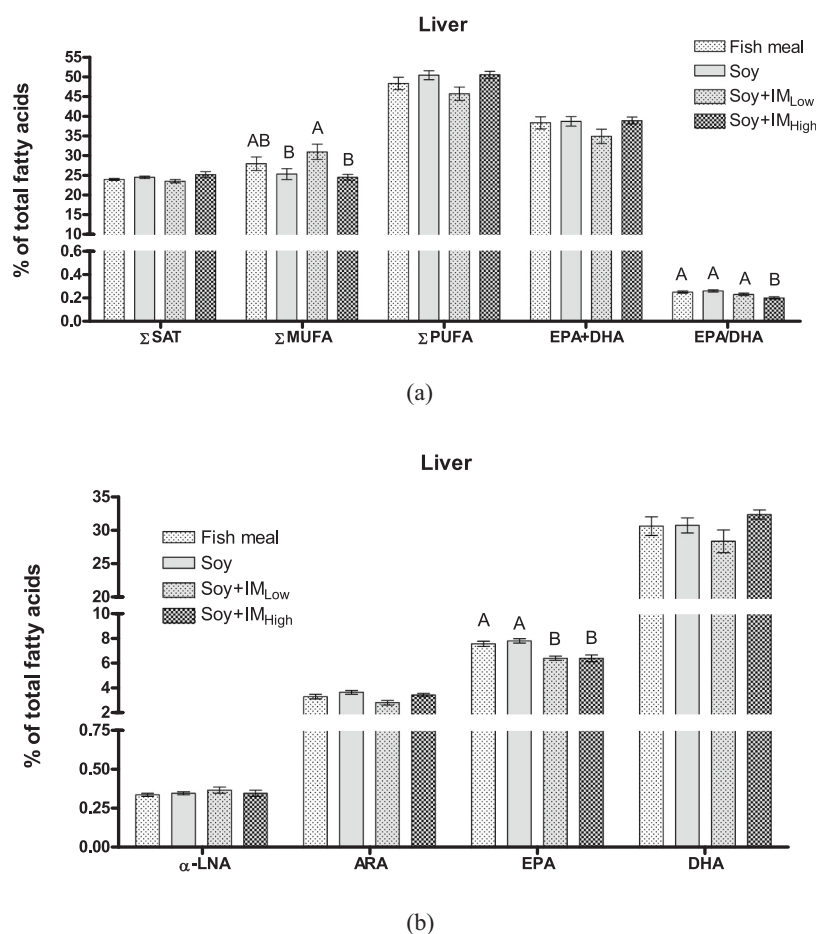


Fig. 3. a,b. Liver fatty acids composition (% of total fatty acids) in rainbow trout fed test diets for 10 weeks. Values with different letters are significantly different ($P < 0.05$). Error bars denote standard error.

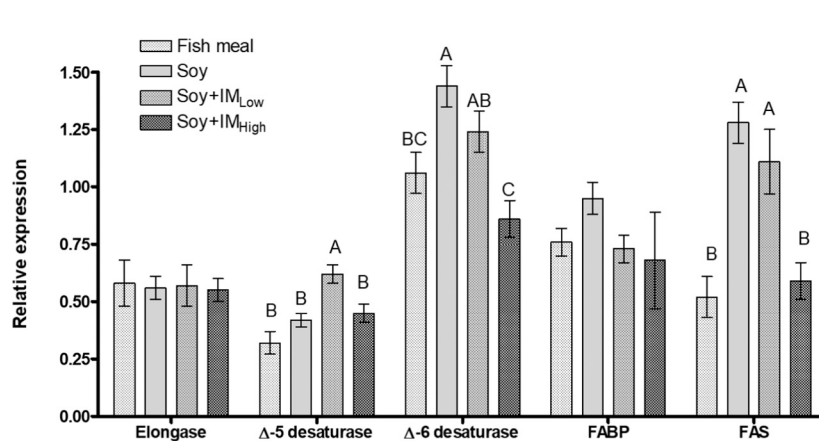


Fig. 4. Relative expression of hepatic lipid metabolism-related genes in rainbow trout fed test diets for 10 weeks. Values with different letters are significantly different ($P < 0.05$).

pattern for $\Delta 5$ desaturase was reported in the low BSFL meal incorporated group, while $\Delta 6$ desaturase and FAS were significantly higher in fish fed Soy (basal) and Soy + IM Low diet groups. Finally, we conclude that BSFL meal inclusion at low levels (8%) is beneficial for increased growth and select fatty acid restoration in soy-based diets of rainbow trout.

