

## Validation of the suitability of full fat and defatted black soldier fly larvae meals in diets for rainbow trout (*Oncorhynchus mykiss*)

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**Abstract:** Improved insect stocks, culture methods, and mitigation of safety concerns have increased availability and use of commercial black soldier fly larvae (BSFL) meals as animal feedstuffs. Both full-fat and defatted BSFL meals can have beneficial applications in aquatic animal feeds. The protein fraction in both meal types can serve as a high-quality amino acid source that complements feeds with reduced fish meal and increased amounts of plant ingredients. The lipid fraction of full-fat BSFL meal is rich in medium-chain fatty acids, a readily utilized energy source, and has been associated with immunostimulation in fish. However, differences in BSFL cultivation and processing methods can lead to variation in BSFL meal composition, digestibility, and quality. Thus, to more effectively utilize BSFL meals in rainbow trout (*Oncorhynchus mykiss*) feeds, it is necessary to thoroughly characterize the nutrient content, digestibility, and functional attributes of different types of BSFL meals to validate their suitability.

To accomplish this goal, an *in vivo* digestibility trial was conducted in juvenile trout to determine the available nutrient content of commercially sourced full fat and defatted BSFL meals. Based on the resulting digestibility data, a growth trial was conducted using practical-type rainbow trout diets formulated to contain 0, 5, or 10% full fat or defatted BSFL meal; an additional test diet was created by top-coating the 0% BSFL meal with BSFL lipid at a level equivalent to the 10% full-fat BSFL diet. All diets were formulated to contain 44.8% digestible protein and 15% crude lipid, and balanced to available lysine, methionine, threonine and phosphorus targets of 3.82, 1.30, 2.14 and 0.6, respectively, prior to cooking extrusion. For the growth trial, fifteen rainbow trout ( $10.4 \pm 0.2$  g, initial weight) were randomly stocked into quadruplicate tanks (400 L each) and fed their respective diets for 12 weeks to assess effects on growth performance and immune function.

Growth results indicated no significant effects of BSFL inclusion level, type, or their interaction on final fish weight (184-196 g), weight gain (1,678-1,756 %), feed conversion ratio (0.84-0.87), feed intake (1.76-1.86 % body weight/d), hepatosomatic index (1.2-1.3 %), viscerosomatic index (8.4-10.0%), or fillet ratio (54.0-59.6%).

Evaluation of expression of the immune related genes, IL-10, IL-1 $\beta$ , TNF $\alpha$ , HSP70, Defensin  $\beta$ 3, IL-4 like, and UDP glucose 6-dehydrogenase (UDPG6D) in the intestine revealed no significant changes in the fish fed full-fat or defatted BSF meal. However, expression of IL-10, IL-1 $\beta$ , TNF $\alpha$ , IL-4 like gene, HSP70, and UDPG6D was significantly upregulated in the intestine of fish fed the 0% BSFL meal diet with added BSFL lipid.

These data suggest that both full-fat and defatted BSFL meals are suitable for rainbow trout feeds when diets are formulated on an available nutrient basis that accounts for differences in protein availability and lipid content. The effects of BSFL lipid, but not full-fat BSFL meal, on immune gene expression requires further study but suggests that the immunomodulating components of BSFL lipid may be sensitive to degradation during extrusion.

**Key words:** rainbow trout, black soldier fly, insect proteins

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## Introduction

In aquaculture, feeding constitutes approximately 60% of overall production costs, making it critical for economic viability to utilize low-cost, nutritionally balanced diets (NRC 2011). The efficiency of fish production relies heavily on achieving an advantageous feed conversion ratio while addressing the high costs associated with protein inclusion in fish feed. Primary sources of protein, such as fish meal, offer high digestibility and a superior amino acid profile but are costly with inconsistencies in available supply, prompting the exploration of alternative protein sources (Glencross *et al.* 2007).

In addition to the multitudes of plant protein products that have been evaluated (Gatlin *et al.* 2007), unconventional protein sources such as industrial by-products, insects, seaweed, and former food products have gained attention in recent years for their potential to replace traditional feed ingredients. Insects are part of the natural diet for many fish species, and thus including insect-based ingredients in the diet is promising (Glencross 2020). Several different insect species and product types have been investigated including housefly maggot meal, mealworms, locust meal, silkworm meal, and black soldier fly larvae (BSFL, *Hermetia illucens*) meal (Makkar *et al.* 2014; Henry *et al.* 2015). Among these, utilization of BSFL as both a protein and lipid source is appealing due to their ability to convert organic waste into protein- and lipid-rich biomass with a favorable nutrient profile (English *et al.* 2021; Weththasinghe *et al.* 2022). Concurrently with the acknowledgement of the sustainability value of insects as alternative ingredients, improved insect stocks, modernized culture methods, and mitigation of safety concerns have increased availability of commercial BSFL meals as animal feedstuffs (English *et al.* 2021). BSFL meals have been investigated in various aquaculture species including tilapia (Ylidrium-Askoy *et al.* 2020), Atlantic salmon (Belghit *et al.* 2019), largemouth bass (Peng *et al.* 2021), and others (Mohan *et al.* 2022). However, incorporation of BSFL meals in aquaculture diets has uncovered several challenges related to the level of dietary inclusion (Weththasinghe *et al.* 2022). Specifically, initial processing and treatment of BSFL affects the final quality of the meal and thereby the amount that can be used in feeds (Gasco *et al.* 2024).

Nonetheless, BSFL meals can have beneficial applications in aquatic animal feeds when included in the diet at appropriate levels. Defatted BSFL meal can serve as a high-quality amino acid source that compliments feeds with reduced fish meal and

increased amounts of plant ingredients (English *et al.* 2021), while full-fat BSFL meal are rich in medium-chain fatty acids that are readily utilized as an energy source (Weththasinghe *et al.* 2021) and can upregulate fish immune function (Koutsos *et al.* 2022). Differences in BSFL meal processing methods and BSFL feedstock (i.e., what the BSFL are provided as a growth medium/food source) play key roles in the lipid quality and profile of the resultant BSFL meal (Lalander *et al.* 2019).

To more effectively utilize BSFL meals in aquaculture feeds, it is necessary to thoroughly characterize the nutrient content, digestibility, and functional attributes of different types of BSFL meals to validate their suitability. Accordingly, the current study determined the digestibility of full-fat or defatted BSFL meal in feeds for rainbow trout (*Oncorhynchus mykiss*) and examined the performance and immune function of rainbow trout fed diets containing these ingredients at two different inclusion levels. Additionally, the effect of dietary BSFL lipid inclusion, independent of BSFL meal, was evaluated.

## Materials and Methods

### 1. Digestibility trial

(1) **Experimental design** Two novel, commercially sourced BSFL meals were assessed in an *in vivo* digestibility trial to determine their available nutrient content. The products were full-fat BSFL meal and defatted BSFL meal obtained from Chapul, LLC. (McMinnville, OR, USA). The methods of Cho *et al.* (1982), Bureau *et al.* (1999), and Forster (2001) were used, with modifications for manual feces collection and reference diet formulation, to estimate apparent digestibility coefficients (ADCs) (Table 1). Yttrium oxide was the inert maker. A complete reference diet (Table 1) meeting or exceeding all known nutritional requirements of trout was blended with the test ingredients in a 70:30 ratio (dry-weight basis) to form the test diets.

