Original Research Article

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)

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A B S T R A C T  
Alternative sources of fish oil (FO) are one of the major problems in aquaculture; therefore, the goal of the present study was to examine insect (black soldier fly larvae) oil (BSLO) as a potential replacer of fish/soy oil in juvenile rainbow trout (initial average weight of 32 ± 0.15 g) feed. Four diets were formulated wherein FO (control diet) was completely replaced with either soybean oil (SO) or BSLO, and an additional BSLO-based diet supplemented with 1.5% bile acid (BSLO + BA) were fed to the fish for 10 weeks. Growth performance of the BSLO fed group was similar (P > 0.05) to that of the FO and SO fed groups, however, the fish fed BSLO + BA diet registered the lowest growth (P < 0.05). Oil sources did not (P > 0.05) affect the major nutrient content of whole-body, however, the fatty acid composition of the muscle and liver was influenced (P < 0.05), with the highest 14:0, 16:0, and total saturated fatty acid detected in BSLO or BSLO + BA fed trout compared to the others (P < 0.001). No significant differences were observed in eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) or total n-3 polyunsaturated fatty acid (PUFA) content in muscle among the groups, whereas, the highest EPA:DHA and n-3:n-6 ratios were detected in the FO group. Gene expression for fatty acid binding protein (fabp), fatty acid synthase (fats), and Δ5 desaturase in the liver was lower in FO (P < 0.05), while BSLO + BA registered the highest Δ6 expression (P = 0.006). Supplementation of BA in the BSLO diet increased superoxide dismutase (SOD) and catalase (CAT) activities compared to the other groups (P < 0.05). In conclusion, BSLO could serve as a substitute for FO and SO in rainbow trout diet without negatively impacting growth performance, whole-body composition and nutrient retention, and modulate the expression of fatty acid metabolism-related genes in rainbow trout.

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1. Introduction

The declining trend in capture fisheries has spurred a steady increase in aquaculture production to meet global demands for seafood. However, the sustainability of aquaculture production is hinged on the development of alternative feed ingredients, as the demand for commercial feed increases, while marine resources used in aquafeed manufacturing are becoming increasingly limited. Marine resources, especially fish meal (FM) and fish oil (FO), are the most commonly used protein and lipid source ingredients in fish feed production, especially for carnivorous species (Turchini et al., 2020).
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fish growth, health, and the high contents of long-chain omega-3 polysaturated fatty acids (n-3 LC-PUFA). The increasing demand for aquafeeds and the need to replace FO has resulted in the use of alternative terrestrial oils as lipid sources in commercial fish feed production, however, most of these oils are not sustainable or are deficient in n-3 highly unsaturated fatty acid (Tocher, 2015; Yang et al., 2020). With increasing global limitation of FO availability for use in aquafeed and demand for human consumption of more oil sources in the near future, it is of great importance to find suitable alternative sources of oils.

Insect meals, especially black soldier fly larvae (BSL) are high in protein (40% to 45%) and lipid (26% to 35%) and have been recognized as a promising feed ingredient (Schiavone et al., 2018; Sheppard et al., 1994; Tran et al., 2015). Several studies have investigated the substitution of FM by BSL meal in different fish species including rainbow trout and turbot (Cardinaletti et al., 2019; Elia et al., 2018; Kroeckel et al., 2012; Renna et al., 2017; Stamer et al., 2014), but little attention has been given to BSL oil for possible use in fish feed (Li et al., 2016; Xu et al., 2021). BSL oil is a byproduct of meal production and is known to contain high levels of medium-chain fatty acid, especially lauric acid (21% to 45%) (Li et al., 2016), which is beneficial in the reduction of abdominal fat deposition due to their preferential use as an energy substrate (Wang et al., 2015; Li et al., 2016). In addition, the concentration of unsaturated fatty acid (linoleic, 18:2n-6, and linolenic, 18:3n-3) in BSL oil is closely related to that of soybean oil (SO), and this could be effectively utilized by freshwater fish to biosynthesize n-3 and n-6 LC-PUFA due to their endogenous capability (Tocher, 2003). The nutritional modulation and accumulation of LC-PUFA in fish is related to the biosynthesis and decomposition of fatty acids by enzymes associated with fatty acid metabolism and pertinent transcription factors (Yu et al., 2019). Fatty acid synthase (FAS), one of the main lipogenic enzymes, catalyzes the synthesis of long-chain saturated fatty acids (LC-SFA) from acetyl CoA and malonyl-CoA in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) (Dong et al., 2014), while fatty acid-binding proteins (FABP) aid in the transport of fatty acids into cells (Torstensen et al., 2009). The expression of fatty acid elongase and desaturase genes are known to be influenced by the dietary fatty acid composition (Jordal et al., 2005; Zheng et al., 2004) and the fish species. Although studies on the effect of dietary BSL oil (partial replacement) on growth and nutrient deposition in rainbow trout (Dumas et al., 2018), Jian carp (Li et al., 2016) and mirror carp (Xu et al., 2021) have been reported, empirical data on the dietary effect of dietary BSL oil (with or without BA) as a substitute for FO and SO replacement) on growth and nutrient deposition in rainbow trout. Thus, the objective of this study was to investigate the effect of dietary BSL oil on growth, fatty acid deposition, antioxidant capacity, and expression of fatty acid metabolism-related genes in rainbow trout. This study will help to determine the effectiveness with which rainbow trout utilize BSL oil, as a replacement for FO and SO, to synthesize n-3 HUFA from PUFA and their deposition in muscle.

