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Sensory Analysis of Rainbow Trout, *Oncorhynchus mykiss*, Fed Enriched Black Soldier Fly Prepupae, *Hermetia illucens*

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Abstract

A growth trial and fillet sensory analysis were conducted to examine the effects of replacing dietary fish meal with black soldier fly (BSF) prepupae, *Hermetia illucens*, in rainbow trout, *Oncorhynchus mykiss*. A practical-type trout diet was formulated to contain 45% protein; four test diets were developed by substituting 25 and 50% of the fish meal with normal (BSF) or fish offal-enriched black soldier fly (EBSF) prepupae. Dietary fat was adjusted to approximately 20% lipid using fish oil and poultry fat. Diets were fed to three replicate tanks of fish per treatment (10 fish/tank) for 8 wk. After the trial, three fish per tank were sampled for determination of hepatosomatic index, intraperitoneal fat ratio and muscle ratio, and muscle proximate and fatty acid composition. Fish remaining after sampling were used for sensory evaluation. Growth of fish fed the EBSF diets was not significantly different from those fish fed the fish meal-based control diet, while the growth of fish fed the BSF diets was significantly reduced as compared to the control diet. A group of 30 untrained panelists did not detect a significant difference in a blind comparison of fish fed the fish meal containing control diet as compared to fish fed the EBSF or BSF diets.

Recently, heightened emphasis has been placed on utilization of sustainable plant products as protein sources in aquafeeds (Gatlin et al. 2007). However, some detrimental effects on fish growth and fillet quality have been reported when attempting to replace fish meal protein with alternative animal- or plant-derived ingredients for rainbow trout, *Oncorhynchus mykiss*. Barrows et al. (2007) reported that rainbow trout fed diets where fish meal was replaced with plant meals or plant protein

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concentrates exhibited slightly reduced growth and feed efficiency compared to fish fed commercial formulations. de Francesco et al. (2004) found that replacing all of the dietary fish meal with a plant protein mixture altered the trout fillet chemical composition along with the sensory characteristics of trout fillets. D'Souza et al. (2006) determined that fillet color and oxidative stability were altered when dietary fish meal was replaced with soybean meal. Incomplete data on the nutritive value of plant-based ingredients coupled with a lack of understanding of what defines product quality for consumers are likely reasons that alternative ingredients have generally yielded suboptimal performance and research is ongoing to strengthen this knowledge base (Barrows et al. 2008); however, identification of novel and complimentary alternative ingredients also is necessary.

Insects are a natural part of the rainbow trout diet (Angradi and Griffith 1990) and may be another source of dietary protein if fish growth and fillet quality can be maintained. Insects have the potential to recycle lost nutrients by incorporating residual amino acids and fatty acids present in manure and waste from a variety of animal production facilities into their biomass. This resultant biomass is typically high in protein and fat making it suitable for incorporation into animal feeds. For example, prepupae from the black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) consist of 42% protein and 35% fat (Newton et al. 1977). BSF protein has been successfully used as a feed ingredient for terrestrial animals (Calvert et al. 1969; Newton et al. 1977) and commercially raised fish (Bondari and Sheppard 1981).

A recent study in our laboratory indicated that BSF prepupae could be used to replace a portion (25%) of the fish meal component of a practical grow-out diet for rainbow trout without compromising growth (St-Hilaire et al. 2007a). Further, our previous study indicated that dietary fish oil supplementation may also be reduced with BSF prepupae inclusion due to the prepupae's high fat content; however, there was a slight decrease in the fillet long chain-polyunsaturated fatty acid (LC-PUFA) content. Modifications of fillet fatty acid concentrations are known to affect fish aroma and flavor (Turchini et al. 2003); however, fillet quality was not assessed in our preliminary study.

In an effort to increase the LC-PUFA in BSF prepupae, subsequent to our preliminary trout feeding trial, a larva rearing trial was conducted where prepupae were fed diets containing different proportions of fish offal (St-Hilaire et al. 2007b). This enrichment process resulted in a substantial increase in levels of LC-PUFA in prepupae from negligible levels (0.1%) to approximately 5% of their total fat content. The objective of the current study was to evaluate the value of these LC-PUFA-enriched BSF prepupae as a feed ingredient, and to assess the effects of dietary inclusion of BSF prepupae on rainbow trout fillet quality.