(2) **Diet manufacturing** All diets were mixed and co-ground without fish oil using an air-swept pulverizer (model 18-H, Jacobson, Minneapolis, MN, USA) to a particle size of less than 250  $\mu\text{m}$ . Diets were manufactured using a twin-screw cooking extruder (DNDL-44, Bühler AG, Uzwil, Switzerland) with six-barrel sections and 18-s transit time through the barrel. The material was not steam conditioned prior to extrusion and water was added to barrel section 2. The 3.5 mm floating pellets were dried in a pulse-bed drier (Bühler AG) at 102°C until moisture levels were below 7% and cooled prior to

vacuum oil infusion top-coating (A.J. Mixing, Ontario, Canada). Diets were stored in a cool dry environment until utilized.

(3) **Fish culture and fecal sample collection** Fish weighing an average of 230 g were stocked at a rate of 25 fish per 400-L tank connected to a common bio-filter with spring water inflow of approximately 20% makeup. Water temperature was maintained at 15°C. Lighting was maintained on a 13 :11 h L:D cycle. Each diet was randomly assigned to triplicate tanks of fish. Fish were fed to apparent satiation twice daily for seven days prior to fecal collection.

(4) **Sample collection and analyses** Fecal samples were obtained by manual stripping, 16-18 h post-feeding. For each collection, all fish in the tank were sedated with 50 ppm tricaine methanesulfonate dissolved in a water bath, gently dried, and stripped by applying light pressure to the lower abdominal region, taking care to exclude urinary excretions from the fecal samples. To collect adequate sample volumes for laboratory analyses, feces were collected three times with a minimum recovery period of one day between collections. Fecal samples were pooled by tank, freeze-dried and stored at -20°C until chemical analyses were performed.

Dry matter analysis of ingredients, diets, and feces was performed according to standard methods (AOAC 2012). Yttrium and phosphorus were determined in diets and feces by inductively coupled plasma atomic absorption spectrophotometry (Anderson 1996). Crude protein (N x 6.25) was determined in ingredients, diets and feces by the Dumas method (AOAC 2012) on a Leco TruSpec N nitrogen determinator (LECO Corporation, St. Joseph, MI, USA). Total energy was determined by adiabatic bomb calorimetry (Parr1281, Parr Instrument ADM Inc., Moline, IL, USA). Amino acid analysis was performed by the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) according to in-house protocols. Apparent digestibility coefficients (ADCs) of each nutrient in the test diets were calculated according to established equations (Kleiber 1961; Forster 2001).

## 2. Growth trial

(1) **Experimental design** Based on digestibility data generated in the trial described above, a growth trial was conducted using practical-type rainbow trout diets formulated to contain 0 (Control), 5, or 10% full fat ("FF") or defatted ("DF") BSFL meal; an additional test diet was created by top-coating the

**Table 1** Composition of digestibility trial reference diet

<i>Ingredients</i>	(% dry-weight)
Wheat flour <sup>1</sup>	28.3
Squid meal <sup>2</sup>	25.0
Soy protein concentrate <sup>3</sup>	17.1
Fish oil <sup>4</sup>	13.5
Corn gluten meal <sup>5</sup>	8.3
Soybean meal <sup>6</sup>	4.3
Vitamin premix ARS <sup>7</sup>	1.0
Chromic oxide <sup>8</sup>	1.0
Choline chloride <sup>8</sup>	0.6
Taurine <sup>9</sup>	0.5
Stay-C 35 <sup>10</sup>	0.2
Trace mineral premix <sup>11</sup>	0.1
Yttrium oxide <sup>8</sup>	0.1

<sup>1</sup> Archer Daniels Midland (Decatur, IL, USA), 14 g/kg protein.

<sup>2</sup> Rangen Inc. (Buhl, ID, USA), 810 g/kg protein.

<sup>3</sup> Solae Profine VP (St. Louis, MO, USA), 693 g/kg crude protein.

<sup>4</sup> Omega Proteins Inc (Houston, TX, USA).

<sup>5</sup> Cargill Animal Nutrition (Minneapolis, MN, USA), 601.0 g/kg protein.

<sup>6</sup> Archer Daniels Midland (Decatur, IL, USA), 480 g/kg protein.

<sup>7</sup> Vitamin premix ARS 702 DSM Nutritional Products, Basel, Switzerland. Provided per kg diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D3, 2160 IU; vitamin E (as DL- $\alpha$ -tocopheryl-acetate), 1590 IU; niacin, 990 mg; calcium pantothenate, 480 mg; riboflavin, 240 mg; thiamin mononitrate, 150 mg; pyridoxine hydrochloride, 135 mg; menadione sodium bisulfate, 75 mg; folacin, 39 mg; biotin, 3 mg; vitamin B<sub>12</sub>, 90  $\mu$ g.

<sup>8</sup> Sigma-Aldrich Company (St Louis, MO, USA).

<sup>9</sup> Archer Daniel Midlands (Decatur, IL, USA).

<sup>10</sup> Rovimix Stay-C 35 (DSM).

<sup>11</sup> Contributed, per kg of diet; zinc, 40 mg; manganese, 10 mg; iodine, 5 mg; copper, 9 mg; selenium, 0.4 mg.

Control diet with BSFL lipid sourced from the same commercial supplier at a level equivalent to the 10% FF diet ("BSFL Lipid"). All growth trial diets (Table 2) were formulated to contain 44.8% digestible protein and 15% crude lipid, and balanced to available lysine, methionine, threonine and phosphorus targets of 3.82, 1.30, 2.14 and 0.6, respectively. All other micronutrients were provided at levels above NRC requirements (NRC 2011) and at levels proven beneficial at the Bozeman Fish Technology Center (Bozeman, MT, USA) in the evaluation of alternative proteins. For the feeding trial, 2.5 mm pellets were extrusion manufactured as previously described for the digestibility trial.