2. Materials and methods

2.1. Ethics statement

The experimental protocols together with fish handling and sampling were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Idaho (IACUC-2019-76).

2.2. Diets, feeding trial and sample collection

Four isonitrogenous (44% crude protein) and isolipidic (21% crude lipid) experimental diets were formulated, and differed only in their lipid sources (Table 1). The dietary oils were FO, SO, and black soldier fly larvae oil (BSL) with an additional BSLO-supplemented diet with BA (1.5%; Yamamoto et al., 2007a) (BSL + BA). All feed ingredients were mixed thoroughly in a Hobart mixer, water was added, and then cold pelleted using a

| Table 1 | Ingredients and proximate composition of the trial diets (as-is basis) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item            | FO             | SO             | BSLO            | BSLO + BA       |
| Ingredients, g/kg |          |          |          |          |
| Fish meal, sardine | 240      | 240      | 240     | 240      |
| Poultry meal    | 102        | 102      | 102     | 102      |
| Soy protein concentrate | 60      | 60       | 60      | 60       |
| Wheat gluten meal | 50       | 50       | 50      | 50       |
| Corn protein concentrate | 60     | 60       | 60      | 60       |
| Blood meal      | 50          | 50       | 50      | 50       |
| Wheat flour     | 249         | 249      | 249     | 249      |
| FO             | 196         | 0        | 0       | 0        |
| SO             | 0           | 160      | 0       | 0        |
| BSLO           | 0           | 0        | 160     | 160      |
| Bile salt       | 0           | 0        | 0       | 15       |
| Dicalcium phosphate | 12    | 12       | 12      | 12       |
| Choline chloride (60%) | 6     | 6        | 6       | 6        |
| Vitamin premix  | 8           | 8        | 8       | 8        |
| Trace mineral mixture | 1    | 1        | 1       | 1        |
| Vitamin C (Stay C-35%) | 2    | 2        | 2       | 2        |
| Total           | 1,000       | 1,000    | 1,000   | 1,000    |

Proximate composition, %

| Moisture          | 6.0     | 6.0     | 6.2     | 7.4     |
| Crude protein     | 44.8    | 44.5    | 44.1    | 44.4    |
| Crude lipid       | 21.0    | 21.7    | 21.4    | 20.9    |
| Ash               | 7.6     | 8.4     | 8.6     | 7.8     |

FO = fish oil; SO = soybean oil; BSLO = black soldier fly larvae oil; BA = bile acid.

References

1. Rangen Inc., Buhl, ID, USA.
2. Proline VF, The Solae Company, St. Louis, MO, USA.
3. Empyreal 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
4. EnviroFlight, USA.
5. Oxbile extract powder (cholic acid: 51.2%), Lot No. 786269, Creative enzymes, USA.
6. Vitamin premix supplied the following per kg diet: vitamin A, 2.4 mg; vitamin D, 0.15 mg; vitamin E, 267 mg; vitamin K as menadione sodium bisulfite, 20 µg; thiamin as thiamin mononitrate, 32 mg; riboflavin, 64 mg; pyridoxine as pyridoxine-HCl, 64 mg; pantothenic acid as Ca-d-pantothenate, 192 mg; niacin as niacinamide, 240 mg; biotin, 0.56 mg; folic acid, 12 mg; vitamin B12, 50 µg; and inositol as meco-inositol, 400 mg.
7. US Fish and Wildlife Service Trace Mineral Premix #3. It supplied the following (mg/kg diet): Zn (as ZnSO4 · 7H2O), 75; Mn (as MnSO4), 20; Cu (as CuSO4 · 5H2O), 1.54; I (as KI10), 10.
8. Skretting USA, Tooele, UT, USA.
laboratory pellet mill (California Pellet Mill Company, San Francisco, CA, USA) fitted with a 2.4-mm die at the Hagerman Fish Culture Experiment Station (HFCES), University of Idaho, USA. The feeds were dried in a forced-air dryer set at 40 °C until moisture content was reduced to less than 10%, and then stored at ambient temperature until use. The proximate composition of the experimental diets is provided in Table 1. Fatty acids profile of oil (BSL0, FO and SO) is provided in Table 2. The BSFL0 used in this study was provided by EnviroFlight, USA.