Materials and Methods

Black Soldier Fly Culture and Enrichment

BSF prepupae were reared on dairy cow manure. One group was raised at Idaho State University, Pocatello, Idaho, USA on manure that had been processed through a screen solid separator using specialized containers (ESR International LLC, Dalat, Vietnam). The dry matter content of this manure ranged between 19 and 21%. Larvae were provided manure every other day. During the last month of growth, larvae were fed manure supplemented with between 25 and 50% fish offal. Fish offal consisted of visceral organs and fat from a rainbow trout processing plant. Prepupae were collected with a ramp and collection device as they migrated away from the manure to pupate. All prepupae were frozen and maintained at −20 C until the rainbow trout feed trial. Culture of the prepupae occurred between June 2006 and June 2007. It took approximately 4 mo for eggs to hatch and larvae to develop to prepupae. The second group of BSF prepupae was grown under similar conditions as described above but without supplemental fish offal at the Texas Agrilife Research and Extension Center, Stephenville, Texas, USA. Upon arrival to the University of Idaho Hagerman Fish Culture Experiment Station, BSF prepupae were further processed by freeze grinding in liquid nitrogen and drying at 40 C for 36 h.

Diet Formulation

Amino acid and fatty acid contents of the BSF prepupae used in this trial were analyzed (see proximate analysis method) to assist in diet formulation (Tables 1 and 2, respectively). A practical-type control diet (Hardy 2002) that met or exceeded all the known dietary requirements of rainbow trout (NRC 1993) was formulated with 45% protein and 20% fat (Table 3). For the four test diets, 25 and 50% of the protein content provided by the fish meal was substituted with normal (BSF) or enriched black soldier fly (EBSF) prepupae on equal amino acid content (Table 1). All diets were formulated to meet the amino acid profile of rainbow trout protein (Wilson and Cowey 1984). The total fat content was adjusted using fish oil to provide required levels of essential fatty acids (1% eicosapentaenoic and docosahexaenoic acids) and poultry fat was used to balance total lipid levels. Feeds were produced by compression pelleting without steam using a laboratory-scale pellet mill (California Pellet Mill, San Francisco, CA, USA) at the Bozeman Fish Technology Center, Bozeman, Montana, USA.

TABLE 1. *Amino acid composition of normal and enriched black soldier fly,* Hermetia illucens, *prepupae.*

Amino acids (g/100 g)[1]	Ingredients	
	Normal black soldier fly prepupae	Enriched black soldier fly prepupae
Alanine	2.45	2.55
Arginine	1.78	1.89
Aspartic acid	4.09	4.14
Glutamine	4.42	4.17
Glycine	1.72	1.80
Histidine	0.76	0.82
Isoleucine	1.83	1.89
Leucine	2.66	2.72
Lysine	2.05	2.12
Methionine	0.77	0.79
Phenylalanine	1.83	1.82
Serine	1.37	1.42
Threonine	1.58	1.60
Tyrosine	2.22	2.57
Valine	2.99	3.06
Total	32.78	33.56

[1]All values are reported as means of duplicate analyses on a dry matter basis (g/100 g dry sample).

TABLE 2. *Selected fatty acid composition of normal and enriched black soldier fly,* Hermetia illucens, *prepupae.*[1]

Fatty acids (%)	Ingredients	
	Normal black soldier fly prepupae	Enriched black soldier fly prepupae
12:0	23.6	37.1
14:0	5.1	6.3
16:0	19.8	17.3
16:1n7	6.3	7.6
18:0	6.5	2.0
18:1n9	22.7	18.8
18:2n6	6.8	5.9
18:3n3	0.0	0.5
18:4n3	0.0	0.5
20:5n3	0.1	3.5
22:5n3	0	0.35
22:6n3	0	1.7

[1]All values are reported as means of duplicate analyses.

Rainbow Trout Feeding Trial

All experimental animal protocols were conducted in accordance with the guidelines of the University of Idaho's Animal Use and Care Committee and Idaho State University's Animal Care Committee. Mixed-sex rainbow trout from the College of Southern Idaho Fish Hatchery, Twin Falls, Idaho, USA (House Creek strain) were counted in groups of 10 fish, bulk-weighed, and stocked into 150-L fiberglass tanks. The average initial bulk weight of the tanks was 1458 g (±34). Tanks were supplied with 4–6 L/min of untreated, constant temperature (14.5 C) spring water at the Hagerman Fish Culture Experiment Station. Fish were fed to apparent satiation two times per day, 6 d/wk for 8 wk. The amount of feed provided to each tank of fish was measured and recorded daily. Fish were fed all they would consume in 20 min, three times per day. Each diet was fed to three replicate tanks of trout, and the assignment of diets among tanks was allocated using a randomized design. Fish were bulk-weighed and counted every 2 wk, and weight gain and feed conversion ratios (FCR = total feed fed [dry matter basis]/weight gained) were calculated.

