

## Fly Prepupae as a Feedstuff for Rainbow Trout, *Oncorhynchus mykiss*

SOPHIE ST-HILAIRE<sup>1</sup>

*Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209 USA*

CRAIG SHEPPARD

*Department of Entomology, University of Georgia, Tifton, Georgia 31794 USA*

JEFFERY K. TOMBERLIN

*Texas A & M University, Texas Cooperative Extension, Stephenville, Texas 76401 USA*

STEPHEN IRVING

*CEFAS, Weymouth Laboratory, Weymouth, Dorset DT4 8UB UK*

LARRY NEWTON

*Department of Animal & Dairy Science, University of Georgia, Tifton, Georgia 31794 USA*

MARK A. MCGUIRE AND ERIN E. MOSLEY

*Department of Animal & Veterinary Science, University of Idaho, Moscow, Idaho 83844 USA*

RONALD W. HARDY AND WENDY SEALEY

*Hagerman Fish Culture Experimental Station, University of Idaho, Hagerman, Idaho 83332 USA*

### Abstract

Fly larvae may provide an effective method to mitigate two large and growing global concerns: the use of fish meal derived from capture fisheries in aquaculture diets and manure management in livestock and poultry facilities. A 9-wk feed trial was conducted to determine whether fly larvae could be used as a partial fish meal and fish oil replacement in rainbow trout, *Oncorhynchus mykiss*, diets. A trout diet was formulated to contain 40% crude protein and 15% fat. Sixty-seven percent of the protein in the control diet was derived from fish meal, and all the fat was derived from fish oil. Two of the test diets included using the black soldier fly, *Hermetia illucens*, prepupae, which are 40% protein and 30% fat, as 25 and 50% replacement for the fish meal component of the control diet. The total protein derived from black soldier fly prepupae in these two test diets was 15 and 34%, respectively. A third test diet included using housefly, *Musca domestica*, pupae, which is 70% protein and 16% fat, as 25% replacement for the fish meal component of the control diet. Data suggest that a rainbow trout diet where black soldier fly prepupae or housefly pupae constitute 15% of the total protein has no adverse effect on the feed conversion ratio of fish over a 9-wk feeding period. In addition, the diet with black soldier fly prepupae permitted a 38% reduction in fish oil (i.e., from 13 to 8%); however, fish fed black soldier fly diets low in fish oil had reduced levels of omega-3 fatty acids in their muscle fillets. The findings from this study suggest that either the black soldier fly or the housefly may be a suitable feedstuff for rainbow trout diets.

Extensive research has been done on the potential for manure management in livestock facilities using the black soldier fly, *Hermetia illucens* (Sheppard et al. 1994, 1998; Newton et al. 2005). It has been estimated that this

nonpest fly species can reduce nitrogen and phosphorus waste by up to 75% and the mass of manure residue by 50% in poultry and swine systems (Sheppard et al. 1994, 1998; Newton et al. 2005). The by-product of this bioconversion system is an abundant amount of fly prepupae. These prepupae are approximately 40%

<sup>1</sup> Corresponding author.

protein and 30% fat (Sheppard et al. 1994, 1998; Newton et al. 2005), which makes for a suitable source of food for animals including commercially raised fish (Calvert et al. 1969; Newton et al. 1977; Bondari and Sheppard 1981). The market value of these prepupae is estimated as \$330/1000 kg (Newton et al. 2005).

Practical-type rainbow trout, *Oncorhynchus mykiss*, diets generally consist of approximately 40% protein and 20% fat (Hardy 2002). In many cases, more than 50% of the protein and fat in these diets is derived from wild-caught fish. Feed requirements for an expanding global aquaculture industry place pressure on capture fisheries around the world (Millennium Ecosystem Assessment 2005). In 2003, the Food and Agricultural Organization estimated that 52% of all fish meal and 86% of all fish oil harvested were used in fish diets (Tacon et al. in press). Commensurate with the increase in demand and price for this natural resource is the need to find alternatives for commercial aquaculture diets.

Research into alternate sources of protein and fat has focused on plant-based oils and proteins. Soybean meal is the most commonly utilized alternate protein source, comprising between 10 and 50% of the protein in the diet (Tacon and Akiyama 1997; Hardy 2002). The nutritional requirements of carnivorous fish, such as rainbow trout, have made it difficult to completely eliminate the use of fish meal and fish oil in diets without compromising growth or utilizing cost-prohibitive plant protein concentrates. Total replacement of fish meal as the protein component of diets may be problematic because of the high sensitivity of carnivorous species to dietary imbalances, antinutritional factors present within plant meals (Francis et al. 2001), and palatability problems (Papatryphon and Soares 2001).