Twenty-five juvenile rainbow trout with an initial average weight of 32 ± 0.0 g were randomly stocked into each of the twelve 145-L experimental tanks and supplied with 8 L/min of gravity-fed spring water at constant temperature (14 °C). After a 2-week acclimation period on the control diet, each experimental diet was randomly allocated to 3 replicate tanks of rainbow trout following a completely randomized design, and fish were hand-fed to apparent satiation twice daily (09:00 and 17:00), 6 d per week for 10 weeks. The photoperiod was maintained at 14 h of light and 10 h of dark per day using electric timer-controlled fluorescent lights. Fish were weighed (Mettler Toledo: XS32001L, Switzerland) every 30 d to appraise growth rate and feed efficiency. Trout were fasted for 24 h before each measurement to avoid inclusion of ingested feed in the weight measurement. Before the start of the feeding trial, 12 fish were randomly selected for initial whole-body composition analysis.

At the conclusion of the feeding trial, fish from each tank were fasted for 24 h, counted, and batched weighed to determine the final growth response and nutrient utilization indices as specified below. Three fish were randomly selected per tank (n = 9 fish per diet) and anaesthetized with tricaine methansulphonate (MS-222, 80 mg/L, buffered to pH 7.0) for blood collection using 1 mL hypodermic syringe. The blood was collected via the caudal vein, kept in ice to clot and serum was recovered by centrifugation at 2,000 g for 10 min at 4 °C. The serum samples were stored at −80 °C and later analysed for glucose and antioxidant enzyme activity assays. Then, the sacrificed fish were dissected to remove various tissues for further analysis. Liver and visceral organs were removed for the determination of hepatosomatic index (HSI) and viscerosomatic index (VSI), respectively. A small section of liver and muscle samples were collected, flash-frozen in liquid nitrogen (N2) and stored at −80 °C until use for fatty acid profile analysis. The liver was further sub-sampled into 1.5-mL tubes (RNAase free, AXYGEN) for gene expression analysis, frozen in liquid N2 and stored as described above. Another 3 fish per tank were collected, euthanized with a lethal dose of MS-222 (300 mg/L), and kept at −20 °C for the determination of whole-body proximate composition.

2.3. Biological response indicators

Using the fish weight and feed consumption data, trout growth performance parameters were calculated from the following formulas to determine the effect of experimental diets:

- Weight gain (g) = Final body weight (g) − Initial body weight (g);
- Weight gain (%) = [Weight gain (g)/Initial body weight (g)] × 100;
- Specific growth rate (SGR, %/d) = 100 × [ln Final body weight (g) − ln Initial body weight (g)] / Duration of feeding;
- Feed intake (g/ fish) = Total dry feed given (g)/Number of fish;
- Feed conversion ratio = Feed consumption (g)/[Final biomass (g) − Initial biomass (g) + Dead fish weight (g)];
- Protein efficiency ratio (PER) = Net weight gain (g)/Protein fed (g);
- Nutrient retention (%) = 100 × Nutrient gain (g)/Nutrient consumed (g);
- Condition factor (CF, %) = 100 × Weight of fish / (Length of fish)³;
- HSI (%) = 100 × Wet weight of liver (g)/Whole body weight of fish (g);
- VSI (%) = 100 × Wet weight of visceral (g)/Whole body weight of fish (g);
- Survival (%) = 100 × (Total number of fish harvested/Total number of fish stocked).

2.4. Analysis

2.4.1. Proximate and fatty acid composition

Proximate composition of trout whole-body and experimental diets were conducted per standard AOAC (2000) methods as described previously (Kumar et al., 2020). Briefly, dry matter was determined by drying samples overnight (12 h) in an oven (105 °C) to a constant weight. Crude protein content was determined (total