TABLE 3. *Ingredients and proximate composition of the control diet containing anchovy fish meal and experimental diets containing normal and enriched black soldier fly (BSF),* Hermetia illucens*, prepupae for rainbow trout.*

Ingredients (% dry weight basis)	Diets				
	Control diet	Normal BSF (25%)	Normal BSF (50%)	Enriched BSF (25%)	Enriched BSF (50%)
Fish meal (anchovy)[1]	29.07	21.80	14.54	21.80	14.54
Normal BSF	0	16.4	32.80	0	0
Enriched BSF	0	0	0	18.12	36.24
Corn gluten meal[1]	7.0	7.0	7.0	7.0	7.0
Soybean meal[1]	16.0	16.0	16.0	16.0	16.0
Wheat gluten meal[1]	7.8	7.8	7.8	7.8	7.8
Gem gel[1]	14.19	7.46	0.70	10.12	6.03
Fish oil[1]	2.60	3.20	2.60	3.20	3.80
Poultry fat[1]	14.75	11.8	10.06	7.52	0.29
Vitamin premix[2]	1.5	1.5	1.5	1.5	1.5
Choline chloride[1]	0.5	0.5	0.5	0.5	0.5
Stay-C 35[1]	0.3	0.3	0.3	0.3	0.3
Trace mineral mix[3]	0.1	0.1	0.1	0.1	0.1
Dicalcium phosphate	2.55	2.55	2.55	2.55	2.55
Taurine	0.50	0.50	0.50	0.50	0.50
Lysine HCl	2.33	2.30	2.28	2.25	2.16
Threonine	0.58	0.50	0.43	0.47	0.37
DL-methionine	0.23	0.29	0.35	0.27	0.32
Analyzed composition[4] (SE)					
% Crude protein	46.0 (±0.14)	48.5 (±0.02)	50.4 (±0.19)	51.2 (±0.19)	52.5 (±0.32)
% Lipid	20.9 (±0.17)	20.5 (±0.04)	21.3 (±0.11)	17.5 (±0.12)	19.2 (±0.02)
Gross energy (kcal/g)	5450 (±20)	5524 (±50)	5445 (±10)	5357 (±50)	5579 (±80)
% Ash	7.4 (±0.03)	9.8 (±0.07)	12.2 (±0.04)	8.8 (±0.42)	9.2 (±0.07)
% Moisture	4.9 (±0.19)	7.0 (±0.04)	8.4 (±0.20)	7.9 (±0.28)	9.4 (±0.08)

[1]Origin of ingredients: anchovy meal, corn meal, soybean meal, fish oil, and vitamin C were from Rangen, Buhl, ID, USA. Gem gel (pregelatinized wheat starch) and choline chloride were obtained from Nelson & Sons, Murray, UT, USA.

[2]Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D_3, 720 IU; vitamin E (as DL-%-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 B_{12}, 30 ug.

[3]Contributed in milligram per kilogram of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3.

[4]Means of two replicate samples (±SE) per diet on an as-fed basis.

The individuals feeding the fish were blinded to the diets being fed to the fish.

At the end of the 8-wk feeding trial, three fish per tank were bled and then individually weighed, liver and intraperitoneal fat removed, and hand-filleted for determination of hepatosomatic index, intraperitoneal fat ratio and muscle ratio, respectively. The muscle portion obtained was subsequently utilized for determination of proximate and fatty acid composition as described below. Plasma was stored at −20 C until amino acid determination.

Fish remaining after sampling were pooled by tank, euthanized, and immediately transported on ice to a commercial fish processing facility (Idaho Fish Processors, Hagerman, ID, USA). At the processing facility, fish were individually hand-filleted as gourmet fillets with ribs and pin bones removed. Following filleting, samples were pooled by tank and stored at −20 C until shipment (ca. 1 wk) to Washington State University, Pullman, Washington, USA (WSU) for sensory evaluation.

Sensory Evaluation

Frozen trout fillet prepared from trout that were fed different protein diets were shipped on dry ice overnight from Hagerman, Idaho, USA to WSU. Samples arrived frozen and in good condition. Treatments were identified as: control (A), BSF 25 (B), BSF 50 (C), EBSF 25

(D), and EBSF 50 (E). Fillets had been boned, but not skinned and were packaged in zip closure bags (not vacuum packed). Fillets were stored at −23 C until analysis (ca. 3 wk) at WSU Food Science and Human Nutrition in Pullman, Washington, USA.

Fillets were partially thawed, just enough to cut with a knife. About 10 mm was removed from the head and ventral, and then 9.0 ± 1.0 g serving portions were sliced across the fillet from dorsal to ventral. The skin was peeled from the meat while the tissue was still frozen. Frozen fillet portions (six to nine pieces) were placed in 25 × 30 cm boil-in pouches. Samples in pouches were vacuum packed using the Ultravac 250 sealer (Koch Equipment, Kansas City, MO, USA). Vacuum-packed fillet samples were immediately returned to −23 C until sensory analysis.