(2) **Fish culture** For the growth trial, fifteen rainbow trout (10.4  $\pm$  0.2 g, initial weight; Troutlodge Inc, Sumner, WA, USA) were randomly stocked into quadruplicate 400-L tanks in a recirculating system connected to a common bio-filter with

Table 2 Composition (% dry weight) of growth trial diets containing different levels of full-fat (FF) or defatted (DF) BSFL meals or BSFL lipid

Ingredients	Control	BSFL Lipid	5% FF	10% FF	5%DF	10%DF
Fishmeal <sup>1</sup>	13.32	13.32	12.54	11.76	12.35	11.40
Poultry by-product meal <sup>2</sup>	13.90	13.90	13.09	12.28	12.89	11.90
Full-fat BSFL meal <sup>3</sup>	0	0	5	10	0	0
Defatted BSFL meal <sup>3</sup>	0	0	0	0	5	10
BSFL lipid <sup>3</sup>	0	3.72	0	0	0	0
Menhaden fish oil <sup>1</sup>	4.69	2.83	3.90	3.11	4.47	4.25
Poultry fat <sup>4</sup>	3.84	1.98	3.05	2.25	3.63	3.42
Wheat flour <sup>5</sup>	25.10	25.10	23.26	21.43	22.46	19.79
Lysine HCl <sup>5</sup>	1.29	1.29	1.29	1.28	1.31	1.32
DL-Methionine <sup>5</sup>	0.43	0.43	0.44	0.45	0.44	0.46
Threonine <sup>5</sup>	0.30	0.30	0.30	0.31	0.32	0.33
Blood meal <sup>2</sup>	5	5	5	5	5	5
Feather meal <sup>2</sup>	5	5	5	5	5	5
Corn protein concentrate <sup>6</sup>	5	5	5	5	5	5
Soybean meal <sup>5</sup>	15	15	15	15	15	15
Lecithin <sup>5</sup>	1.5	1.5	1.5	1.5	1.5	1.5
Stay-C 35 <sup>7</sup>	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix ARS 702 <sup>8</sup>	1	1	1	1	1	1
Monocalcium phosphate <sup>5</sup>	2.5	2.5	2.5	2.5	2.5	2.5
Choline Cl 50% <sup>5</sup>	1	1	1	1	1	1
Taurine <sup>5</sup>	0.5	0.5	0.5	0.5	0.5	0.5
TM ARS 1440 <sup>8</sup>	0.1	0.1	0.1	0.1	0.1	0.1
Astaxanthin <sup>7</sup>	0.08	0.08	0.08	0.08	0.08	0.08
Calcium propionate <sup>2</sup>	0.15	0.15	0.15	0.15	0.15	0.15
Yttrium oxide <sup>9</sup>	0.1	0.1	0.1	0.1	0.1	0.1

<sup>1</sup> Omega Protein Corp., Menhaden fish meal, special select (700 g/kg protein), Hammond, LA, USA; Menhaden fish oil, Virginia Prime, Reed, Virginia, USA.

<sup>2</sup> Rangen Inc. (Buhl, ID, USA), Poultry by-product, pet food grade, 670 g/kg protein; Blood meal, 960 g/kg protein; calcium propionate.

<sup>3</sup> Chapul, LLC. (McMinnville, Oregon, USA).

<sup>4</sup> Tyson Foods, Springdale, AR, USA.

<sup>5</sup> Archer Daniels Midland Alliance Nutrition (Twin Falls, ID, USA), wheat flour 14 g/kg protein; L-lysine hydrochloride; DL-methionine; threonine; soybean meal, 460 g/kg protein; lecithin - Yelkinol AC dry lecithin; monocalcium phosphate; choline chloride 50%, taurine.

<sup>6</sup> CPC Empyreal 75, Cargill Corn Milling, Blair, NE, USA.

<sup>7</sup> Ascorbyl polyphosphate Rovimix Stay-C 35; Carophyll pink, DSM Nutritional Products Ltd., Basel, Switzerland.

<sup>8</sup> Star Milling, Peris, CA, USA. ARS 702 contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D3, 720 IU; vitamin E (as DL- $\alpha$ -tocopheryl-acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B12, 30  $\mu$ g; ARS 1440 contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3; selenium, 0.4.

<sup>9</sup> Sigma-Aldrich Company, St Louis, MO, USA.

spring water inflow of approximately 20% makeup. Water temperature was maintained at 15°C. Fish were fed twice daily to apparent satiation. Apparent satiation was defined as all the feed the fish would consume in a 30-min period. Feed consumption was recorded weekly. All fish in each tank were counted and group-weighted every three weeks and fed their respective diets for 12 weeks to assess effects on growth

performance and immune function.

(3) **Sample collection and analyses** At the termination of the 12-week trial, all fish were counted and weighed. Three fish per tank were randomly selected, euthanized by submersion in buffered tricaine methanesulfonate (350 mg/L) for 10 min and then dissected to collect tissue samples and determine the

following organosomatic indices:

Fillet Ratio (FR) = fillet mass with ribs (g) \* 100/fish mass (g)

Hepatosomatic Index (HSI) = liver mass (g) \* 100/fish mass (g)

Viscerosomatic Index (VSI) = viscera mass (g) \* 100/fish mass (g)

Fillet samples from these three fish per tank were analyzed to determine dietary effects on fillet fatty acid profile. Approximately 0.2 g of freeze-dried tissue from each sample was added to a 2 mL microcentrifuge tube containing 3 stainless-steel balls. A 3:1 chloroform: methanol solution was then added to the tubes in sufficient volume to fill them. The tubes were shaken on a bead beater (Mini-Bead Beater 24, BioSpec Products, Bartlesville, OK, USA) for three 30 s intervals, allowing 2 min between each shake. The tubes were then transferred to a microcentrifuge (Microfuge 18, Beckman Coulter, Brea, CA, USA) and spun down at 15,000 g for 20 min. Standards for calibration (CRM18918, CRM47570, CRM47571, Supelco, Bellefonte, PA, USA) were prepared in similar solvent and combined into a single fatty acid mixture. Pasteur pipettes were used to transfer the supernatant from the 2 mL tubes into labeled 10 mL round bottom glass tubes. An auto-pipette was then used to add 2 mL 1.25 M HCl-methanol solution to the supernatant in the 10 mL tubes which were then incubated (Isotemp 11-715-125D, Fisher Scientific, Pittsburg, PA, USA) for 0.5 h at 70°C. Following incubation, the tubes were allowed to cool to room temperature before 4 mL of distilled water was added to each. The tubes were then vortexed for 2-3 s and spun down for 5 min in a centrifuge (Sorvall ST16, Thermo Scientific, Waltham, MA, USA) at 500 g to separate the aqueous and non-aqueous layers. The upper aqueous layer from each tube was removed using suction and discarded. The lower chloroform layer was transferred to pre-labeled GC vials containing inserts. The methylated samples were analyzed using a Trace 1300 GC system (Thermo Scientific) supplied with a flame ionization detector set at 325°C and an Omegawax 250 capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas. Detector gas flows were as follows: Air, 350.0 mL min<sup>-1</sup>; Hydrogen, 35.0 mL min<sup>-1</sup>; makeup gas, 45.0 mL min<sup>-1</sup>. Total flow rate at the split vent was 480 mL/min, and flow rate through the column was 2.4 mL min<sup>-1</sup>. The split/splitless injector was set at 235°C with a split ratio of 200:1, and 0.20 µL injections were made. The following oven temperature program was used: initial temperature 170°C, ramp 1.3°C min<sup>-1</sup> to 200°C followed by ramp 1.3°C min<sup>-1</sup> to 225°C. The total run time was 42.31 min. Results were