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Table 2

| Item | Name | FO | SO | BSLO |
|------|------|----|----|------|
| C8:0 | Caprylic acid | – | – | 0.02 |
| C10:0 | Capric acid | – | – | 0.92 |
| C12:0 | Lauric acid | – | – | 40.1 |
| C13:0 | Tridecyl acid | – | 0.01 | 9.88 |
| C14:0 | Myristic acid | 4.51 | – | 0.01 |
| C14:1 | Myristoleic acid | – | – | 0.13 |
| C15:0 | Pentadecylic acid | 0.61 | – | 0.08 |
| C16:0 | Palmitic acid | 15.71 | 11.01 | 13.1 |
| C16:1 | Palmitoleic acid | 5.76 | – | 1.54 |
| C16:2 | Palmitelaidic acid | – | – | 0.36 |
| C17:0 | Heptadecanoic acid | – | – | 0.12 |
| C18:0 | Stearic acid | 3.72 | 3.25 | 2.12 |
| C18:1 | Elaidic acid | 4.34 | – | 0.02 |
| C18:1n-9 | Oleic acid | 19.31 | 25.05 | 12.0 |
| C18:2n-6 | Linoleic acid | 5.71 | 54.55 | 0.01 |
| C18:3n-3 | α-Linolenic acid | 2.17 | 6.21 | – |
| C20:0 | Arachidic acid | 0.24 | – | 0.07 |
| C20:1n-9 | 11-Eicosenoic acid or Conidoic acid | 4.87 | – | 0.07 |
| C20:2n-6 | Eicosadienoic acid | 2.11 | – | 0.32 |
| C20:3n-6 | Dihomo-γ-linolenic acid | 0.17 | – | – |
| C20:4n-6 | Arachidonic acid | 0.95 | – | 0.07 |
| C20:5n-3 | Eicosapentaenoic acid | 1.19 | – | – |
| C20:5n-3 | Eicosapentaenoic acid | 7.91 | – | – |
| C22:0 | Behenic acid | 0.15 | – | 0.03 |
| C22:1n-9 | Erucic acid | 5.06 | – | 0.05 |
| C22:5n-3 | Docosapentaenoic acid | 2.26 | – | – |
| C22:6n-3 | Docosahexaenoic acid | 10.45 | – | – |
| C24:0 | Lignoceric acid | 0.08 | – | – |

FO – fish oil; SO – soybean oil; BSLO – black soldier fly oil; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; LC-PUFA – long chain polyunsaturated fatty acid.

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nitrogen × 6.25) by combustion method with a nitrogen determinator (Elementar- rapid N, Exceed, Germany). Crude fat content was determined using an ANKOM XT 15 extractor (ANKOM Technology, Macedon, NY, USA) with petroleum ether as the extracting solvent. Ash content was determined by incineration at 600 °C in a muffle furnace for 4 h. The fatty acid composition of the oil (BSLO, SO and FO), liver and muscle samples were determined using a modified A0AC method 991.39 (AOAC, 1995). Briefly, samples were dried for 5 to 6 h under an N2 stream at 50 °C (OA-SYS heating system, Organization Associates, Inc., Berlin, MA, USA). Thereafter, 2 mL of 0.5 mol/L NaOH was added for sample saponification at 70 °C for 60 min. Following sample cooling, free fatty acids were methylated by the addition of 2 mL 14% BF3 (Boron trifluoride, Sigma–Aldrich) in methanol and incubated at 70 °C for 60 min. After the samples were cooled, 2 mL of hexane were added, inverted repeatedly for 60 s, and 1 mL of saturated NaCl was added. Samples were again inverted repeatedly for 60 s and then centrifuged at 2,000 × g for 5 min. An aliquot (100 μL) of the clarified hexane extract was diluted in hexane (1:10) and placed into autosampler vials for gas chromatography/mass spectrometry (GC/MS) analysis. The injection mode, helium flow rate, and the column temperature were according to Overtuft et al. (2013).

2.4.2. Hepatic gene expression

Total RNA was isolated from collected liver samples (n = 9 fish per diet) using TRIzol reagent (Invitrogen, USA) extraction method and performed following the manufacturer’s protocol. The RNA purity and quantity of samples were determined by measuring absorbance at 260 and 280 nm (NanoDrop, 2000 spectrophotometer, Thermo Scientific, USA). To ascertain the level of lipid metabolism-related gene expression, a quantitative real-time (RT)-PCR was performed with an ABI QuantStudio Real-Time PCR system using the TaqMan One-Step RT-PCR Master Mix Reagents kit with the procedure provided by ABI (Foster City, CA, USA). The concentration of each reaction was: Master Mix, 1 × (contains AmpliTag Gold enzyme, dNTP including dUTP, a passive reference, and buffer components); MultiScribe reverse transcriptase, 0.25 U/μL; RNase inhibitor mix, 0.4 U/μL; forward primer 600 nmol/L; reverse primer 600 nmol/L; probe, 250 nmol/L; total RNA, 75 ng. The primer sequences of each gene, accession numbers, and probes are given in Table 3. The β-actin was used as the reference gene. The thermocycler 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 (fas, fabp, elongase, Δ5-and Δ6-desaturases), and the values were normalized by 2^−ΔΔCT method (Livak and Schmittgen, 2001) with β-actin as the reference gene.