Vacuum-packed frozen fillet samples were thawed in room temperature water prior to poaching. Samples were poached for 6 min at 74 C in a Hobart model CF 11 water bath, then removed from the boiling pouch and held under radiant heat (Hatco Glo-Ray Food Warmer Model CRAH-48, Hatco Corporation, WI, USA) for a maximum of 15 min before serving to panelists.

Poached trout fillet was first evaluated by an informal panel to determine if there was a notable difference in fillets from trout raised in different tanks (blocks) but fed the same diet. Five panelists (untrained and experienced) evaluated the poached trout for differences within the three blocks of each of five treatments. In a blind test, panelists did not consistently identify differences between treatment blocks, so blocks were combined for formal sensory tests.

Thirty volunteers were recruited to serve on the formal panel. Panelists were from a diverse ethnic background and most were from the WSU community. The panel consisted of 14 males and 16 females ranging in age from 18 to 65.

Sensory testing was conducted in the WSU Food Science sensory laboratory and data were collected and analyzed using Compusense 5 software (Compusense, Inc., Guelph, Ontario, Canada). Treatment comparisons were presented in random order. Samples were served under red lights to minimize the influence of appearance of the cooked fillet. Panelists were provided with water and unsalted top saltine crackers for rinsing the palate between samples.

In a triangle difference test, 30 consumers (untrained volunteers) were asked to indicate the odd sample in a set of three coded samples. Panelists were asked to comment on why they selected a particular sample as different from the other two to aid in determining what attributes may contribute to perceived differences. The level of significance was set at $P < 0.05$. Results were analyzed using the tables of Roessler et al. (1978).

Proximate Composition Analysis

Muscle samples were pooled by tank, ground for homogeneity, and frozen at −20 C until analysis. Muscle and diet samples were dried, and analyzed in duplicate assays using standard AOAC (1995) methods for proximate composition. Protein was calculated from sample nitrogen content determined using a LECO TRUSPEC nitrogen analyzer (TruspecN, Leco Corporation, St. Joseph, MI, USA) and lipid using a Foss Tecator Soxtec HT Solvent Extractor (Model Soxtec HT6, Höganäs, Sweden). Gross energy was determined by adiabatic bomb calorimetry (Parr 1281, Parr Instrument Company, Inc., Moline, IL, USA).

Amino Acid Analysis

Plasma and ingredient amino acids were quantified according to Fleming et al. (1992) utilizing an Agilent 1100 series HPLC. To determine ingredient amino acids, samples were capped with nitrogen and hydrolyzed in 6 M HCl at 110 C for 16 h (AOAC 1995). For plasma amino acids, plasma proteins were precipitated with 1.5 M perchloric acid, followed by centrifugation at 17,500×g for 5 min. All samples were derivatized with *o*-phthaldialdehyde (P0532, Sigma-Aldrich Co., St. Louis, MO, USA) immediately prior to injection on a 5-μm Agilent Hypersil AA ODS column (part number 79916AA-572;

Agilent Technologies, Palo Alto, CA, USA) using an automated injection sequence. Separation conditions were as described by Fleming et al. (1992).

Fatty Acid Analysis

BSF prepupae (normal and enriched), fish fillets, oil sources, and the five diets were freeze dried before lipids were extracted using chloroform : methanol (2:1) (Clark et al. 1982). Direct methylation of extracted lipids from the prepupae was conducted (Kramer et al. 1997). Lipids from the fish fillets and the diets were methylated using base-catalyzed transesterification (Christie 1982) with a reaction time of 10 min. The fatty acid methyl esters (FAMEs) were analyzed on a gas chromatograph (Hewlett-Packard 6890 Series with auto injector) fitted with a flame ionization detector and a 100 m × 0.25 mm, with 0.2-µm film capillary column coated with CP-Sil 88 (Chrompack, Middelburg, The Netherlands). Initially, the oven temperature was 70 C (for 3 min) and then increased to 175 C at a rate of 3 C/min and held for 3 min. Oven temperature was then increased to 185 C at a rate of 1 C/min and held for 20 min, increased to 215 C at a rate of 3 C/min, and then increased to 230 C at a rate of 10 C/min and held for 5 min. Response correction factors determined by the analysis of a butter oil standard with certified values (CRM 164; European Community Bureau of Reference, Brussels, The Netherlands) were used to quantify fatty acids.

Statistical Analyses

The PROC MIXED procedure, SAS Software Version 7.00 (SAS Institute, Inc., Cary, NC, USA) was used to conduct an analysis of variance for a mixed effects model (Ott 1977) in which diet was defined as a fixed effect and tanks within treatments were defined as a random effect. Binomial data were transformed using the arcsine transformation prior to analysis. Differences among treatment means were determined using the Tukey procedure for pair-wise comparisons. Treatment effects in all statistical analyses in this project were considered different when P values are less than 0.05.