Total replacement of fish oil has also not been feasible, especially with carnivorous species because of their specific dietary requirements for long-chain unsaturated fatty acids. Partial replacement of fish oil has been reported using plant and animal oils (Koshio et al. 1994; Balfry et al. 2002); however, more research is required concerning the use of finishing diets to manipulate the final tissue fatty acid profile and

product quality (Bell et al. 2004; Morris et al. 2005).

Limited work has been done on the use of insects for fish meal and fish oil replacement in rainbow trout diets, despite the fact that insects make up part of their natural diet (Angradi and Griffith 1990). The objective of this preliminary study was to determine if black soldier fly prepupae and housefly, *Musca domestica*, pupae could be used as a partial replacement for fish meal and fish oil in rainbow trout diets.

## Materials and Methods

### *Diet Formulation*

Black soldier fly prepupae and domestic housefly pupae were used as a partial protein replacement in rainbow trout diets. Black soldier fly prepupae were reared on swine manure as described by Newton et al. (2005) in "Swine System Two." Briefly, larvae were reared on passively dewatered manure directly under the pigs. Prepupae were collected with a ramp and gutter system as they migrated away from the manure to pupate. These were collected between July and September 2004 and dried in a forced-air electric drying oven at 80 C. The dried product was stored in paper bags in an unheated, uncooled shed until May 2005 when they were shipped to Idaho State University, Pocatello, ID. Then, the prepupae were transported frozen to the Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, ID.

Housefly pupae were grown by a commercial manufacturer Skipio's™ (Tillamook, OR, USA) on cow manure, harvested as pupae, and shipped live to the Hagerman Fish Culture Experiment Station, University of Idaho. Upon arrival, both black soldier fly prepupae and housefly pupae were further processed by freeze grinding in liquid nitrogen and drying at 40 C for 36 h.

Proximate composition of the flies and other protein feedstuffs used in this trial was analyzed (Table 1) to assist in diet formulation (Table 2). Samples were dried and analyzed in duplicate assays using standard AOAC (1995) methods for proximate composition, with the exception of protein and crude lipid. Protein was calculated from sample nitrogen content determined

TABLE 1. Proximate composition of the protein feedstuffs evaluated in this study.<sup>a</sup> Percentages are reported on a dry-matter basis (standard error).

	Feedstuff				
	Fish meal (anchovy)	Black soldier fly prepupae	Domestic housefly pupae	Corn gluten meal	Soybean meal
% Dry matter	91.5 (0.1)	91.6 (0.1)	88.1 (0.4)	88.7 (0.1)	88.1 (0.01)
% Crude protein	72.5 (0.1)	43.6 (0.2)	70.4 (1.8)	64.0 (0.01)	54.4 (0.2)
% Lipid	12.0 (0.1)	33.1 (0.1)	16.1 (0.3)	5.7 (0.2)	3.7 (0.1)
% Ash	19.2 (0.01)	15.5 (0.03)	9.8 (0.1)	2.2 (0.1)	7.4 (0.01)

<sup>a</sup> Means of two replicate analyses per feedstuff on a dry-matter basis.

using a nitrogen analyzer (TruSpec N; LECO Corporation, St. Joseph, MI, USA) and lipid using a Foss Tecator Soxtec HT Solvent Extractor, Model Soxtec HT6 (Foss Tecator AB, Höganäs, Sweden). Gross energy was determined by adiabatic bomb calorimetry (Parr 1281; Parr Instrument Company Inc., Moline, IL, USA). Specific amino acids analyses were conducted by the AOAC-approved method described at [http://www.eurofinsus.com/Item.html\\_itemServices/Services%20\(2005-01-01.html](http://www.eurofinsus.com/Item.html_itemServices/Services%20(2005-01-01.html) (Eurofins US, Petaluma,

CA, USA), and the amino acid composition of the two fly ingredients is presented in Table 3.

A control diet (Hardy 2002) that met or exceeded all the known dietary requirements of rainbow trout (NRC 1993) was formulated with 40% protein and 15% fat (Table 2). Approximately 67% of the protein was derived from fish meal and the remaining 33% was derived from plant sources (Tables 1 and 2). For two of the test diets, 25 and 50% of the fish meal component were substituted for black soldier fly prepupae

TABLE 2. Ingredients and proximate composition of control diet containing anchovy fish meal and experimental diets containing black soldier fly, *Hermetia illucens*, prepupae and domestic housefly, *Musca domestica*, pupae for rainbow trout. Ingredients are reported as percentage of total diet on a dry-matter basis. Proximate composition is reported on a dry-matter basis (% fish meal replaced).