Credit author statement

Vikas Kumar: designed the research project, diet formulation, interpretation of results and corrected the draft manuscript. Md. S. Hossain: Original draft preparation, conducted the fish feeding trial and lab work. Femi. J. Fawole and Shyam. N. Labh: Helped for the analytical lab work and reviewed the manuscript. Brian C. Small and Ken Overturf:

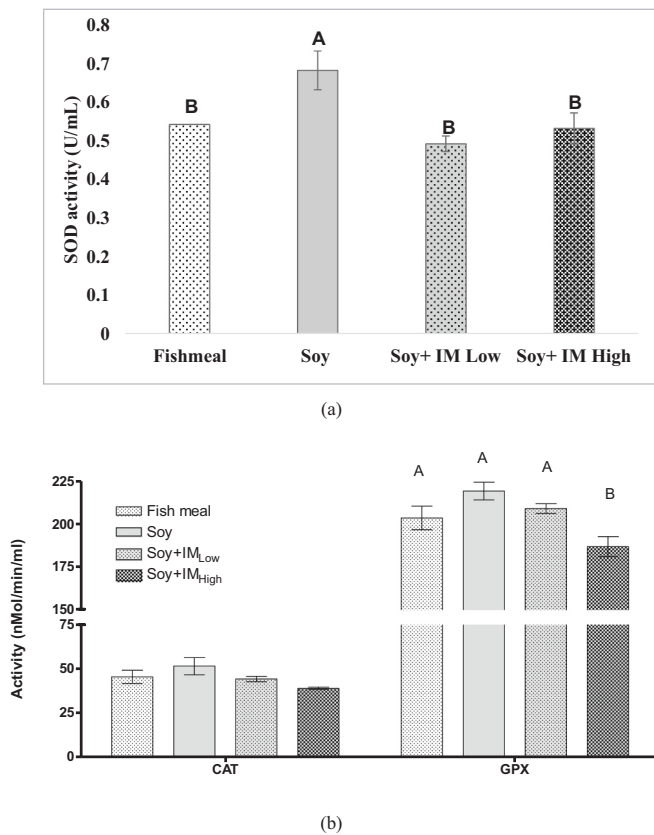


Fig. 5. a,b. Antioxidant potential (Superoxide dismutase activity, Fig. 4a; Catalase and glutathione peroxidase activity, Fig. 4e) in rainbow trout fed test diets for 10 weeks. Values with different letters are significantly different ($P < 0.05$).

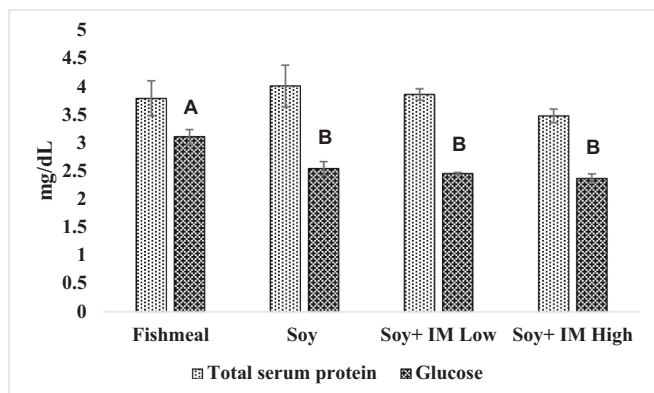


Fig. 6. Total serum protein and serum glucose content of rainbow trout fed experimental diets for 10 weeks. Values with different letters are significantly different ($P < 0.05$).

contributed to planning of the experiment and interpretation of results and reviewed the manuscript. All authors read the draft, corrected and approved the final manuscript.