Results

Rainbow Trout Feeding Trial

Analyzed composition of the test diets generally reflected formulation goals and analyzed content of dietary ingredients (Tables 3 and 4, respectively). Growth of fish fed the EBSF

TABLE 4. *Analyzed selected fatty acid composition of the control diet containing anchovy fish meal and experimental diets containing normal and enriched black soldier fly (BSF),* Hermetia illucens.[1]

Fatty acids (%) total	Diets: Control diet	Normal BSF (25%)	Normal BSF (50%)	Enriched BSF (25%)	Enriched BSF (50%)
12:0	0.22	3.08	6.67	8.98	16.92
14:0	2.76	3.50	3.35	4.77	5.62
16:0	20.91	20.84	20.55	18.72	16.95
16:1n7	5.85	6.31	5.83	6.60	7.03
18:0	5.35	5.33	5.65	3.60	2.59
18:1n7t	0.48	0.39	0.27	0.52	0.44
18:1n9	28.00	25.69	25.83	19.12	15.75
18:1n7	2.20	1.99	1.76	2.01	1.74
18:2n6	12.77	11.26	10.33	8.46	6.28
18:3n3	1.55	1.41	1.23	1.28	1.05
18:2n6t	0.41	0.38	0.32	0.47	0.45
20:4n6	0.64	0.57	0.46	0.73	0.66
20:5n3	4.47	4.32	3.15	7.12	6.73
22:6n3	2.01	1.80	1.31	2.92	2.51

[1]All values are reported as means of duplicate analyses.

TABLE 5. *Growth performance of rainbow trout fed a control diet containing anchovy meal and experimental diets containing enriched and normal black soldier fly (BSF),* Hermetia illucens, *prepupae pupae for 8 wk.*[1]

	Fish groups						
	Control diet	Normal BSF (25%)	Normal BSF (50%)	Enriched BSF (25%)	Enriched BSF (50%)	Pooled SEM	Pr > *F*
Weight gain[2] (% increase)	123[a]	85[b]	93[b]	104[ab]	102[ab]	6.60	0.0211
Feed conversion ratio[3]	1.2	1.0	1.0	1.0	1.1	0.06	0.1748
Feed consumption[4]	1.7	1.6	1.7	1.7	1.7	0.05	0.6716
Muscle ratio[5]	51.4	53.9	51.2	52.6	52.7	1.69	0.3544
Intraperitoneal fat ratio[6]	1.9[a]	1.4[b]	1.3[b]	1.1[b]	1.2[b]	0.29	0.0138
Hepatosomatic index[7]	1.3[a]	1.1[b]	1.1[b]	1.2[ab]	1.2[ab]	0.09	0.0138

[1]Means of three replicate tanks (10 fish/tank). Values within rows with different superscript symbols differ significantly at $P \leq 0.05$ based on the Tukey multiple comparison test.

[2]Final average fish weight – initial average fish weight/initial average fish weight × 100.

[3]Feed conversion ratio = grams of feed fed (dry)/grams of weight gained (wet).

[4]Feed consumption = grams feed consumed per 100 g body weight per day.

[5]Muscle ratio = fillet weight × 2/total weight × 100.

[6]Intraperitoneal fat ratio = intraperitoneal fat weight/total weight × 100.

[7]Hepatosomatic index = liver weight/total weight × 100.

diets was lower but not significantly different from those fish fed the fish meal-based control diet, while the growth of fish fed the BSF diets was reduced as compared to the control diet (Table 5). Hepatosomatic index displayed a similar trend. Intraperitoneal fat ratio was significantly decreased in fish fed diets containing BSF prepupae. In contrast, neither feed conversion ratios which ranged from 1.0 to 1.3 nor feed consumption which ranged from 1.6 to 1.7 was significantly altered by diet. Muscle ratio was similarly unaffected by diet.

Proximate Composition Analyses

Muscle moisture and lipid composition were significantly altered by replacement of dietary fish meal with BSF prepupae (Table 6). Fish fed diets containing BSF prepupae had significantly greater muscle moisture and lower lipid than those fish fed the control diet. No significant effects of diet were observed for muscle protein, energy, or ash content.

No significant differences in plasma amino acid content were observed for any of the examined amino acids (Table 7). In contrast, muscle fatty acid profile was significantly altered by diet in a fatty acid-specific manner (Table 8). Lauric acid content was increased in fish fed all BSF diets, whereas only fish fed the EBSF diets had similar linoleic, eicosapentaenoic acid, and docosahexaenoic acid content compared to the controls (Table 8).