	Diets			
	Control diet	Housefly pupae (25%)	Black soldier fly prepupae (25%)	Black soldier fly prepupae (50%)
<b>Ingredients (%)</b>				
Fish meal (anchovy) <sup>a</sup>	36.0	27.0	27.0	18.0
Black soldier fly prepupae	0	0	14.9	29.8
Housefly pupae	0	9.2	0	0
Corn gluten meal <sup>a</sup>	8.0	8.0	8.0	8.0
Soybean meal <sup>a</sup>	16.0	16.0	16.0	16.0
Gem Gel <sup>TMa</sup>	24.6	24.6	23.4	22.2
Fish oil <sup>a</sup>	13.0	12.8	8.3	3.6
Vitamin premix <sup>b</sup>	1.5	1.5	1.5	1.5
Choline chloride <sup>a</sup>	0.5	0.5	0.5	0.5
Vitamin C <sup>a</sup>	0.3	0.3	0.3	0.3
Trace mineral mix <sup>b</sup>	0.1	0.1	0.1	0.1
<b>Analyzed composition<sup>c</sup> (SE)</b>				
% Crude protein	39.1 (0.1)	41.0 (0.2)	37.4 (0.2)	37.5 (0.1)
% Lipid	15.1 (0.2)	14.8 (0.1)	13.9 (0.2)	13.0 (0.3)
Gross energy (kcal/g)	5784 (44)	5910 (42)	5568 (6)	5606 (35)
% Ash	8.1 (0.2)	7.3 (0.03)	8.4 (0.02)	8.6 (0.7)
% Moisture	8.0	8.9	5.7	7.8

<sup>a</sup> Origin of ingredients: anchovy meal, corn meal, soybean meal, fish oil, and vitamin C were from Rangen, Buhl, ID, USA. Gem Gel<sup>TM</sup> and choline chloride were obtained from Nelson & Sons, Murray, UT, USA.

<sup>b</sup> Same as Cheng et al. (2003).

<sup>c</sup> Means of two replicate samples per diet.

TABLE 3. *Amino acid composition of black soldier fly, Hermetia illucens, prepupae and domestic housefly, Musca domestica, pupae. All percentages are reported on a dry-matter basis.*

Amino acid	Black soldier fly prepupae	Domestic housefly pupae
Alanine	3.02	3.10
Arginine	2.65	3.22
Aspartic acid	3.72	4.55
Glutamic acid	3.78	5.52
Glycine	2.28	3.04
Histidine	1.18	1.63
Isoleucine	2.03	2.26
Leucine	3.10	3.43
Lysine	2.62	3.38
Methionine	0.74	1.04
Phenylalanine	2.00	2.62
Proline	2.39	2.58
Serine	1.68	1.88
Threonine	1.78	2.30
Tyrosine	3.08	3.22
Valine	2.79	3.26
Total	38.85	47.06

(Table 2). The total protein in these two diets derived from fly prepupae was 15 and 34%, respectively. In the third test diet, 25% of the fish meal was substituted for housefly pupae (Table 2).

The total fat content was adjusted to approximately 15% using fish oil. Feeds were produced

by compression pelleting without steam using a laboratory-scale pellet mill (California Pellet Mill; San Francisco, CA, USA) at the Hagerman Fish Culture Experiment Station, Hagerman, ID. A proximate analysis, as described above for the protein feed ingredients, was conducted on each of the diets (Table 2), and fatty acid analyses were done on each of the diets at the end of the study (see Fatty Acid Analysis section) (Table 4).

#### *Rainbow Trout Feed Trial*

Mixed-sex rainbow trout from the College of Southern Idaho Fish Hatchery (House Creek strain) were counted in groups of 15 fish, bulk weighed, and stocked into 150-L fiberglass tanks. The average bulk weight of the tanks was 339.4 g (range 321.9–356.5 g). Tanks were supplied with 4–6 L/min of untreated, constant temperature (14.5 C) springwater at the Hagerman Fish Culture Experiment Station. Fish were fed to apparent satiation three times per day, 6 d/wk, for 9 wk. The amount of diet provided to each tank of fish was measured and recorded daily. Each diet was fed to four replicate tanks of trout, and the assignment of diets among tanks was done using a randomized design. Fish were bulk weighed and counted every 3 wk, and weight gain and feed conversion ratios (FCRs =

TABLE 4. *Selected fatty acid composition of black soldier fly, Hermetia illucens, prepupae (BSF) and domestic housefly, Musca domestica, pupae (HF) and four diets used in the feed trial expressed on a weight percent of total lipid (% fish meal replaced).*