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Declaration of Competing Interest

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

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References

- Agbohossou, P.S., Mandiki, S.N.M., Goughbédji, A., Megido, R.C., Hossain, M.S., Jaeger, P. D., Larondelle, Y., Francis, F., Lalèye, P.A., Kestemont, P., 2021. Total replacement of fish meal by enriched-fatty acid *Hermetia illucens* meal did not substantially affect growth parameters or innate immune status and improved whole body biochemical quality of Nile tilapia juveniles. *Aquac. Nutr.* 27 (3), 880–896. <https://doi.org/10.1111/anu.13232>.
- AOAC, 1995. (Association of Official Analytical Chemists) Official methods of analysis, 16th edn. AOAC, Washington, DC.
- Askarian, F., Zhou, Z., Olsen, R.E., Sperstad, S., Ringø, E., 2012. Culturable autochthonous gut bacteria in Atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and invitro growth inhibition of four fish pathogens. *Aquaculture* 326–329, 1–8.
- Baeverfjord, G., Kroghdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *J. Fish Dis.* 19, 375–387.
- 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, 193–201.
- Belghit, I., Liland, N.S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Kroghdahl, Å., Lock, E.-J., 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 491, 72–81.
- Belghit, I., Liland, N.S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Kroghdahl, Å., Lock, E.-J., 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture* 503, 609–619.
- Bondari, K., Sheppard, D.C., 1987. Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). *Aquac. Res.* 18 (3), 209–220.
- Burrells, C., Williams, P.D., Southgate, P.J., Crampton, V.O., 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentrations of soybean proteins. *Vet. Immunol. Immunopathol.* 72, 277–288.
- Castro, C., Corraze, G., Panserat, S., Oliva-Teles, A., 2015. Effects of fish oil replacement by a vegetable oil blend on digestibility, postprandial serum metabolite profile, lipid and glucose metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. *Aquac. Nutr.* 21, 592–603.
- Chen, W., Ai, Q.H., Mai, K.S., Xu, W., Liufu, Z., Zhang, W., Cai, Y., 2011. Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 318, 95–100.
- Cummins, V.C., Rawles, S.D., Thompson, K.R., Velasquez, A., Kobayashi, Y., Hager, J., Webster, C.D., 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture* 473, 337–344.
- Deng, J., Mai, K., Ai, Q., Zhang, W., Wang, X., Xu, W., Liufu, Z., 2006. Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* 258, 503–513.
- Devic, E., Leschen, W., Murray, F., Little, D.C., 2018. Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier Fly (*Hermetia illucens*) larval meal. *Aquac. Nutr.* 24 (1), 416–423.
- Diener, S., Zurbrugg, C., Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Manag. Res.* 27, 603–610.
- 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.