TABLE 6. *Muscle proximate composition of rainbow trout fed a control diet containing anchovy meal and experimental diets containing normal and enriched black soldier fly (BSF),* Hermetia illucens, *prepupae pupae for 8 wk.*[1]

	Fish groups						
	Control diet	Normal BSF (25%)	Normal BSF (50%)	Enriched BSF (25%)	Enriched BSF (50%)	Pooled SEM	Pr > *F*
Moisture (%)	73.2[b]	75.9[a]	75.4[a]	75.6[a]	75.1[a]	0.48	0.0157
Protein (%)	21.7	21.7	22.5	22.2	23.3	0.52	0.2886
Lipid (%)	4.7[a]	3.4[b]	2.3[b]	3.0[b]	2.4[b]	0.42	0.0145
Ash (%)	1.6	1.6	1.6	1.6	1.6	0.08	0.8547
Energy (kcal/g)	1653[a]	1426[b]	1476[b]	1494[b]	1484[b]	48	0.0659

[1]Means of three replicate tanks. Values within rows with different superscript symbols differ significantly at $P \leq 0.05$ based on the Tukey multiple comparison test.

TABLE 7. *Analyzed amino acid composition of plasma of rainbow trout fed a control diet containing anchovy meal and experimental diets containing normal and enriched black soldier fly (BSF),* Hermetia illucens*, prepupae pupae for 8 wk.*[1]

	Fish groups						
Amino acids (nmol/mL)	Control diet	Normal BSF (25%)	Normal BSF (50%)	Enriched BSF (25%)	Enriched BSF (50%)	Pooled SEM	Pr > F
Alanine	410.3	343.6	389.2	473.6	451.9	42.4	0.3417
Arginine	155.4	161.7	150.9	132.6	144.8	17.3	0.8515
Asparagine	77.2	85.1	81.5	93.2	83.4	14.4	0.9661
Aspartic acid	14.4	18.5	16.9	16.9	21.5	3.3	0.6525
Glutamate	67.1	69.6	109.5	64.5	81.3	22.5	0.6435
Glutamine	296.6	320.0	320.5	278.9	263.4	56.8	0.9349
Glycine	534.4	522.8	443.2	410.7	573.8	73.8	0.6001
Histidine	90.1	115.0	97.1	87.7	90.5	10.0	0.3872
Isoleucine	217.7	231.8	282.3	183.6	183.3	21.8	0.0642
Leucine	369.5	426.8	505.9	359.4	361.4	39.3	0.1201
Lysine	376.4	395.5	348.3	341.6	360.5	42.0	0.9063
Methionine	139.1	194.0	165.1	192.8	219.8	31.1	0.4650
Phenylalanine	144.9	163.1	145.0	155.3	205.1	18.0	0.1939
Serine	79.81	84.19	74.52	80.51	87.06	12.0	0.9547
Taurine	912.2	823.2	1489.8	726.2	976.1	501.1	0.8521
Threonine	277.9	357.3	318.6	258.8	295.0	37.6	0.4940
Tyrosine	67.6	76.6	71.7	75.4	84.5	12.4	0.8927
Valine	581.6	614.6	729.7	529.2	490.7	52.5	0.0819
Sum	4812.1	4997.3	5739.8	4460.8	4974.3	650.3	0.7656

[1]Means of three replicate tanks. Values within rows with different superscript symbols differ significantly at $P \leq 0.05$ based on the Tukey multiple comparison test.

TABLE 8. *Selected fatty acid composition of muscle fillets of rainbow trout fed a control diet containing anchovy meal and experimental diets containing normal and enriched black soldier fly (BSF),* Hermetia illucens*, prepupae pupae for 8 wk.*[1]

	Fish groups						
Fatty acids total (%)	Control diet	Normal BSF (25%)	Normal BSF (50%)	Enriched BSF (25%)	Enriched BSF (50%)	Pooled SEM	Pr > F
12:0	0.06[e]	0.61[d]	1.31[c]	2.35[b]	4.01[a]	0.16	<.0001
14:0	2.01[c]	1.86[c]	1.90[c]	2.57[b]	2.84[a]	0.08	<0.0001
16:0	16.81[a]	14.41[b]	12.90[b]	14.41[b]	13.41[b]	0.69	0.0020
18:0	4.77[a]	3.65[c]	3.57[c]	3.89[b]	3.55[c]	0.08	<0.0001
18:1n7t	0.20	0.19	0.20	0.21	0.24	0.02	0.5290
18:1n9	28.56[a]	27.29[a]	28.26[a]	25.73[b]	22.36[c]	0.48	<0.0001
18:1n7	2.87[b]	2.86[b]	2.85[b]	3.03[a]	2.88[b]	0.04	0.0407
18:2n6	10.15[a]	10.32[a]	8.79[ab]	8.04[b]	7.04[b]	0.60	0.0013
18:3n3	1.53[b]	1.85[a]	1.81[a]	1.72[ab]	1.63[ab]	0.08	0.0235
18:2 n6t	1.15	1.11	1.20	1.21	1.15	0.04	0.3303
20:4n6	0.90[c]	1.09[a]	1.02[abc]	0.96[c]	1.04[ab]	0.04	0.0159
20:5n3	2.64[c]	3.18[b]	2.59[c]	3.62[a]	3.95[a]	0.14	<0.0001
22:6n3	8.29	11.8	11.0	10.50	11.52	0.96	0.0982

[1]Means of three replicate tanks. Values within rows with different superscript symbols differ significantly at $P \leq 0.05$ based on the Tukey multiple comparison.