Fatty acid	Feedstuff and diets					
	BSF raised on swine manure	HF raised on cow manure	Control diet	HF diet (25%)	BSF diet (25%)	BSF diet (50%)
12:0	49.34	0.18	0.11	0.13	17.1	33.3
14:0	6.83	2.56	8.76	8.38	8.29	7.35
16:0	10.48	26.40	20.05	20.74	17.17	14.03
16:1n7	3.45	13.56	10.26	10.53	7.83	5.20
18:0	2.78	4.77	3.69	3.79	3.48	3.24
18:1n9	11.81	19.17	6.7	7.58	8.24	9.7
18:2n6	3.68	17.83	3.95	5.32	4.84	5.56
18:3n3	0.08	0.87	2.33	2.27	0.60	1.04
18:4n3	0	0	0.02	0.19	0.13	0.06
20:4n6	0	0.07	1.47	1.23	0.86	0.40
20:5n3	0	0.05	12.5	11.45	8.34	3.85
22:4n6	0	0	0.61	0.56	0.39	0.18
22:5n3	0	0	2.38	2.18	0.10	0.69
22:6n3	0	0	10.32	9.10	7.02	3.61

total feed fed [dry-matter basis]/weight gained) were calculated. Mortality was monitored, and fish weights were adjusted for any fish that died. The individuals feeding the fish were blinded to the diets being fed to the fish.

Fish were sampled prior to the start ( $n = 10$ ) and at the end of the feed trial ( $n = 3/\text{tank}$ ) were sampled for determination of proximate composition as described above. At the end of the study, fatty acid analysis was done on three fish from each of the tanks.

#### *Fatty Acid Analysis*

Black soldier fly prepupae, housefly pupae, fish fillets, and the four fish 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 and housefly pupae 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 were analyzed on a gas chromatograph (Hewlett-Packard 6890 Series with auto injector, Agilent, Warwick, RI, USA) fitted with a flame ionization detector and a  $100\text{-m} \times 0.25\text{-mm}$ , with  $0.2\text{-}\mu\text{m}$  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, Belgium) were used to quantify fatty acids.

#### *Statistical Analyses*

The total kilocalorie fed to each treatment tank was calculated by multiplying the mean gross energy (kcal/g) by the total grams of diet fed to each tank. The FCR, weight gain, total kilocalorie fed, and mortality of fish on different diets were compared using a Kruskal–Wallis test. Total body lipid, gross energy, and protein

content of fish on different diets were compared using a Kruskal–Wallis test. The total lipid and the percentage of  $\alpha$ -linolenic acid (18:3n3; ALA), eicosapentaenoic acid (20:5n3; EPA), and docosahexaenoic acid (22:6n3; DHA) in the fillets of fish on different diets were compared using an ANOVA. Regression diagnostics were used to test the assumptions of parametric tests. When these were not met, the data were compared using a Kruskal–Wallis test. If the Kruskal–Wallis statistic or the ANOVA test was significant ( $P < 0.05$ ), then Tukey tests were used for multiple comparisons. All statistical tests were done using SPSS v.14.0 for Windows (SPSS, Inc., Chicago, IL, USA).  $P$  values of less than 0.05 were considered significant.

#### **Results**

The proximate analysis for the protein diet ingredients used in this study indicated that black soldier fly prepupae were higher in fat and lower in protein compared to the anchovy fish meal (Table 1). The housefly pupae were very similar to the fish meal in fat and protein content (Table 1). The amino acid profiles for the housefly pupae and black soldier fly prepupae used in the diets were similar (Table 3). The protein content of diets ranged between 41.0 and 37.4%, and the lipid content ranged between 13.0 and 15.1% (Table 2). The gross energy of the diets ranged between 5569 kcal/g for the 25% black soldier fly diet and 5910 kcal/g for the housefly diet (Table 2). This difference was not statistically significant ( $H = 6.17$ ,  $P = 0.104$ ). Over the 9-wk trial period, the fish in the control diet were fed approximately  $14.0 \times 10^6$  kcal ( $6.9 \times 10^5$ ). This was approximately  $2 \times 10^6$  kcal more than the other three diets; however, this difference was not statistically significant ( $H = 3.93$ ,  $P = 0.27$ ).

One fish, fed the diet with black soldier fly as 34% of the total protein (or 50% replacement of the fish meal), died during the course of the study ( $H = 3.0$ ,  $P = 0.392$ ). There was no significant difference in total weight gain between the fish fed the control diet and those fed the 25% black soldier fly diet; however, total weight gain for fish on diets containing 50% black soldier fly prepupae and 25% housefly pupae was

TABLE 5. Growth performance of rainbow trout fed a control diet containing anchovy meal and experimental diets containing black soldier fly, *Hermetia illucens*, prepupae (BSF) and domestic housefly, *Musca domestica*, pupae (HF) for 9 wk.<sup>1</sup> Feed intake and feed conversion ratio (FCR) are reported on a dry-matter basis (% fish meal replaced).