- Ekpo, K.E., Onigbinde, A.O., 2007. Characterization of lipids in winged reproductives of the termite *Macrotermis bellicosus*. Pak. J. Nutr. 6 (3), 247–251.
- Elia, A.C., Capucchio, M.T., Caldaroni, B., Magara, G., Dörr, A.J.M., Biasato, I., Biasibetti, E., Righetti, M., Pastorino, P., Prearo, M., Gai, F., Schiavone, A., Gasco, L., 2018. Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 496, 50–57.
- 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, 734849.
- Fisher, H.J., Collins, S.A., Hanson, C., Mason, B., Colombo, S.M., Anderson, D.M., 2020. Black soldier fly larvae meal as a protein source in low fish meal diets for Atlantic salmon (*Salmo salar*). Aquaculture 521, 734978.
- Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E., Mola, P., Chatzifotis, S., 2016. *Tenebrio molitor* meal in diets for European seabass (*Dicentrarchus labrax* L.) juveniles: growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34–45.
- Hardy, R.W., Barrows, F.T., 2002. Diet formulation and manufacturing. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, San Diego, CA, USA, pp. 505–600.
- Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203, 1–22.
- Hossain, M.S., Koshio, S., 2017. Dietary substitution of fishmeal by alternative protein with guanosine monophosphate supplementation influences growth, digestibility, blood chemistry profile, immunity and stress resistance of red sea bream, *Pagrus major*. Fish Physiol. Biochem. 43, 1629–1644.
- Hossain, M.S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N.M., Islam, M.J., Maekawa, M., Fujieda, T., 2018. Substitution of dietary fishmeal by soybean meal with inosine administration influences growth, digestibility, immunity, stress resistance and gut morphology of juvenile amberjack *Seriola dumerili*. Aquaculture 488, 174–188.
- Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 54 (4), 287–293.
- Jordal, A.-E.O., Torstensen, B.E., Tsoi, S., Tocher, D.R., Lall, S.P., Douglas, S.E., 2005. Dietary rapeseed oil affects the expression of genes involved in hepatic lipid metabolism in Atlantic salmon (*Salmo salar* L.). J. Nutr. 135, 2355–2361.
- Karlsen, Ø., Amlund, H., Berg, A., Olsen, R.E., 2017. The effect of dietary chitin on growth and nutrient digestibility in farmed Atlantic cod, Atlantic salmon and Atlantic halibut. Aquac. Res. 48, 123–133.
- Katayama, N., Ishikawa, Y., Takaoki, M., Yamashita, M., Nakayama, S., Kiguchi, K., Force, S.A.T., 2008. Entomophagy: a key to space agriculture. Adv. Space Res. 41 (5), 701–705.
- Kroeckel, S., Harjes, A.G., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C., 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute-Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364, 345–352.
- Krogdahl, A., Bakke-Mckellep, A., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 9, 361–371.
- Li, Y., Ai, Q., Mai, K., Xu, W., Cheng, Z., 2012. Effects of the partial substitution of dietary fish meal by two types of soybean meals on the growth performance of juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier 1828). Aquac. Res. 43, 458–466.
- 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., Ji, H., Zhang, B., Zhou, J., Yu, H., 2017. Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological. Aquaculture 477, 62–70.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ΔΔCT method. Methods 25, 402–408.
- Lock, E.-J., Arsiwalla, T., Waagbø, R., 2016. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) post-smolt. Aquac. Nutr. 22, 1202–1213.
- Madibana, M.J., Mwanza, M., Lewis, B.R., Fouché, C.H., Toefy, R., Mlambo, V., 2020. Black soldier fly larvae meal as a fishmeal substitute in juvenile dusky kob diets: effect on feed utilization, growth performance, and blood parameters. Sustainability 2020 (12), 9460. <https://doi.org/10.3390/su12229460>.
- Magalhães, R., Sánchez-López, A., Leal, R.S., Martínez-Llorens, S., Oliva-Teles, A., Peres, H., 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476, 79–85.
- Makkar, H.P., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33.
- McCarthy, D.H., Stevenson, J.P., Roberts, M.S., 1973. Some blood parameters of the rainbow trout (*Salmo gairdneri* Richardson): I. The Kamloops variety. J. Fish Biol. 5, 1–8.
