JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 38, No. 2
June, 2007

Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids

SOPHIE ST-HILAIRE[1] AND KATIE CRANFILL
Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209 USA

MARK A. MCGUIRE AND ERIN E. MOSLEY
Department of Animal & Veterinary Science, University of Idaho, Moscow, Idaho 83844 USA

JEFFERY K. TOMBERLIN
Texas A&M University, Texas Cooperative Extension, Stephenville, Texas 76401 USA

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

WENDY SEALEY
Hagerman Fish Culture Experimental Station, University of Idaho,
Hagerman, Idaho 83332 USA

CRAIG SHEPPARD
CEFAS, Weymouth Laboratory, Weymouth, Dorset DT4 8UB UK

STEPHEN IRVING
Department of Entomology, University of Georgia, Tifton, Georgia 31794 USA

Abstract.—The black soldier fly, *Hermetia illucens*, has the potential to reduce animal waste on livestock facilities and produce an animal-grade feedstuff high in protein and fat. The lipid content of insects is largely dependent on their diet. Data from this study suggest that black soldier fly prepupae incorporate α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) when fish offal is included in their diet. Fly larvae were fed three different proportions of fish offal and cow manure diets over a 21-d trial. An additional group of larvae were fed 22% fish offal diet within 24 h of their pupation. Larvae fed fish offal were, on average, 30% lipid, which was 43% more than the controls fed cow manure only, and approximately 3% of this lipid was omega-3 fatty acids (EPA, DHA, and ALA). Furthermore, this concentration of omega-3 fatty acids was achieved within 24 h of feeding fish offal. These omega-3 fatty-acid-enhanced prepupae may be a suitable fish meal and fish oil replacement for carnivorous fish and other animal diets. In addition, they may provide a method of reducing and recycling fish offal from processing plants.

Manure management is a growing concern in intensive livestock facilities. The black soldier fly, *Hermetia illucens*, has been investigated for its manure bioconversion capabilities (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 over 50% in poultry and swine systems (Sheppard et al. 1994, 1998; Newton et al. 2005). Further, the prepupae are approximately 40% protein and 30% fat (Sheppard et al. 1994, 1998; Newton et al. 2005), which makes for a suitable source of food for animals (Newton et al. 1977; Bondari and Sheppard 1981, 1987).

In a recent study where rainbow trout, *Oncorhynchus mykiss*, were fed diets containing black soldier fly prepupae, it was determined that replacement of 25% of the fish meal and 38% of the fish oil components of a commercial diet (with 40% protein – 67% of which was derived from fish meal and the other 33% from plant origin, and 15% lipid – all derived from fish oil) had no effect on the feed conversion

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[1] Corresponding author.

ratio (St-Hilaire et al. 2007). However, the fillets of fish fed black soldier fly prepupae were lower in omega-3 fatty acids than the controls, possibly because of lower levels of long-chain unsaturated fatty acids in their diets.

The lipid content of insects is largely dependant on their diets and stage of development (Stanley-Samuelson and Dadd 1983) and many insects have naturally occurring long-chain unsaturated fatty acids (Thompson 1973). The objective of this study was to determine if the omega-3 fatty acid content of black soldier fly prepupae could be increased by feeding them fish waste from processing plants.

Materials and Methods

Approximately 1000 black soldier fly eggs were collected from an established colony at Texas A&M University Extension service (Stephenville, TX, USA) and sent to Idaho State University, Pocatello, Idaho, USA. The eggs were hatched and grown on cow manure at room temperature (~22 C). After 37 d, 50 larvae were counted into 1 of 12 containers and weighed (average total weight in containers 4.68 g; SE = 0.141 g and range 3.73–5.42 g). Containers were randomly allocated to four diets: manure only, 50/50 mixture of cow manure and fish offal, 25% fish offal mixed with 75% cow manure, and 10% fish offal mixed with 90% cow manure. The fish offal included in the larval diets consisted of homogenized heads, viscera, and some bony structures from rainbow trout processed at a plant in Idaho, USA.

Larvae were fed 3 d a week. A total of 150 g wet weight of food was provided to each container over a 21-d period. Approximately 1 mL of water was sprayed into the containers when the diet was dry (five times during the trial period). At the end of the 21-d study, the prepupae were collected, dried with a paper towel, counted, weighed, and frozen for future fatty acid analysis.

A sample of larvae ($n = 5$) from two of the three control groups (fed cow manure only) were transferred into two new containers and fed 21 g of material containing 22% fish offal and 78% cow manure. The prepupae/larvae were harvested within 24 h, dried with a paper towel, weighted, and frozen for fatty acid analysis. Prepupae were sent frozen to the Department of Animal & Veterinary Science at University of Idaho, Moscow, Idaho, USA, for fatty acid analysis.

Fatty Acid Analyses

Prepupae were freeze dried before lipids were extracted using chloroform : methanol (2:1) (Clark et al. 1982). Direct methylation of extracted lipids was conducted (Kramer et al. 1997). The fatty acid methyl esters were analyzed on a gas chromatograph (Hewlett-Packard 6890 Series with autoinjector) fitted with a flame ionization detector and a 100 × 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, Belgium) were used to quantify fatty acids. Fatty acids were expressed as a weight percentage of total lipid on a dry matter basis.

Statistical Analyses

The average weight of larvae and prepupae were compared using an ANOVA. If the ANOVA statistic was significant ($P < 0.05$), then Tukey tests were used for multiple comparisons. The total percent lipid and proportion of specific fatty acids of prepupae fed different diets were compared using the Kruskal–Wallis statistic. This statistical test was used because the number of replicates in each group was low ($n = 3$), and for seven of the 12 fatty acids assessed, the parametric test assumption of homoscedasticity was violated. The Kruskal–Wallis tests and all descriptive statistics were done using MINITAB® Statistical Software (Mintab Inc., State College, PA, USA). The ANOVA and Tukey 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 average weight of the larvae in each of the diet treatments at the start of the 21-d trial was similar (range 0.088–0.096 g) ($F_{3,8} = 0.377$, $P = 0.772$) (Table 1). The final average weight of the prepupae fed cow manure only was lower than the other diet groups fed a mixture of manure and fish offal ($F_{3,8} = 13.29$, $P = 0.002$) (Table 1).