calculated on a relative percent concentration basis by dividing the response factor (RF)-corrected areas of individual fatty acid peaks by the total peak area. The identity and elution order of additional fatty acids were determined similarly utilizing a mass spectrometer detector in electron ionization (70 eV) mode with the following settings: MS transfer line temperature of 250°C, ion source temperature of 200°C, mass range of 50-400 amu, scan time of 0.25 s and start time of 2.1 min. The FAME calibration standards eluted in the following order: methyl octanoate (C8:0), methyl decanoate (C10:0), methyl laurate (C12:0), methyl tetradecanoate (C14:0), methyl palmitate (C16:0), methyl palmitoleate (C16:1 n-9z), methyl octadecanoate (C18:0), cis-9-oleic methyl ester (C18:1 n-9z), methyl linoleate (C18:2 n-6,9zz), methyl linolenate (C18:3 n-3,6,9zzz), methyl eicosapentaenoate (EPA) (C20:5 n-3,6,9,12,15zzzzz), methyl arachidate (C20:0), methyl docosanoate (C22:0), methyl erucate (C22:1 n-9z), methyl lignocerate (C24:0), and methyl docosahexaenoate (DHA) (C22:6 n-3,6,9,12,15,18).

An additional three fish per tank were randomly selected and euthanized as described above to determine whole body composition. Moisture, crude protein, gross energy, and crude lipid of whole-body samples were determined according to the standard methods (AOAC 2012) described for the digestibility trial. Protein retention efficiency values were calculated as follows:

$$\text{Protein retention efficiency (PRE)} \\ = \text{protein gain (g)} * 100 / \text{protein fed (g)}$$

To assess the effects of BSFL meal and BSFL lipid inclusion on rainbow trout immune responses, a panel of genes known to undergo changes in expression during inflammation were measured in the intestine of three fish from each replicate tank. Briefly, a section of the distal intestine was dissected from each fish and frozen in liquid nitrogen. Total RNA was isolated from the samples using TRIzol according to the manufacturer's protocol (Invitrogen, Rockville, MD, USA). RNA quantity and quality were determined spectrophotometrically using a nanodrop (ThermoFisher, Waltham, MA, USA). Trace amounts of DNA were removed from all samples by treatment with DNase I (Invitrogen) and samples were assessed by quantitative real time RT-PCR as previously described by Johansen and Overturf (2005) using a Quantstudio 6 (ThermoFisher). Pro-inflammatory and immune-responsive genes examined included IL-10, IL-1β, TNFα, HSP70, Defensin β3, IL-4 like, and UDP glucose 6-dehydrogenase (UDPG6D). All the treatment values were standardized to a β-

actin control for both CT values and relative numerical values providing a numerical value for the control and each treatment from which the relative change was calculated (Johansen and Overturf 2005). Accession numbers for genes analyzed along with primers and probes used and efficiency for each gene are provided in Table 3.

### 3. Statistical analyses

Pooled SEMs from tank means are considered as the best estimate of variation inherent in this type of experiment and were used as an indicator of variation among treatments. Results were tested for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test). Normally distributed data was examined for significance of diet using ANOVA. Differences with  $P < 0.05$  were considered significant for all analyses. Means were compared with the control (Menhaden fish meal for the digestibility trial and the control diet for the feeding trial) using Dunnett's test. All data analyses were performed with JMP 15.1 (SAS Institute, Cary, NC, USA).

## Results and Discussion

BSFL meals have previously been reported to contain approximately 50-60% crude protein and 30-35% crude lipids for full-fat meals on an as-fed basis (Tomberlin *et al.* 2002). However, nutrient bioavailability of BSFL meals is less consistent due, in part, to their variable chitin content, which salmonids cannot digest (Olsen *et al.* 2006), and different processing methods that are used during lipid extraction and drying that can compromise amino acid availability (English *et al.* 2021).

In the current study, nutrient and energy ADCs for full-fat and defatted BSFL meals were not significantly different from each other or menhaden fish meal, with the exception of phosphorus (Table 4). Phosphorus digestibility was significantly higher for BSFL meals (57.9 - 58.0%) compared to menhaden fish meal (33.4%). Similarly, ADCs of aspartic acid, proline and tyrosine were significantly higher than menhaden fish meal while ADC of ornithine was significantly lower. The ADC

**Table 3** Gene names, accession numbers, probe and primer sequences and PCR reaction efficiencies for the quantitative PCR analysis performed on the fish samples

Gene Name	Accession Number	Probe and Primer Sequences (Listed 5'-3')	PCR reaction efficiency
B-actin	AF254414	BactinF: CCCTCTTCCAGCCCTCCTT BactinR: AGTTGTAGGTGGTCTCGTGGATA BactinMGB: 6FAM-CCGCAAGACTCCATACCGA-NFQ	97.4
Defensin $\beta$ 3	NM_001195183	Def $\beta$ 3F: ACGGAGGGTCATATTCATCAAATCAAA Def $\beta$ 3R: GCAATGACTAAAAGAGCCACTAGCA Def $\beta$ 3MGB: CTGCCTGATGATCTTC-NFQ	94.6
HSP70	AB062281	HSP70F: ACAGAGACAGGCCACTAAGGA HSP70R: CTCGTTGATGATCCTCAGCACAT HSP70MGB: TCAGCCCAGCGATCAC-NFQ	97.2
Interleukin-1 $\beta$	AJ004821	IL1 $\beta$ F- GTAATATTTCTCTTCCCCTGTGTGT IL1 $\beta$ R- CCCTGAGGCAGCTTGGA IL1 $\beta$ MGB- CATGCTGCACTTTCAG-NFQ	94.7
Interleukin-4	XM_036961927	IL4F- TCTTTCACAATTCCAGATTTTCAGCTGTA IL4R- GCCCAGCAAATAGATGACAACACT IL4MGB- ACACGCACATTAATTC-NFQ	93.0
Interleukin-10	AB118099	IL10F: AGTAGCTCAACGGGTAGAGAGAA IL10R: AATTCCTTGTCGTCAGTGAGTGT IL10MGB: TCTGGTCCCCAAGATC-NFQ	95.5
TNF $\alpha$	NM_001124357	TNF $\alpha$ F: GAATACAATCCTAATCTTTCCGCTGACA TNF $\alpha$ R: GAACCCGCCCTGGGAAAA TNF $\alpha$ MGB: CTGGCCGTCATCCTTT-NFQ	92.0
UDP glucose 6-dehydrogenase	XM_036934411	UDPG6DF: CCAGGGTTGAAGGAGGTAGTG UDPG6DR: TCTCTGATGGCGGAATCTATATCTGT UDPG6DMGB: TCCCTCGGCATGACTC-NFQ	95.6

**Table 4** Apparent digestibility coefficients (ADCs, %) for menhaden fish meal, full-fat BSFL meal, and defatted BSFL meal