Sensory Testing

A group of 30 untrained panelists did not detect a significant difference in a blind comparison of fish fed the fish meal containing control diet as compared to any of the experimental dietary treatments (Table 9). Fifteen correct choices were required for significance at $P \leq 0.05$.

TABLE 9. *Panelist responses for triangle difference test of muscle fillets from rainbow trout fed a control diet containing anchovy meal and experimental diets containing normal and enriched black soldier fly (BSF),* Hermetia illucens, *prepupae pupae for 8 wk.*[1]

	Dietary comparisons			
Reponses	Control versus normal BSF (25%)	Control versus normal BSF (50%)	Control versus enriched BSF (25%)	Control versus enriched BSF (50%)
Incorrect	20	19	18	16
Correct	10	11	12	14
Total	30	30	30	30
Significance (P-value)	0.568	0.415	0.276	0.090

[1]Fifteen correct responses required for significance at $P < 0.05$ ($N = 30$ panelists).

Discussion

The search for sustainable ingredients to replace fish meals has been ongoing for the rainbow trout industry. In Idaho, one product of interest is BSF prepupae due to its ability to ameliorate dairy waste disposal as well as potentially providing a valuable source of nutrients for rainbow trout. BSF larvae or prepupae have been fed experimentally to several animal species including cockerels, *Gallus domesticus* (Hale 1973); pigs, *Sus scrofa domestica* (Newton et al. 1977); catfish, *Ictalurus punctatus* (Bondari and Sheppard 1981); and tilapia, *Oreochromis aureus* (Bondari and Sheppard 1987).

Previous research in our laboratory also indicated that potential exists for the utilization of BSF prepupae as a feed ingredient for trout (St-Hillare et al. 2007a, 2007b), although some nutritional limitations were identified. In the present feeding trial, growth and compositional analyses of the fish fed the BSF diets showed similar responses to increasing levels of prepupae inclusion as were observed in the previous trial. The reduction in growth did not appear to be due to reduced feed consumption or feed conversions. Reduced feed consumption often is responsible for reduced growth rates when many alternative ingredients are utilized in lieu of fish meal (Gatlin et al. 2007), but neither feed consumption nor conversion efficiency was affected by BSF prepupae inclusion levels in the present trial. Alternatively, the differences may be attributable to decreased nutrient availability in the BSF prepupae. Lending support for this hypothesis is the lower muscle lipid and intraperitoneal fat observed in fish fed the BSF or EBSF diets relative to fish fed the fish meal diet. However, in contrast to fish fed BSF diets, reduced growth performance was not observed in fish fed the EBSF diets. This reduction suggests that enrichment of the BSF prepupae with trout offal increased nutrient availability such that fish fed the EBSF diets displayed more lean growth than fish fed the fish meal diet. However, digestibility studies are necessary to fully dissect the reasons for altered energy storage.

A limitation of our previous trial was that diets were formulated to be isonitrogenous, thereby effectively reducing the levels of digestible protein or, more specifically, available amino acids of fish fed the BSF prepupae diets relative to those fed the fish meal diet due to the BSF prepupae chitin content (Sheppard et al. 1994). Chitin, an unbranched polymer of N-acetylglucosamine, is a primary component of invertebrate exoskeletons and indigestible to many fish species because they lack chitonase activity (Rust 2002). For this reason, care was taken in the current trial to balance for reported dietary amino acids needs and mimic ideal amino acid profile for rainbow trout (Wilson and Cowey 1984; NRC 1993) when fish meal protein was replaced with either normal or enriched BSF prepupae. Plasma amino acid profiles can be used to verify dietary sufficiency (Gaylord and Barrows 2008) and indicate that equivalent levels of dietary amino acids were available in the current study. Thus, the observed growth reductions were most likely not attributable to amino acid deficiencies in the BSF diets.