2.4.3. Antioxidant enzyme and glucose assay

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

2.5. Statistical analysis

All data were tested for normality and homogeneity of variances before one-way ANOVA analysis to test for the significant difference in the means using IBM SPSS version 22 package. When significant difference was observed among the means (F-test), data were subjected to Duncan’s multiple range test to determine specific mean differences at a confidence level of P ≤ 0.05. Principal component analysis (PCA) was also used to relate dietary oil sources with tissue fatty acid composition in order to emphasize differences among the groups. The correlation matrix was used, and the first two principal components were plotted.

3. Results

3.1. Growth performance, feed efficiency, and somatic indices

The BA supplemented group (BSLO + BA) recorded the lowest growth performance (weight gain [g and %] and SGR) compared with other groups (P = 0.009; P = 0.007, respectively), whereas other groups exhibited similar growth performance. Fish grew nearly 6-fold of initial body weight when fed the FO, SO, or BSLO diet (Table 4) for 10 weeks. There was no significant difference noticed in feed intake, feed conversion ratio, or PER values among the tested dietary groups (P > 0.05). Highest VSI was observed in BSLO and BSLO + BA fed fish compared to the lowest values recorded in the FO and SO groups (P = 0.004). No significant differences were observed in HSI, CF, or survival of fish fed different dietary oil sources (P > 0.05).

3.2. Whole-body composition and nutrient retention

The whole-body composition and nutrient retention of rainbow trout fed different oil sources are shown in Table 5. Crude protein, lipid, and ash contents were not significantly different (P > 0.05); however, fish fed the FO-based diet had the lowest moisture content compared to the SO, BSLO, and BSLO + BA groups (P = 0.018). The efficiency of protein and lipid retentions among the dietary groups was unaffected by the dietary oils (P = 0.091 and P = 0.189, respectively).

3.3. Fatty acid composition of muscle and hepatic tissue

The fatty composition of the muscle and liver were influenced by the oil sources of the diet (Tables 6 and 7, respectively). The muscle and liver 14:0, 16:0, and total SFA proportion of rainbow trout fed BSLO or BSLO + BA diets were significantly higher than those fed FO and SO (P < 0.001). The highest total MUFA and PUFA proportion in the muscle, which was similar to liver deposition, was

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Table 3

| Genes                     | Gene symbols | Forward primers (5′-3′) | Reverse primers (5′-3′) | Probes                        | Accession number |
|---------------------------|--------------|-------------------------|-------------------------|-------------------------------|------------------|
| Fatty acid synthase       | Fas          | GCCAGGCTGCGATCTCC       | GGGGCCGTGAACTCAAAAGA    | CCAACGACCCTCC                 | XM-02157622     |
| Fatty acid binding protein | Fadh         | CCAAGAGTCTCCCAATCACAGA  | CTGTTATCCTGGTAGGGGTT    | TCACTCGGCCCTCAAC             | XM-020900137.2  |
| Fatty acid elongase       | Fac          | CTGTTATCCTGGGATCTCTCC   | AACTGATACGCTGCTTGTTGAGTGA | CCAAGGCCAGGGCTCTAG          | CTCCGGCGCTTCACT  |
| Delta-5- desaturase       | D5s          | GTGCCCACACATCC          | CCAAGGACGAGGCTCTAG      | CACGAGAAGCCAGAAGTGAAAGCTCA  | XM-01470388     |
| Delta-6- desaturase       | D6s          | CAGATTTCCCAACACCCGATCT | AGGTTAGGTTAGGCTTGAGATA  | AGGTTAGGTTAGGCTTGGGATT      | XM-01470388     |
| β-actin                   | bact         | CCTCTTCCAGCTCCCTCTCT   | CCAAGGCCAGGGCTCTAG      | CACGAGAAGCCAGAAGTGAAAGCTCA  | \[44] \[44] \[44] \[44] |