	Menhaden Fish Meal	Full fat BSFL Meal	Defatted BSFL Meal	<i>Pooled SEM</i>	<i>Prob &gt; F</i>
ADCs					
Dry matter	72.1	71.2	65.6	3.5	0.1279
Crude lipid	93.1	89.4	89.0	2.5	0.6505
Crude protein	72.9	71.4	70.4	1.9	0.3639
Gross energy	78.5	77.3	73.0	2.9	0.1187
Phosphorus	33.4	57.9*	58.0*	7.4	0.0098
Amino Acids					
ALA	81.0	84.2	85.4	1.9	0.0741
ARG	85.7	88.0	88.7	1.9	0.2193
ASP	70.1	79.1*	78.9*	3.8	0.0432
CYS	57.7	52.7	54.7	9.4	0.8151
GLU	82.3	78.5	80.5	3.2	0.4021
GLY	70.0	75.1	76.4	2.9	0.0796
HIS	79.7	80.2	80.7	2.6	0.8965
HYD-LYS	57.9	88.6*	84.3*	6.8	0.0342
HYD-PRO	52.9	68.4	69.1	9.5	0.5330
ILE	83.3	83.9	83.9	3.0	0.9686
LEU	84.2	80.0	81.1	2.5	0.1901
LYS	85.6	88.0	86.4	2.1	0.4091
MET	81.4	85.6	84.8	4.6	0.5323
ORN	75.1	37.4*	37.6*	3.9	<0.0001
PHE	81.8	80.6	80.3	2.6	0.7497
PRO	72.3	80.4*	83.5*	2.4	0.0029
SER	76.8	78.5	79.3	3.1	0.6131
THR	79.2	80.4	79.7	2.5	0.8472
TRP	85.2	88.6	87.7	2.5	0.2968
TYR	85.0	89.6*	89.8*	1.5	0.0134
VAL	83.2	88.0	90.3	3.4	0.1055

Values are means ( $n = 3$ ). ADCs in the same row with an asterisk are significantly different from ADC of menhaden fish meal as determined by Dunnett's test ( $P < 0.05$ ).

values reported here are consistent with Dumas *et al.* (2018), who reported the ADCs of protein and amino acids in defatted BSFL meal varied between 87 and 93%, whereas digestibility of lipid and dry matter in BSFL meal were relatively low at 73 and 75%, respectively. Dry matter and gross energy digestibility in the current study was lower for defatted BSFL meal but lipid ADCs were higher for BSFL meals (Table 4) than those previously reported. The current digestibility findings also fall on the upper end of the ranges reported for other BSFL meals in rainbow trout in a recent literature review by Mohan *et al.* (2022). Taken together, these data indicate the novel BSFL meals investigated in the current study are high quality products, and the bioavailability of the nutrients present in these products were not damaged during fat extraction or drying.

The growth performance observed in the current trial also points the suitability of the tested BSFL meals as ingredients in rainbow trout feeds, achieving approximately 1,700% weight

gain during the trial (Table 5). Additionally, the feeds were all readily accepted, and no mortality was observed during the 12-week feeding study (Table 4). Statistical analysis of the growth results indicated no significant effects of BSFL meal or BSFL lipid inclusion on final fish weight ( $P = 0.7321$ ), weight gain as a percent increase ( $P = 0.9513$ ), feed conversion ratio ( $P = 0.8333$ ), or feed intake ( $P = 0.5885$ ; Table 5). These results are in alignment with early research examining the potential of black soldier fly meal as feed ingredients in rainbow trout at inclusion levels of 15 to 18% that found no significant effects on growth and feed efficiency (St-Hilaire *et al.* 2007b; Sealey *et al.* 2011). More recent work has recapitulated the successful incorporation of BSFL meal at levels up to approximately 15% in feeds for rainbow trout (Renna *et al.* 2017; Dumas *et al.* 2018; Caimi *et al.* 2021).

Organosomatic indices are valuable indicators of general health and the nutritional adequacy of a fish's diet (Hoque *et al.* 1998). In the current study, hepatosomatic index ( $P =$

0.6808), viserosomatic index ( $P = 0.5200$ ), and fillet ratio ( $P = 0.3959$ ) were not significantly affected by BSFL meal or BSFL lipid inclusion (Table 5). These results are aligned with other studies that have examined the effects of BSFL on rainbow trout organosomatic indices (Renna *et al.* 2017; Caimi *et al.* 2021) and fall within previously reported ranges for rainbow trout of this size (Sealey and Gaylord 2025).

Proximate composition data provides insight into an animal's growth efficiency and, by proxy, nutrient utilization. In the current study, BSFL meal or BSFL lipid inclusion did not affect rainbow trout whole body moisture ( $P = 0.3282$ ), lipid ( $P = 0.4544$ ) or protein ( $P = 0.3280$ ) content (Table 6).

In contrast, a significant effect of diet level was observed for fillet moisture content ( $P = 0.0298$ ), where in rainbow trout fed 10% BSFL fillet moisture decreased by approximately 0.5% (Table 7). Although statistically significant, the difference in fillet moisture content in the current study in the absence of other significant alterations such as corresponding significant increases in fillet lipid content likely have minimal physiological impacts. Notably, previous research by Mancini *et al.* (2018) and Huyben *et al.* (2019) reported that the dietary inclusion of BSFL meal had no adverse effects on rainbow trout fillet quality.

Previous research examining the lipid content of BSFL has

**Table 5** Growth performance and organosomatic indices of juvenile rainbow trout fed diets containing different inclusions of full-fat (FF) or defatted (DF) BSFL meals and/or BSFL lipid (BSFL Lipid) for 12 weeks

	Final Fish Wt <sup>1</sup>	Weight Gain <sup>2</sup>	FCR <sup>3</sup>	Feed Intake <sup>4</sup>	Viscero-somatic Index <sup>5</sup>	Fillet Ratio <sup>6</sup>	Hepatosomatic Index <sup>7</sup>
	(g)	%	feed g/gain g	% bw	%	%	%
Control	194	1,755	0.86	1.84	10.0	57.8	1.3
BSFL Lipid	184	1,687	0.87	1.81	9.8	58.5	1.3
5% FF	184	1,678	0.86	1.79	10.0	55.3	1.3
10% FF	189	1,756	0.88	1.83	10.4	58.2	1.2
5% DF	196	1,718	0.84	1.76	8.4	54.0	1.2
10% DF	193	1,729	0.87	1.86	9.7	59.6	1.2
<i>Pooled SEM</i>	13	143	0.04	0.08	1.31	3.78	0.17
<i>Prob &gt; F</i>	0.7321	0.9513	0.8333	0.5885	0.5200	0.3959	0.6808

<sup>1</sup> Final tank weight (g) / number of fish in the tank. <sup>2</sup> Percent increase (%) = (final weight – initial weight) x 100 / initial weight. <sup>3</sup> FCR, feed conversion ratio = g feed consumed / g weight gained. <sup>4</sup> Feed intake (%) = g dry feed consumed/average fish biomass (g) /culture days x 100. <sup>5</sup> Viscerosomatic index (%) = viscera mass x 100 / fish mass. <sup>6</sup> Fillet ratio (%) = fillet with rib mass x 100 / fish mass. <sup>7</sup> Hepatosomatic index (%) = liver mass x 100 / fish mass.