Fatty acid content of the diets differed depending on both prepupae inclusion level and enrichment protocol (Table 8) as was expected given the analyzed fatty acid profiles of the ingredient (Table 2). It is by and large observed that fish fatty acid profiles generally reflect dietary fatty acids content (Turchini et al. 2009). Results from this study are in general agreement with this dogma in that differences in the fatty acid composition of muscle lipids reflected differences in diet; however, observed muscle fatty acid concentrations were attenuated relative to dietary differences. For example, dietary lauric acid ranged from 0.22 to 16.9% of the dietary lipids, but only ranged from 0.06 to 4.0% of the muscle fatty acids. Higher concentrations of docosahexaenoic acid were detected in the muscle fatty acids relative to the diet, while eicosapentaenoic acid appeared reduced in muscle fatty acids compared to dietary concentrations. The reasons for the differences between fed and retained fatty acid levels are unclear as trout appear to have limited ability to elongate and desaturate dietary fatty acids into DHA (Hardy 2002). Other researchers have reported similar results with similar fatty acid sources (Ballestrazzi et al. 2006).

Another potential explanation for the observed differences in growth between the BSF prepupae fed fish is that the increased lauric acid content in the EBSF diets may have allowed for increased lipid oxidation of the lauric acid as an energy substrate. Medium chain-length saturated fatty acids supplied through coconut oil (43% lauric acid) have been shown to be viable energy sources for many fish species including red drum, *Sciaenops ocellatus;* African catfish, *Heterobranchus lonifilis*; and common carp, *Cyprinus carpio* L. (Craig and Gatlin 1995; Legendre et al. 1995; Fontagné et al. 1999). More recently, Ballestrazzi et al. (2006) reported that rainbow trout fed up to 13% coconut oil as a replacement for fish oil maintain growth performance when fish meal levels were maintained at 23%. Olsen et al. (1998) reported that, in Arctic charr, *Salvelinus alpinus*, the lauric acid present in coconut oil was more efficiently absorbed than other longer saturated fatty acids in the pyloric caeca and gut. Thus, the rate of absorption of lauric acid in the present study may have increased energy utilization and thus improved performance of fish fed the EBSF diets. However, other physiological mechanisms also likely play a role. Buhler et al. (1995) reported that trout microsomes preferentially hydroxylate (oxidize) lauric acid at a much higher rate than other longer-chain saturated fatty acids and may, at least in part, explain why fish fed the BSF diet did not perform as well as fish fed the EBSF diet.

The value of an alternative ingredient cannot be simply defined by its ability to maintain growth because the market value of fish cultured for human consumption depends, in large part, on the perceived quality (Amberg and Hall 2008). In the current study, fish muscle proximate composition, specifically lipid content, and fatty acid profile differed substantially between the dietary treatments as previously described. Modifications of fillet lipid and fatty acid concentrations directly affect the total volatile compounds present and thus fish aroma and flavor (Turchini et al. 2003). It is therefore somewhat surprising that consumers participating in a blind taste test were unable to differentiate between fish fed the BSF prepupae and those fed the fish meal control diet. Although not statistically significant, fish fed the EBSF 50% diet yielded 14 correct choices approaching the required 15 for significance. A review of comments indicated that the panelists were divided in their reasons for choosing the odd sample as different. Consumer comments ranged from statements such as "milder taste" to "tastes fishier than the other." Fresh fish flavor is reported to be due to volatile aldehydes and alcohols, which are mainly derived from the oxidative deterioration of LC-PUFA (Kawai 1996); thus, the increased level of eicosapentaenoic acid in the muscle of fish fed the EBSF diets relative to the control diet may explain the taste test results. Alternatively, the drastically different muscle lauric acid concentrations may have contributed to the lack of significant differences between the groups. Volatile compound analyses of brown trout fillets from fish fed a diet with the refined palm oil, oleine oil, identified the presence of dodecanoic acid or lauric acid as its primary volatile compound (Turchini et al. 2003). Lauric acid is described as a faint odor of bay oil or soap. However, the lauric acid hypothesis is weakened by the fact that in the present study, none of the panelists used either of these descriptors or their derivates as a criterion for choosing the EBSF fed fillets as different. Alternatively, the detailed comments may simply reflect differences in individual consumer opinion and preference. Previous research examining the effects of alternative ingredients on fillet quality has shown that although fillet color and oxidative stability were altered when fish meal was replaced with soybean meal, no discernable differences were noted for taste panel acceptability of the products (D'Souza et al. 2006).

Conclusions

Research on fish meal and fish oil replacements has identified a variety of potential ingredients that when used in appropriate mixtures can promote good growth results for rainbow trout. The present study indicates that BSF prepupae reared on dairy cattle manure and trout offal can be used to replace up to 50% of fish meal portion of a practical trout diet for 8 wk without significantly affecting fish growth or the sensory quality of rainbow trout fillets. However, the reduced growth observed in the current study, albeit nonsignificant, indicates that additional research is needed to further identify nutritional limitations of this ingredient.

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