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observed in fish fed FO and SO diets, respectively (P < 0.001). Dietary SO resulted in higher 18:2n-6 and 18:3n-3 deposition in the liver and muscle compared to other groups, however, the BSLO-based groups had a higher 18:2n-6 deposition than FO-fed fish. The muscle 20:4n-6 deposition in BSLO fish was higher than in FO and SO fed fish while BSLO and BSLO + BA were found to be similar (P < 0.001; Table 6). However, no difference was found in liver 20:4n-6 content of trout fed SO, BSLO, or BSLO + BA, but the FO group recorded significantly lower deposition (P < 0.001; Table 7). Fish fed the FO diet had a significantly higher eicosapentaenoic acid (EPA; 20:5n-3) content in both the muscle and liver than those fed SO and BSLO-based diets (P < 0.001), while EPA in the liver of the BSLO groups was found to be lower than that in SO fed fish. The highest fillet docosahexaenoic acid (DHA; 22:6n-3) deposition and DHA:EPA ratio occurred in the BSLO and BSLO + BA groups followed by SO and FO (Table 6), whereas in the liver, trout fed FO had the highest DHA and the lowest DHA:EPA ratio. The highest ratio of EPA to DHA or n-3:n-6 ratio in both the muscle and liver was detected in FO fed fish (P < 0.001), but no significant difference was recorded among the other dietary groups. In the liver, fish fed FO diet had the highest total n-3 PUFA compared to the other dietary groups (P < 0.001), and no significant difference was found between the SO and BSLO + BA groups. Muscle EPA + DHA showed no differences among the dietary groups (P = 0.233), whereas significant variation was seen in the liver with the highest value observed in FO fish (P < 0.001). Higher total n-6 PUFA content was observed in the SO group followed by BSLO, BSLO + BA, and FO in both the muscle and liver (P < 0.001).

### 3.4. Principal component analysis of the liver and muscle fatty acid profile

The PCA analysis showed that the two components (PC1 and PC2) represented 77% of the total variance in rainbow trout liver and muscle fatty acid composition (Fig. 1). Several fatty acids and LC-PUFA in the liver and muscle of fish fed either SO or BSLO-based diets were found to be grouped together and are in close proximity to each other, which accounted for 47% of the total variance (PC1). However, FO was found to be separated from other oil sources and accounted for 30% (PC2) of the total variance.

### 3.5. Expression of hepatic lipid metabolism-related genes

The relative expression of hepatic fabp and fas in rainbow trout fed the different dietary oils is shown in Fig. 2. Fish fed the FO diet had significantly lower fabp expression compared to those given the SO and BSLO diets (P = 0.02) but was similar to the BSLO + BA group (Fig. 2). Hepatic fas expression in trout fed SO was higher.
than that fed the FO diet; however, no statistically significant difference was observed when compared to fish fed BSLO and BSLO + BA based diets. There were no significant differences (P = 0.37) in the mRNA expression level of fatty acid elongase among the dietary oil groups (Fig. 2). The relative mRNA expression level of hepatic \( \Delta 5 \)-desaturase in trout fed the FO diet was not significantly different from that fed the FO diet at P < 0.05. Initial average weight of fish is 32 ± 0.0 g.
significantly lower than those fed the other dietary oils \((P = 0.005)\); whereas, no difference was observed among fish fed the SO, BSLO, or BSLO + BA diets. Expression of the \(\Delta 6\)-desaturase was significantly higher in the BSLO + BA-fed fish, which was similar to the BSLO group but differed significantly from those fed the FO or SO diet \((P = 0.006)\).

### 3.6. Antioxidant enzyme and glucose assay

SOD enzyme activity in serum was found to be higher in fish fed the BSLO + BA diet with the lowest activity seen in the FO and SO groups \((P < 0.001)\) (Fig. 3). Serum CAT activity of trout fed the FO, SO and BSLO diets were significantly lower than those fed the BSLO + BA diet \((P = 0.03)\); whereas, no significant difference was recorded in the GPx activity among the dietary groups \((P = 0.001;\) Fig. 5).

### 4. Discussion

The complete replacement of FO and SO with BSLO in the diet of rainbow trout is possible without any significant alteration in the growth performance and nutrient retention efficiency. Our results are consistent with those of Li et al. (2016) in Jian carp in which black soldier fly oil was found to have no negative effects on growth response indices when completely replacing SO. Similarly, Dumas et al. (2018) reported that rainbow trout could utilize 10% BSLO without adversely impacting growth or nutrient utilization and deposition. The authors, however, hypothesized that higher dietary inclusion levels could be possible, and this study has revealed that rainbow trout can utilize up to 160 g/kg BSLO. Numerous studies have reported that replacement of FO with an alternative oil in which the essential fatty acid requirements are met does not impair the growth performance of fish (Hixson et al., 2017; Luo et al., 2014; Peng et al., 2017; Turchini et al., 2010). Thus, it can be inferred that the level of BSLO added in this study was not beyond the tolerance level of rainbow trout and the fatty acids requirements were not compromised. Furthermore, the voluntary feed intake among the treatment groups was similar indicating that diet palatability/acceptability was unaffected by oil source, and this further supports the suitability of BSLO as full replacement of FO or SO in rainbow trout diets.