Values are means ( $n = 4$ ).

**Table 6** Whole body proximate composition<sup>1</sup> and protein retention efficiency<sup>2</sup> of juvenile rainbow trout fed diets containing different inclusions of full-fat (FF) or defatted (DF) BSFL meals and/or BSFL lipid (BSFL Lipid) for 12 weeks

	Moisture	Lipid	Protein	Protein Retention Efficiency
Control	69.4	6.8	17.3	42.9
BSFL Lipid	69.4	6.5	17.5	43.3
5% FF	69.8	7.0	16.9	41.7
10% FF	69.9	6.6	17.5	42.4
5% DF	68.8	7.1	17.7	45.3
10% DF	68.9	6.9	17.9	43.4
<i>Pooled SEM</i>	0.78	0.44	0.62	2.25
<i>Prob &gt; F</i>	0.3282	0.4544	0.3280	0.4697

<sup>1</sup> Whole body composition based on three fish composite sample per tank. <sup>2</sup> PRE, protein retention efficiency = g protein gain x 100/g protein fed.

Values are means ( $n = 4$ ).

**Table 7** Fillet moisture, lipid and fatty acid (%) composition of juvenile rainbow trout fed test diets containing different inclusions of full-fat (FF) or defatted (DF) BSFL meals and/or BSFL lipid (BSFL Lipid) for 12 weeks

	Moisture	Lipid	12:0	14:0	16:0	16:1 n-7	18:0	18:1 n-9	18:1 n-5	18:2 n-6	20:4 n-6	20:5 n-3	22:6 n-3
Control	72.1	5.4	0.1	2.5	22.1	8.2	5.1	24.6	5.6	12.7	0.8	2.0	9.2
BSFL Lipid	71.3	5.5	4.2*	3.3*	21.5	7.0*	4.8	22.7	5.3	12.2	0.7	1.3*	8.0*
5% FF	72.6	4.6	2.5*	2.9	21.5	7.1*	5.0	23.1	5.1	13.7*	0.9	1.8	10.0
10% FF	71.1*	6.2	4.8*	3.0*	19.9*	7.0*	5.0	24.3	5.1	14.5*	0.8	1.2*	7.7*
5% DF	71.7	5.1	2.0*	2.9	19.8*	6.9*	5.1	24.3	6.2	14.3*	0.9	1.8	9.0
10% DF	71.3	5.8	3.7*	3.3*	21.0	7.4*	5.0	23.2	5.3	14.1*	0.8	1.7	8.4
<i>Pooled SEM</i>	0.63	0.77	0.71	0.20	0.74	0.47	0.24	1.02	0.97	0.60	0.11	0.33	0.76
<i>Prob &gt; F</i>	0.0298	0.1137	<0.0001	0.0006	0.0374	0.0275	0.3214	0.0847	0.4213	0.0003	0.5208	0.0255	0.0036

Values are means ( $n = 4$ ). Values in the same row with an asterisk are significantly different from the value of Control as determined by Dunnett's test ( $P < 0.05$ ).

**Table 8** Fatty acid (%) composition of test diets containing different inclusions of full-fat (FF) or defatted (DF) BSFL meals and/or BSFL lipid (BSFL Lipid) for 12 weeks of growth trial

	12:0	14:0	16:0	16:1 n-7	18:0	18:1 n-9	18:1 n-5	18:2 n-6	20:4 n-6	20:5 n-3	22:6 n-3
Control	0.1	3.4	22.0	7.7	5.7	21.8	2.5	16.9	0.9	5.7	5.5
BSFL Lipid	9.7	4.2	19.7	5.9	4.8	20.0	2.1	19.6	0.6	3.9	3.7
5% FF	5.3	3.9	21.7	7.1	5.5	21.0	2.5	17.6	0.7	4.7	4.6
10% FF	9.4	3.9	20.1	6.1	5.1	20.7	2.1	18.6	0.6	3.7	3.7
5% DF	3.7	3.7	21.0	7.4	5.4	21.5	2.5	17.0	0.8	4.9	4.6
10% DF	7.5	4.3	21.0	7.0	5.2	20.1	2.2	16.9	0.7	4.7	4.4

Means of duplicate analyses on a dry matter basis.

suggested that the lipid component of BSFL meal can pose challenges in feed formulation as it may lead to an imbalanced fat profile in fish (Eide *et al.* 2024) due to the large variation in black soldier fly larvae saturated fatty acids levels (58-72%) and mono and polyunsaturated fatty acids (19-40%) that have been reported (Kroeckel *et al.* 2012). In the current study feeding diets containing BSFL meal or BSFL lipid significantly increased the levels of lauric acid (12:0) in the diets (Table 8) and subsequently, rainbow trout fillets particularly when fish were fed 10% BSFL meal or BSFL lipid diets (Table 8). Conversely, rainbow trout fed the control diet containing no BSFL meal or BSFL lipid had higher fillet levels of palmitic (16:0) and palmitoleic (16:1 n-7) acid. Importantly, rainbow trout fillet polyunsaturated fatty acids levels were altered by BSFL meal inclusion level in that linoleic acid (C18:2 n-6), an omega-6 fatty acid significantly increased with BSFL meal supplementation while docosahexaenoic acid (DHA, 22:6 n-3), and eicosapentaenoic acid (20:5 n-3) significantly decreased in rainbow trout fed the 10% FF BSFL meal or the BSFL lipid diets (Table 7). These results agree with those reported by St-

Hilaire *et al.* (2007a, b) and Melenchón *et al.* (2021) who found that feeding high inclusion levels of BSFL meal reduced the omega 3 fatty acid contents in rainbow trout fillets. However, the present results differ from those of Drosdowech *et al.* (2024), who reported that BSFL meal inclusion had no significant effect on DHA levels in rainbow trout fillets. These differing results are likely explained by differences in the total lipid content and fatty acid profiles of the BSFL meals used in the various studies (St-Hilaire *et al.* 2007a, b; Melenchón *et al.* 2021).