	Diet				Pooled SE	P value for Kruskal–Wallis test
	Fish meal diet (control)	HF (25%)	BSF (25%)	BSF (50%)		
Initial tank weight (g)	342 <sup>a</sup>	333 <sup>a</sup>	343 <sup>a</sup>	340 <sup>a</sup>	9.5	0.446
Final tank weight (g)	2396 <sup>a</sup>	2072 <sup>b</sup>	2159 <sup>ab</sup>	1899 <sup>b</sup>	143.5	0.027
Weight gain <sup>2</sup> (g)	2054 <sup>a</sup>	1739 <sup>b</sup>	1815 <sup>ab</sup>	1559 <sup>b</sup>	141.5	0.027
Total feed intake (g)	2427 <sup>a</sup>	2155 <sup>a</sup>	2217 <sup>a</sup>	2286 <sup>a</sup>	176.4	0.501
FCR <sup>3</sup>	1.18 <sup>a</sup>	1.22 <sup>a</sup>	1.22 <sup>a</sup>	1.47 <sup>b</sup>	0.07	0.033

<sup>1</sup> Means of four replicate tanks (15 fish/tank). Means within each row with different superscripts were found to differ at the  $P = 0.05$  probability level based on the Tukey multiple comparison test.

<sup>2</sup> Final tank weight – initial tank weight.

<sup>3</sup> FCR = gram of diet fed/gram weight gained.

lower than the control diet (Table 5). Diet intake was not affected by diets (Table 5). The FCRs of fish fed diets with 25% housefly pupae and 25% black soldier fly prepupae were not significantly different from one another or the control diet (Table 5). The fish fed the diet containing 50% black soldier fly prepupae as replacement for the fish meal component of the diet had a statistically higher FCR compared to all other diets (Table 5).

Fatty acid profiles suggest that fly larvae grown on manure do not have significant amounts of long-chain unsaturated fatty acids (Table 4). Fish fed diets with black soldier fly prepupae were slightly lower in total body lipid content ( $H = 11.93$ ,  $P = 0.008$ ; Table 6), and the lipid content of the fillets from fish fed the diet with 50% black soldier fly as protein

replacement was significantly lower than the fillet lipid content of the fish on the housefly diet ( $F = 3.19$ ,  $P = 0.033$ ; Table 7). However, the lipid content of fish fed the other two experimental diets was not significantly different from one another or the fish fed the control diet (Table 7). Key fatty acids were affected by diets with proportionally less ALA ( $F = 58.07$ ,  $P < 0.001$ ), EPA ( $H = 33.11$ ,  $P < 0.001$ ), and DHA ( $H = 45.92$ ,  $P < 0.001$ ) (Table 7) in fish fed the black soldier fly diets.

## Discussion

Data from this feed trial indicate that insects may be suitable partial replacement feedstuff for fish meal and fish oil in rainbow trout diets. Replacement of 25% of the fish meal component with black soldier fly prepupae reduced

TABLE 6. Whole-body proximate composition of rainbow trout fed a control diet containing anchovy meal and experimental diets containing black soldier fly, *Hermetia illucens*, prepupae (BSF) and domestic housefly, *Musca domestica*, pupae (HF) for 9 wk.<sup>1</sup> All values are reported on a wet basis (% fish meal replaced).

	Fish group				Pooled SE	P value for Kruskal–Wallis statistic
	Fish fed fish meal diet (control)	Fish fed HF pupae (25%)	Fish fed BSF prepupae (25%)	Fish fed BSF prepupae (50%)		
Moisture (%)	70.5 <sup>a</sup>	71.6 <sup>ab</sup>	72.6 <sup>b</sup>	73.1 <sup>b</sup>	0.79	0.02
Protein (%)	15.7 <sup>a</sup>	15.7 <sup>a</sup>	15.5 <sup>a</sup>	15.4 <sup>a</sup>	0.41	0.571
Lipid (%)	11.8 <sup>a</sup>	11.0 <sup>ab</sup>	9.9 <sup>b</sup>	9.3 <sup>b</sup>	0.69	0.008
Ash (%)	2.2 <sup>a</sup>	2.1 <sup>a</sup>	2.1 <sup>a</sup>	2.3 <sup>a</sup>	0.11	0.08
Energy (kcal/g)	6848 <sup>a</sup>	6805 <sup>a</sup>	6652 <sup>b</sup>	6537 <sup>b</sup>	70	0.006

<sup>1</sup> Means of four replicate tanks. Values within each row with different superscripts were found to differ at the  $P = 0.05$  probability level based on the Tukey multiple comparison test.