BA or its salt has been reported to enhance growth performance in rainbow trout (Iwashita et al., 2008; Yamamoto et al., 2007b), yellow croaker (Ding et al., 2020), and largemouth bass (Guo et al., 2020) due to their emulsifying effect on lipids for digestion and absorption. On the contrary, supplementation of BA to BSLO in the current study resulted in growth depression, and this noticeable effect could be attributed to the level of BA which appear to be more than the level tolerated by rainbow trout. Excessive addition of BA has been reported to cause growth depression, gallstone formation, and vacuolization of hepatocyte in genetically improved farmed tilapia (GIFT) (Jiang et al., 2018). Also, trout fed the BA-based diet showed a slow response to feeding (visual observation) compared
Average weight of fish was not affected by the dietary lipid source and was similar among experimental diets. The similarity of lipid retention among diets indicates that the quantity of ingested lipid was comparable in trout fed FO, SO, or BSLO-based diets implies that the 18:3n-3 or n-3 LC-PUFA in the diets were sufficient. The similarity of HSI of fish fed the SO and BSLO-based diets having the highest 18:2n-6 and 18:3n-3 and 14:0, 16:0, and total SFA, respectively, than trout fed the FO diet. Dietary FO resulted in higher muscle deposition of EPA and a higher n-3:n-6 ratio, but the BSLO-based diet produced the highest DHA which invariably led to similarities in muscle EPA + DHA. The highest deposition of ARA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) occurred in the muscle of the BSLO-fed fish despite low dietary levels of 18:2n-6 and 18:3n-3 compared to the SO diet which signifies the high capacity of rainbow trout to biosynthesize LC-PUFA (i.e. ARA, EPA and DHA) from their specific substrate when black soldier fly oil is fed. This was further revealed by Δ6-fatty acid desaturase activity which was higher in the BSLO groups. In a similar study, Liet al. (2016) observed that the levels of ARA and DHA in the muscle of Jian carp fed 100% BSLO were significantly higher than in those fed a SO diet. Also, turbot fed a palm oil-based diet had a higher deposition of ARA and DHA than SO fed fish (Peng et al., 2017). Contrarily, replacement of FO with linseed oil (LO) resulted in a lower whole-body of LC-PUFA in Manchurian trout (Yu et al., 2019). The lower EPA and n-3:n-6 ratio seen in the BSLO groups compared to FO are consistent with other studies where fish were fed vegetable oil (Mu et al., 2020; Peng et al., 2017; Yang et al., 2020; Yu et al., 2019). Furthermore, the close proximity in PCA of fish fed SO and BSLO signifies that BSLO has a comparable fatty acids composition and is suitable for use in place of SO in trout feeds.

FABP are intracellular cytoplasmic proteins that are active in the transport of fatty acids and other lipid-soluble substances through the cytoplasm (Castro et al., 2015; Torstensen et al., 2009). In this study, the relative mRNA expression of the fabp was significantly up-regulated in fish fed the SO and BSLO-based diet compared with the FO group, which indicates increased fatty acid uptake and
transport in the hepatic cells. In contrast, the expression of *fabp* in the white muscle of Atlantic salmon fed a vegetable oil blend was down-regulated (Torstensen et al., 2009), while no significant differences were noticed in the hepatic *fabp* gene expression of European seabass fed a vegetable oil blend and FO (Castro et al., 2015). The differences noticed in mRNA expression of *fabp* in this study compared to previous findings could be due to the type of oil used, species of fish, as well as the tissue assayed. FAS plays a crucial role in the synthesis of fatty acids and its activity could be correlated with the dietary saturated fatty acid composition. Peng et al. (2017) reported that substituting FO with palm oil or rapeseed oil resulted in higher *fas* expression in turbot, and this was related to the higher SFA contents in the diets, which is consistent with our results. Thus, the higher hepatic total SFA contents observed in BSLO-fed groups is related to the up-regulation of *fas* expression in the liver.