Studies have shown that incorporating BSFL products can positively affect immune gene expression and alter inflammatory responses in fish (Elia *et al.* 2018; Koutsos *et al.* 2022; Wethasinghe *et al.* 2022). The current study examined expression of genes that have critical functions in the rainbow trout immune system and provide an indication of overall fish health. Specifically, tumor necrosis factor alpha (TNF $\alpha$ ) and interleukin1  $\beta$  (IL-1 $\beta$ ) are pro-inflammatory cytokines whereas interleukin-10 (IL-10) and interleukin-4 like (IL-4 like) are anti-inflammatory cytokines. Defensin  $\beta$

is an antimicrobial peptide that helps with wound healing and can inhibit the accumulation of TNF $\alpha$ . Heat shock protein 70 (HSP70) is a molecular chaperone that is expressed in response to stress and UDP-glucose 6-dehydrogenase (UDPG6D) prevents cellular damage from reactive oxygen species. In the current study, no significant effects of BSFL meal on intestinal expression of IL-1 $\beta$  (Fig.1A), TNF $\alpha$  (Fig.1B), IL-10 (Fig.1C),

HSP70 (Fig.1D), IL-4 like (Fig.1E), and UDPG6D (Fig.1G) were observed. However, fish fed the BSFL lipid diet exhibited significant upregulation of IL-1 $\beta$  (Fig.1A), TNF $\alpha$  (Fig.1B), IL-4 like gene (Fig.1E), and UDPG6D (Fig.1G) expression and significant down-regulation of IL-10 (Fig.1C) and HSP70 (Fig.1D). Cardinaletti *et al.* (2019) previously reported upregulated expression of IL-10 and TNF $\alpha$  in the

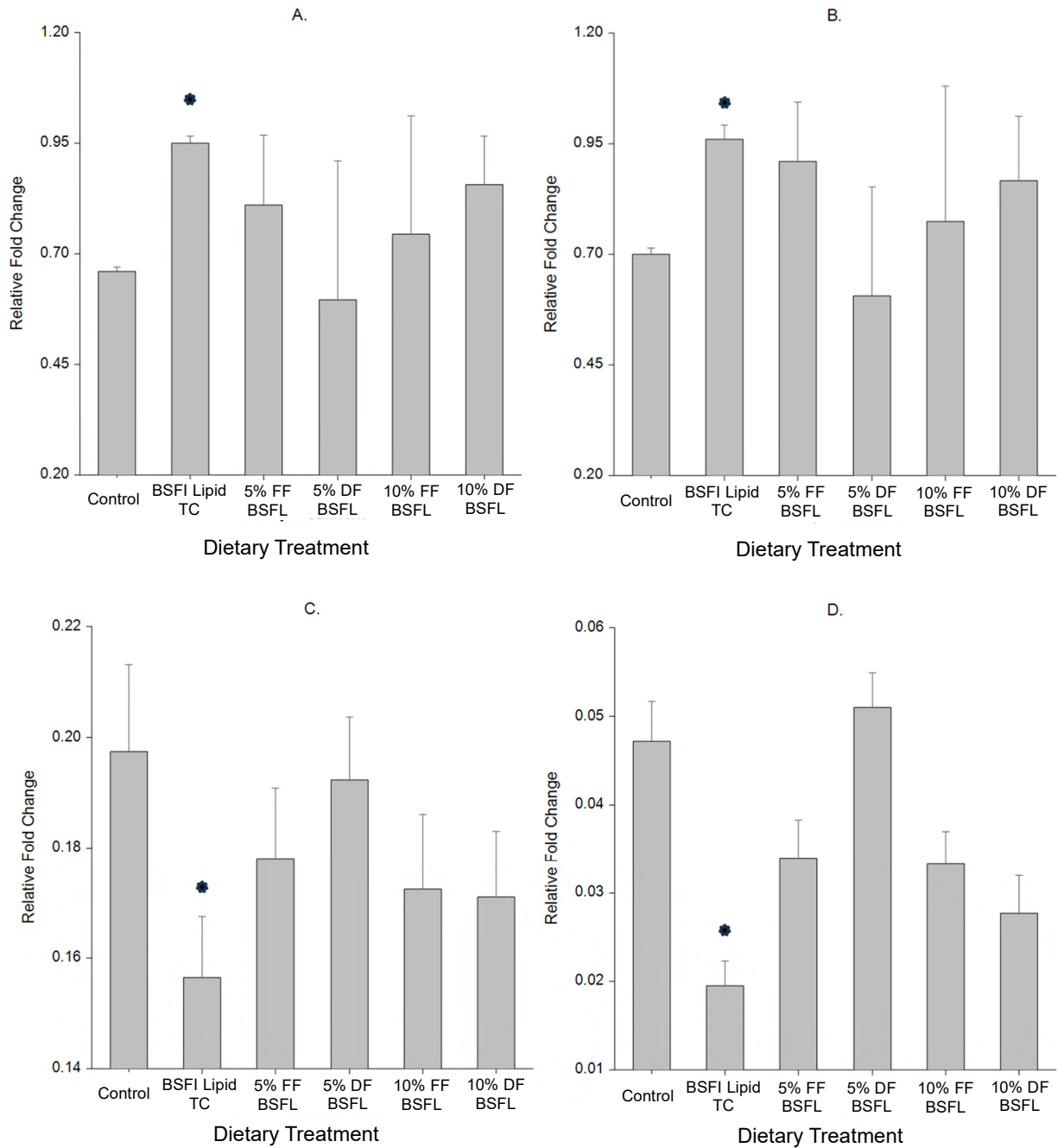


Fig.1 Expression of the intestinal immune related genes IL-1 $\beta$  (A), TNF $\alpha$  (B), IL-10 (C) and HSP70 (D) in rainbow trout fed diets containing different inclusions of full-fat (FF) or defatted (DF) BSFL meals and/or BSFL lipid (BSFL Lipid)

An asterisk indicates that rainbow trout fed the various diets are significantly different from the rainbow trout fed the Control diet as determined by Dunnet's test ( $P < 0.05$ ).