TABLE 7. Total lipid (% of total dry weight) and selected fatty acid composition of muscle fillets of rainbow trout fed different diets. Mean value (standard error) is based on 12 samples and is expressed on a weight percent of total lipid<sup>1</sup> (% fish meal replaced).

Fatty acid	Fish on control diet	Fish on HF diet (25%)	Fish on BSF diet (25%)	Fish on BSF diet (50%)
Total lipid	26.63 (1.29) <sup>ab</sup>	27.36 (0.95) <sup>b</sup>	24.98 (0.63) <sup>ab</sup>	23.4 (0.96) <sup>a</sup>
12:0	0.89 (0.76) <sup>a</sup>	0.14 (0.01) <sup>a</sup>	9.35 (0.48) <sup>b</sup>	14.2 (1.42) <sup>c</sup>
14:0	7.32 (0.12) <sup>a</sup>	6.97 (0.11) <sup>a</sup>	5.81 (0.11) <sup>b</sup>	5.6 (0.13) <sup>b</sup>
16:0	21.16 (0.34) <sup>a</sup>	19.73 (0.26) <sup>b</sup>	17.31 (0.16) <sup>b</sup>	16.1 (0.42) <sup>c</sup>
16:1n7	14.23 (0.31) <sup>a</sup>	15.15 (0.17) <sup>a</sup>	12.10 (0.21) <sup>b</sup>	10.6 (0.48) <sup>c</sup>
18:0	4.83 (0.09) <sup>a</sup>	4.75 (0.11) <sup>ab</sup>	4.55 (0.07) <sup>ab</sup>	4.5 (0.05) <sup>b</sup>
18:1c9	17.15 (0.44) <sup>a</sup>	17.81 (0.51) <sup>a</sup>	20.58 (0.49) <sup>b</sup>	23.6 (0.51) <sup>c</sup>
18:2n6	4.55 (0.17) <sup>a</sup>	5.66 (0.16) <sup>b</sup>	5.46 (0.07) <sup>b</sup>	6.0 (0.14) <sup>b</sup>
18:3n3	2.01 (0.03) <sup>a</sup>	2.11 (0.03) <sup>a</sup>	1.67 (0.03) <sup>b</sup>	1.4 (0.07) <sup>c</sup>
18:4n3	0.22 (0.01) <sup>a</sup>	0.21 (0.01) <sup>a</sup>	0.15 (0.004) <sup>b</sup>	0.13 (0.01) <sup>b</sup>
20:4n6	0.67 (0.02) <sup>a</sup>	0.67 (0.01) <sup>a</sup>	0.51 (0.02) <sup>b</sup>	0.32 (0.03) <sup>c</sup>
20:5n3	3.85 (0.17) <sup>a</sup>	4.02 (0.09) <sup>a</sup>	2.50 (0.15) <sup>b</sup>	1.22 (0.26) <sup>c</sup>
22:4n6	0.13 (0.005) <sup>a</sup>	0.13 (0.003) <sup>a</sup>	0.10 (0.004) <sup>b</sup>	0.065 (0.01) <sup>c</sup>
22:5n3	1.60 (0.06) <sup>a</sup>	1.57 (0.04) <sup>a</sup>	1.17 (0.12) <sup>b</sup>	0.73 (0.08) <sup>c</sup>
22:6n3	7.88 (0.22) <sup>a</sup>	7.51 (0.16) <sup>a</sup>	6.49 (0.22) <sup>b</sup>	4.39 (0.30) <sup>c</sup>

BSF = black soldier fly; HF = housefly.

<sup>1</sup> Means within each row with different superscripts were found to differ at the  $P = 0.05$  probability level based on the Tukey multiple comparison test.

the percentage of fish meal in the diet from 36 to 27% of the total diet with no adverse effect on the FCR of the fish. In addition, this permitted a 38% reduction in the fish oil component of the diet, which reduced the total percentage of fish oil in the diet from 13 to approximately 8%.

The growth and FCR data of the control fish in this study were consistent with previous studies in our laboratory using rainbow trout of approximately the same size and fed similar diets (Stone et al. 2005; Johansen et al. 2006). The altered growth efficiency of fish fed the diet with high (34% of the total protein) levels of black soldier fly prepupae has also been reported in studies on channel catfish, *Ictalurus punctatus*, and blue tilapia, *Oreochromis aureus* (Bondari and Sheppard 1981, 1987; Newton et al. 2005). It is unknown why the average FCR of fish on this diet was significantly higher than that of fish fed the other diets. It is possible that this diet contained more chitin than the other diets (given the higher levels of prepupae), and this may have affected its digestibility. Chitin, an unbranched polymer of *N*-acetylglucosamine, is a primary component of invertebrate exoskeletons and indigestible to many fish species because they lack chitinase activity (Rust

2002). The digestibility data to support this hypothesis were not collected.