- Mikolajczak, Z., Rawski, M., Mazurkiewicz, J., Kieron'czyk, B., Józefiak, D., 2020. The effect of hydrolyzed insect meals in sea trout fingerling (*Salmo trutta m. trutta*) diets on growth performance, microbiota and biochemical blood parameters. Animals 10, 1031. <https://doi.org/10.3390/ani10061031>.
- National Research Council, 2011. Nutrient requirements of fish and shrimp. National academies press, Washington, D.C, p. 70, 327–347.
- Ng, W.K., Liew, F.L., Ang, L.P., Wong, K.W., 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. Aquac. Res. 32 (Suppl. 1), 273–280.
- Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., Józefiak, A., 2019. Insect meals in fish nutrition. Rev. Aquac. 11 (4), 1080–1103.
- Oliva-Teles, A., 2012. Nutrition and health of aquaculture fish. J. Fish Dis. 35 (2), 83–108.
- Overturf, K., Welker, T., Barrows, F., Towner, R., Schneider, R., LaPatra, S., 2013. Variation in rainbow trout, *Oncorhynchus mykiss*, to biosynthesize eicosapentaenoic acid and docosahexaenoic acid when reared on plant oil replacement feeds. J. World Aquacult. Soc. 44, 326–337.
- Panettieri, V., Chatzifotis, S., Messina, C.M., Olivetto, I., Manuguerra, S., Randazzo, B., Ariano, A., Bovera, F., Santulli, A., Severino, L., Piccolo, G., 2020. Honeybee pollen in meagre (*Argyrosomus regius*) juvenile diets: effects on growth, diet digestibility, intestinal traits, and biochemical markers related to health and stress. Animals 10 (2), 231.
- Peng, M., Xu, W., Tan, P., Du, J.L., Mai, K.S., Zhou, H.H., Zhang, Y.J., Rui, N., Benoit, M., Aiet, Q.H., 2017. Effect of dietary fatty acid composition on growth, fatty acids composition and hepatic lipid metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed diets with required n3 LC-PUFAs. Aquaculture 479, 591–600.
- Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., DeMarco, M., Brugiapaglia Zoccarato, A., Gasco, L., 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. J. Anim. Sci. Biotechnol. 8, 57.
- Sanchez-Muros, M.-J., Barroso, F.G., Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. J. Clean. Prod. 65, 16–27.
- Sánchez-Muros, M., De Haro, C., Sanz, A., Trenzado, C.E., Villareces, S., Barroso, F.G., 2016. Nutritional evaluation of *Tenebrio molitor* meal as fishmeal substitute for tilapia (*Oreochromis niloticus*) diet. Aquac. Nutr. 22 (5), 943–955.
- Sheppard, D.C., Newton, G.L., Thompson, S.A., Savage, S., 1994. A value-added manure management system using the black soldier fly. Bioresour. Technol. 50, 275–279.
- St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W., 2007. Fly Prepupae as a feed stuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquacult. Soc. 38 (1), 59–67.
- Teoh, C.-Y., Ng, W.-K., 2016. The implications of substituting dietary fish oil with vegetable oils on the growth performance, fillet fatty acid profile and modulation of the fatty acid elongase, desaturase and oxidation activities of red hybrid tilapia, *Oreochromis* sp. Aquaculture 465, 311–322.
- Tocher, D.R., Bell, J.G., Dick, J.R., Henderson, R.J., McGhee, F., Michell, D., Morris, P.C., 2000. Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. 23, 59–73.
- Torstensen, B., Nanton, D., Olsvik, P., Sundvold, H., Stubhaug, I., 2009. Gene expression of fatty acid-binding proteins, fatty acid transport proteins (cd36 and FATP) and β -oxidation-related genes in Atlantic salmon (*Salmo salar* L.) fed fish oil or vegetable oil. Aquac. Nutr. 15, 440–451.
- Tran, G., Heuzé, V., Makkar, H.P.S., 2015. Insects in fish diets. Anim. Front. 5 (2), 37–44.
- Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1, 10–57.
- Wang, Y.S., Shelomi, M., 2017. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 6, 1–23. <https://doi.org/10.3390/foods6100091>.
- Yildirim-Aksoy, M., Eljack, R., Beck, B.H., 2020. Nutritional value of frass from black soldier fly larvae, *Hermetia illucens*, in a channel catfish, *Ictalurus punctatus*, diet. Aquac. Nutr. 26, 812–819. <https://doi.org/10.1111/anu.13040>.
- Yu, J., Li, S., Niu, H., Chang, J., Hu, Z., Han, Y., 2019. Influence of dietary linseed oil as substitution of fish oil on whole fish fatty acid composition, lipid metabolism and oxidative status of juvenile Manchurian trout, *Brachymystax lenok*. Sci. Rep. 9, 1–10.