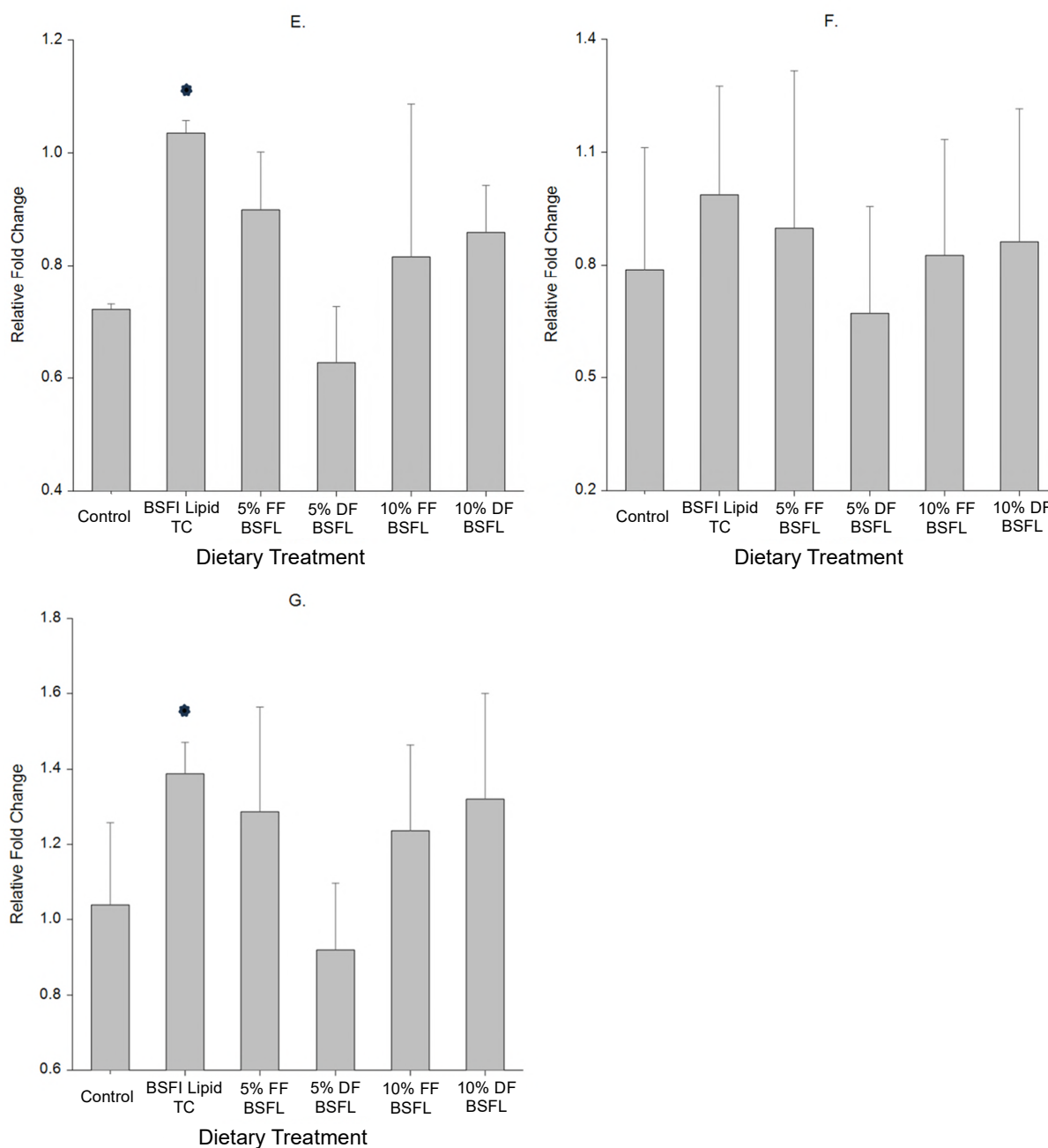


Fig.1 Continued

Expression of the intestinal immune related genes IL-4 like (E), Defensin  $\beta$ 3 (F), and UDP glucose 6-dehydrogenase (UDPG6D; G) in rainbow trout fed diets containing different inclusions of full-fat (FF) or defatted (DF) BSFL meals and/or BSFL lipid (BSFL Lipid)

An asterisk indicates that rainbow trout fed the various diets are significantly different from the rainbow trout fed the Control diet as determined by Dunnet's test ( $P < 0.05$ ).

intestine of rainbow trout fed 10.5 or 21% BSFL meal, as well as upregulation of HSP70 in the liver of fish fed 21% BSFL meal. Similarly, Cho *et al.* (2022) reported significantly higher expression of IL-1 $\beta$  in rainbow trout fed 5% BSFL meal. Gaudioso *et al.* (2021) reported different expression patterns of selected markers in the midgut and head kidney of rainbow trout fed BSFL meal. Specifically, significantly higher

expression of the pro-inflammatory cytokine IL-1 $\beta$  was observed in head kidney tissues of fish fed BSFL meal, whereas the opposite trend was observed in midgut tissues. The current results suggest that BSFL meals can be used at the tested inclusion rates without negatively affecting immune factors. However, top-coating feed with BSFL lipid resulted in significant modulation of a number of immunoregulatory

factors. This is somewhat similar to the findings of Borland *et al.* (2024) except their diets included supplemental chitin in addition to BSFL meal. Dietary upregulation of immune function is thought to be a positive attribute of some dietary components used as immunostimulants, assuming the animals do not become less responsive to the ingredient over time. The full extent of the effects of BSFL lipid on the rainbow trout immune system have yet to be elucidated, but the effects observed herein are considered modestly immunostimulative suggesting that the ingredient may be potentially useful in that regard.

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## Annotated Bibliography of Key Works

- (1) English G, Wanger G, Colombo S (2021) A review of advancements in black soldier fly (*Hermetia illucens*) production for dietary inclusion in salmonid feeds. *J. Agric. Food Res.*, **5**, 100164. <https://doi.org/10.1016/j.jafr.2021.100164>

The authors summarize the main findings on the advancement on black soldier fly (BSF) production methods and their use in salmonid aquaculture and highlight the importance of BSF rearing procedures and processing on the suitability of BSF as a nutritious protein source for salmonids. Further, the authors identified areas for future research regarding optimizing rearing and processing procedures for BSF destined for aquafeeds.

- (2) Gasco L, Bellezza Oddon S, Vandenberg GW, Veldkamp T, Biasato I (2024) Factors affecting the decision-making process of using insect-based products in animal feed formulations. *J. Insects Food Feed*, **10**, 1707-1718. [https://brill.com/view/journals/jiff/10/10/article-p1707\\_5.xml?srsltid=AfmBOoptqHMf9FusU8AQCMepGdGliOCmGduiB yrAqwbeSKofIUad7Psk](https://brill.com/view/journals/jiff/10/10/article-p1707_5.xml?srsltid=AfmBOoptqHMf9FusU8AQCMepGdGliOCmGduiB yrAqwbeSKofIUad7Psk)

The authors discuss the various factors to consider when including insect-based products in animal feeds. In particular, the authors identified the importance of insect meal form, insect species, rearing substrates and production processes. The authors highlighted how the increased use of additives during insect processing has created additional sources of variation in commercial insect products. The authors also discussed how feed manufacturing method was an important consideration when using insect-based products in animal feeds.

- (3) Koutsos E, Modica B, Freel T (2022) Immunomodulatory potential of black soldier fly larvae: applications beyond nutrition in animal feeding programs. *Transl. Anim. Sci.*, **6**, txac084. <https://doi.org/10.1093/tas/txac084>

The authors review recent research that demonstrates the potential for the immunomodulatory activity of various components of black soldier fly (BSF)-derived ingredients. The authors focus this review on the actions of three important BSF components that have been shown to alter immune function and disease resistance in companion animals, poultry and livestock including: antimicrobial peptides that are present in BSF hemolymph; lauric acid, a predominant BSF fatty acid, and the chitin/chitosan components of the insect exoskeleton.

(4) Weththasinghe P, Øvrum Hansen J, Torunn Mydland L, Øverland M (2022) A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids. *Rev. Aquac.*, **14**, 938-956. <https://doi.org/10.1111/raq.12635>

The authors discuss how black soldier fly (BSF) has gained attention as a sustainable novel protein source in fish feed due to its high nutritional value and low environmental impacts and conducted a meta-analysis to compile and systematically quantify the effect of BSF in diets for salmonids

on growth performance and nutrient utilization. The authors present results that demonstrate that overall dietary inclusion of BSF did not compromise the specific growth rate, feed conversion ratio, feed intake, protein digestibility and protein efficiency ratio in salmonids. Importantly, the authors report that when the published literature reviewed was sorted according to the replaced protein source(s), replacing fishmeal by BSF decreased growth rate and feed intake in salmonids but replacing non-fishmeal sources improved growth rate and feed conversion.