The diets were formulated to contain equal amounts of protein and lipid, but retrospective proximate analysis suggested that slight differences existed in the gross energy, and protein and lipid content of the diets. Specifically, on a dry-matter basis, the control fish consumed approximately  $2 \times 10^6$  kcal more gross energy than the fish fed the experimental diets over the 9-wk trial period; thus, small differences in dietary formulations could have contributed to the altered FCR.

There was 2% less lipid (20% reduction) in fish fed lower levels of fish oil (i.e., the black soldier fly diets) compared to fish fed the control diet. Furthermore, the percentage of omega-3 fatty acids was significantly lower in these fish. This is consistent with the fatty acid profile of the different diets; however, it could be problematic for consumers who desire fish with high concentrations of long-chain unsaturated fatty acids. Modifications to the diet of the fly larvae may improve the digestibility and fatty acid content of prepupae, which may enhance the fatty acid profile of fish fed fly prepupae. Recent laboratory research to alter the fatty acid profile of black soldier fly prepupae suggests that larvae

fed a combination diet of fish offal (from processing plants) and cow manure incorporate omega-3 fatty acids (St-Hilaire et al. unpublished data). Work is underway to determine whether these omega-3 fatty acid-enhanced prepupae will permit a higher inclusion level in rainbow trout diets with no deleterious results to the growth rate but improved fatty acid profile of the fish.

Although preliminary results suggest that replacing 25% of the fish meal component of rainbow trout diets with black soldier fly prepupae did not affect the weight gain and FCR, the low number of replicates per diet ( $n = 4$ ) in this trial limited the power of the study to detect small differences in these parameters. Furthermore, the duration of the trial was limited to 9 wk, and in other studies, the FCR of fish fed black soldier fly increased over time (Bondari and Sheppard 1987). Before fly prepupae are used commercially as a partial replacement for fish meal and fish oil in rainbow trout diets, a larger trial done over a longer period of time should be conducted to confirm the results of this initial study. Further research is also required to determine the shelf life of the prepupae and processing requirements for utilizing this feedstuff on a commercial basis.

With the increasing price of fish meal and fish oil, fly prepupae may be an economical and sustainable feedstuff for carnivorous fish diets. In areas where there is an aquaculture industry in close proximity to intensive agriculture facilities, fly larvae could be used to reduce animal waste (fish offal and manure) and provide a good-quality protein and fat source for the aquaculture industry.

### Acknowledgments

This research was funded by Idaho State University (Faculty Research Committee grant number 951). Housefly prepupae were kindly donated by Skipio's™ (Tillamook, OR, USA), and black soldier fly larvae were donated by L. Newton (University of Georgia). All fish handling and experimental 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. We thank W. Chalmers and T. G. Gaylord for their editorial comments and Mike Casten and Carol Hoffman for their assistance with the feed trial.

### Literature Cited

- Angradi, T. R. and J. S. Griffith.** 1990. Diel feeding chronology and diet selection of rainbow trout (*Oncorhynchus mykiss*) in the Henry's Fork of the Snake River, Idaho. *Canadian Journal of Fisheries and Aquatic Sciences* 47:199–209.
- AOAC (Association of Official Analytical Chemists).** 1995. Official methods of analysis of the Association of Official Analytical Chemists, 15th edition. Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.
- Balfry, S., D. Higgs, N. Richardson, and S. P. Lall.** 2002. The influence of alternate supplemental dietary lipids on the growth and health of Atlantic salmon in seawater. Abstract in Symposium Proceedings, Biochemical and Physiological Advances in Finfish Aquaculture. International Congress on the Biology of Fish, 2002, University of British Columbia, Vancouver, BC, Canada.
- Bell, J. G., R. J. Henderson, D. R. Tocher, and J. R. Sargent.** 2004. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids* 39:223–232.
- Bondari, K. and D. C. Sheppard.** 1981. Black soldier fly larvae as a feed in commercial fish production. *Aquaculture* 24:103–109.
- Bondari, K. and D. C. Sheppard.** 1987. Soldier fly *Hermetia illucens* L., as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). *Aquaculture and Fisheries Management* 18:209–220.
- Calvert, G. A., R. D. Martin, and N. O. Martin.** 1969. House fly pupae as food for poultry. *Journal of Economic Entomology* 62:938–939.
- Cheng, Z. J., R. W. Hardy, and J. L. Usry.** 2003. Plant protein ingredients with lysine supplementation reduce dietary protein level in rainbow trout (*Oncorhynchus mykiss*) diets, and reduce ammonia nitrogen and soluble phosphorus excretion. *Aquaculture* 218:553–565.
- Christie, W. W.** 1982. A simple procedure for rapid transmethylolation of glycerolipids and cholesteryl esters. *Journal of Lipid Research* 23:1072–1075.
- Clark, R. M., A. M. Ferris, M. Fey, P. B. Brown, K. E. Hundricser, and R. G. Jensen.** 1982. Changes in the lipids of human milk from 2–16 weeks postpartum. *Journal of Pediatric Gastroenterology and Nutrition* 1:311–315.
- Francis, G., H. P. S. Makkar, and K. Becker.** 2001. Antinutritional factors present in plant-derived