The expression of fatty acid elongase and desaturase genes are known to be influenced by the dietary fatty acid composition (Jordal et al., 2005; Zheng et al., 2004). Different studies have reported increased expression of Δ6-and Δ5-desaturases, and elongase activities when an alternative oil replaced FO in fish diet (Alhazzaa et al., 2011; Bell et al., 2001; Izquierdo et al., 2008; Teoh and Ng, 2016). In the current study, feeding BSLO resulted in increased hepatic and muscle accumulation of 18:2n-6, 20:3n-6, 20:4n-6, and muscle 22:6n-3 compared to FO fed fish. Except for 18:2n-6, all other mentioned fatty acids found at higher concentration in the fish tissues were all at low levels in the experimental diets, which indicates that upregulation of Δ6- and Δ5-desaturases in the BSLO-fed groups aided in the biosynthesis of LC-PUFA (Fig. 2). However, expression of fatty acid elongase, responsible for chain elongation, did not differ significantly among the dietary groups; but the BSLO-fed fish recorded the highest numerical value. The higher gene expression of Δ6- and Δ5-desaturase enzymes suggest that the fish made use of the LC-PUFA precursor (18:2n-6 and 18:3n-3) effectively to synthesize and tissue-accumulate greater levels of DHA in the fillet. Similar results were reported in rainbow trout (GÜLER and Yildiz, 2011; Turchini and Francis, 2009), hybrid tilapia (Teoh and Ng, 2016), gilthead seabream larvae (Izquierdo et al., 2008), and Lates calcarifer (Alhazzaa et al., 2011) fed vegetable oil as a substitute for FO. The high level of EPA and DHA in the FO diet could be the reason for decreased mRNA expression levels of Δ5- and Δ6-desaturases in FO fed fish due to a feedback inhibition. High dietary concentrations of EPA and DHA have been reported to inhibit the pathway responsible for LC-PUFA synthesis in fish (Jordal et al., 2005; Teoh and Ng, 2016; Tocher et al., 2000).

Dietary BSLO caused a significant reduction in serum glucose concentration in rainbow trout compared to the FO group, and this is similar to the observation of Dumas et al. (2018) who reported that BSLO might have antihyperglycemic effect in fasted rainbow trout. On the other hand, no effect on glucose concentration was recorded when Jian carp was fed BSLO as replacement for SO (Li et al., 2016), and this may be due to use of plant oil as a control, which possibly has bioactive compounds. SOD, CAT, and GPx are important antioxidant defense enzymes which help to protect cells against reactive oxygen species-triggered damage in fish (Fawole et al., 2020; Shamma et al., 2020; Yu et al., 2019). In the present study, the activity of SOD was found to be higher in BSLO + BA fed trout compared to the other dietary groups. The increased activity which indicates some level of oxidative stress may be associated with dietary addition of BA. BA help to improve digestion and absorption capacity of lipids in fish; however, it causes a cytotoxic effect to hepatocytes when supplemented in excess (Jiang et al., 2018), and this may induce production of reactive oxygen species and damage to liver cells. This was further supported by the CAT activity, which was significantly higher in the BSLO + BA group. The function of CAT and GPx enzymes are to decompose hydrogen peroxide formed by the action of SOD to a non-toxic constituent (Yu et al., 2019; Fawole et al., 2020), which is likely the reason for the higher CAT activity observed in BSLO + BA fed fish. Although the SOD activity in BSLO group was higher than those fed FO and SO, however, no effect was noticed on the CAT and GPx activities. Hence, the higher response noticed could not be related to stress, however, this require further study.

In conclusion, dietary black solider fly larvae oil (BSLO) has proven to be a suitable alternative lipid source in rainbow trout diets without adversely affecting growth performance, feed efficiency, nutrient retention and survival. Higher DHA accumulation and DHA:EPA ratio, and similar EPA + DHA and total n-3 PUFA in muscle correlated with up-regulated mRNA expression of genes related to LC-PUFA biosynthesis. Furthermore, the lower serum glucose levels and antioxidant enzyme activities demonstrated that replacement of FO with BSLO did not elicit oxidative-induced stress. Thus, it could be inferred that the addition of 160 g/kg BSLO in place of either FO or SO in the rainbow trout diet did not cause any deleterious effect on the fish performance and muscle deposition of essential fatty acid and could help reduce competition for vegetable oil used in human food, since the majority of the commercial aquafeed industry now makes use of high levels of terrestrial oils in feed production. Notably, it was observed that bile acid supplementation had no impact on the fatty acid deposition nor mRNA gene expression, but a negative impact was observed on growth performance. This observation requires further study to determine if BA at lower supplementation could provide a beneficial effect when added as a supplement in BSLO-based diet.

**Author contributions**

Vikas Kumar designed the research project, diet formulation and corrected the draft manuscript. Md. Sakhawat Hossain contributed to diet formulation and conducted the feeding trial. Femi J. Fawole, Md. Sakhawat Hossain and Shyam N. Labh contributed to the design and planning of the experiment, fish sampling, proximate composition, fatty acid composition, and gene expression analyses. Femi J. Fawole performed the statistical analysis and wrote the manuscript. Ken Overturf, Brian C. Small, Thomas L. Welker and Ronald W. Hardy, contributed to planning of the experiment and interpretation of results. All authors read the draft, corrected, and approved the final manuscript.

**Conflict of interest**

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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