- alternate fish feeding ingredients and their effects in fish. *Aquaculture* 199:197–227.
- Hardy, R. W.** 2002. Rainbow trout, *Oncorhynchus mykiss*. Pages 184–202 in C. D. Webster and C. E. Lim, editors. Nutrient requirements and feeding of finfish for aquaculture. The Haworth Press, Binghamton, New York, USA.
- Johansen, K. A., W. M. Sealey, and K. Overturf.** 2006. The effects of chronic immune stimulation on muscle growth in rainbow trout. *Comparative Biochemistry and Physiology Part B. Biochemistry and Molecular Biology* 144:520–531.
- Koshio, S., R. G. Ackman, and S. P. Lall.** 1994. Effects of oxidized herring and canola oils in diets on growth, survival, and flavor of Atlantic salmon, *Salmo salar*. *Journal of Agricultural and Food Chemistry* 42:1164–1169.
- Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz.** 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* 32:1219–1228.
- Millennium Ecosystem Assessment.** 2005. United Nations Report. Accessed January 22, 2007, from <http://www.maweb.org/en/index.aspx>
- Morris, P. C., P. Houghton, A. Black, G. Hide, P. Gallimore, and J. Handley.** 2005. Full-fat soya for rainbow trout (*Oncorhynchus mykiss*) in freshwater: effects on performance, composition and flesh fatty acid profile in absence of hind gut enteritis. *Aquaculture* 248:147–161.
- National Research Council.** 1993. Nutrient Requirements of Fish. National Academy Press, Washington, D.C., USA. p. 114.
- Newton, G. L., C. V. Booram, R. W. Barker, and O. M. Hale.** 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. *Journal of Animal Science* 44:395–400.
- Newton, G. L., D. C. Sheppard, D. W. Watson, G. J. Burtle, C. R. Dove, J. K. Tomberlin, and E. E. Thelen.** 2005. The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Symposium on the State of the Science of Animal Manure and Waste Management. January 5–7, 2005, San Antonio, Texas, USA. URL [http://www.cals.ncsu.edu/waste\\_mgt/natlcenter/sanantonio/proceedings.htm](http://www.cals.ncsu.edu/waste_mgt/natlcenter/sanantonio/proceedings.htm)
- Papatryphon, E. and J. H. Soares, Jr.** 2001. Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, *Morone saxatilis*. *Aquaculture* 202:279–288.
- Rust, M. B.** 2002. Nutritional physiology. Pages 368–446 in J. E. Halver and R. W. Hardy, editors. Fish nutrition, 3rd edition. The Academic Press, New York, New York, USA.
- Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage.** 1994. A value added manure management system using the black soldier fly. *Bioresource Technology* 50:275–279.
- Sheppard, D. C., G. L. Newton, S. A. Thompson, J. Davis, G. Gasho, and K. Bramwell.** 1998. Using soldier flies as a manure management tool for volume reduction, house fly control and feedstuff production. Page 51 in G. Roland, editor. Sustainable agriculture research and education, southern region, 1998. Annual Report. Sustainable Agriculture Research and Education, Southern Region, Georgia Station, Griffin, Georgia, USA.
- Stone, D. A. J., R. W. Hardy, F. T. Barrows, and Z. J. Cheng.** 2005. Effects of extrusion on nutritional value of diets containing corn gluten meal and corn distiller's grain for rainbow trout, *Oncorhynchus mykiss*. *Journal of Applied Aquaculture* 17:1–20.
- Tacon, A. G. J. and D. M. Akiyama.** 1997. Feed ingredients for crustaceans. Pages 411–472 in L. R. D'Abramo, D. E. Conklin, and D. M. Akiyama, editors. Crustacean nutrition, advances in world aquaculture, volume 6. World Aquaculture Society, Baton Rouge, Louisiana, USA.
- Tacon, A. G. J., M. R. Hasan, and R. P. Subasinghe.** 2006. Use of fishery resources as feed inputs for aquaculture development: trends and policy implications. FAO Fisheries Circular no. 1018, FAO. 2006.99, Rome, Italy.