There was approximately a 43% increase (from 21 to 30%) in total lipid of black soldier flies fed different proportions of fish offal during a 21-d period compared to the prepupae fed only cow manure ($H = 9.27$, $P = 0.055$) (Table 2). The percent total lipid was not affected by the proportion of fish offal in the larval diet (Table 2). There was no difference between the percent lipid of prepupae fed fish offal for only 24 h and the control prepupae (Table 2).

The proportion of the omega-3 fatty acids in the prepupae was increased when they were fed fish offal (Table 2). Approximately 3% of the lipid in the prepupae fed fish offal was omega-3 fatty acids (Table 2). This proportion of omega-3 fatty acids was similar regardless of the concentration of fish offal in the diet or the length of time the larvae were fed fish offal (Table 2). Approximately 0.7% of the lipid in fish offal fed prepupae was α-linolenic acid (18:3n3; ALA), 1.7% eicosapentaenoic acid (20:5n3; EPA), and 0.5% docosahexaenoic acid (22:6n3; DHA) when the prepupae were fed any proportion of fish offal for 21 d (Table 2). Given the average prepupae (fed fish offal for 21 d) was 30% lipid, approximately 1% of its total dry weight was omega-3 fatty acids (Fig. 1). Greater weight percentages of ALA, EPA, and DHA were also detected in prepupae fed 22% fish offal for 24 h; however, total lipid was not changed compared to the cow-manure-only control so the quantity of omega-3 fatty acids on a percent dry weight basis was 0.8% (Fig. 1).

Discussion

Black soldier fly larvae were able to "recycle" a proportion of the fish oils from processing plant fish offal. Prepupae fed as little as 10% fish offal had approximately 43% more lipid than prepupae fed cow manure without fish offal, and went from negligible amounts of omega-3 fatty acids to approximately 3% of the lipid consisting of these long-chain unsaturated fatty acids (Table 2). Furthermore, it may be possible to attain these levels of omega-3 fatty acids in prepupae by incorporating a small amount of fish waste in their diet within 24 h of pupation.

Currently, much of the rainbow trout fish offal in Idaho is sold for the pet food industry or as compost. This study suggests it could be made into a value-added insect product potentially suitable for animal diets. Black soldier fly raised on poultry manure have been fed to several commercial species including poultry; catfish, *Ictalurus punctatus*; blue tilapia, *Oreochromis aureus*; and swine (Newton et al. 1977; Bondari and Sheppard 1981, 1987; Newton et al. 2005). Increasing the omega-3 fatty acid content of prepupae should increase the value of this product as a diet ingredient.

The growth of prepupae on cow manure was slower than what has been reported for poultry

TABLE 1. *Average weight (g) of black soldier fly,* Hermetia illucens, *larvae at the start and prepupae at the end of the trial (SE).*[1]

Group	Average weight of larvae at the start of the trial (g)	Average weight of larvae at the end of the study (g)
Cow manure only	0.09 (0.003)	0.10 (0.008)^{a}
10% fish offal/90% cow manure	0.10 (0.005)	0.14 (0.003)^{b}
25% fish offal/75% cow manure	0.09 (0.008)	0.16 (0.004)^{b}
50% fish offal/50% cow manure	0.10 (0.006)	0.15 (0.010)^{b}
P value associated with the ANOVA statistic	0.772	0.002

[1] Values within columns with a common superscript do not differ ($P > 0.05$) based on the Tukey multiple comparison test.

TABLE 2. *Mean ± SE of selected fatty acid composition of black soldier fly,* Hermetia illucens, *prepupae fed diets with varying proportions of fish offal and cow manure. All proportions are expressed as a weight percentage of the total lipid on a dry matter basis.*

	Black soldier fly prepupae fed					
Fatty acid	Cow manure	10% fish offal and 90% cow manure	25% fish offal and 75% cow manure	50% fish offal and 50% cow manure	24 h 22% fish offal and 78% cow manure	*P* value associated with the Kruskal–Wallis test
Total lipid – % of total dry weight	21.42 ± 0.17	30.38 ± 0.62	28.82 ± 1.46	30.44 ± 1.17	20.28 ± 2.95	0.055
12:0	20.92 ± 1.27	34.10 ± 1.33	41.00 ± 2.01	42.57 ± 0.51	14.72 ± 0.75	0.019
14:0	2.85 ± 0.07	6.46 ± 0.04	6.67 ± 0.16	6.91 ± 0.04	4.51 ± 0.42	0.023
16:0	16.05 ± 0.06	14.30 ± 0.37	12.08 ± 0.53	11.14 ± 0.24	16.50 ± 0.65	0.019
18:0	5.68 ± 0.06	2.35 ± 0.06	1.64 ± 0.09	1.29 ± 0.06	6.22 ± 0.35	0.014
18:1c9	32.11 ± 1.01	16.52 ± 0.34	13.96 ± 0.48	12.28 ± 0.10	27.00 ± 0.65	0.014
18:2n6	4.51 ± 0.56	3.96 ± 0.21	3.22 ± 0.16	3.57 ± 0.09	3.89 ± 0.33	0.060
18:3n3; ALA	0.19 ± 0.01	0.74 ± 0.02	0.71 ± 0.05	0.74 ± 0.03	0.86 ± 0.10	0.091
20:4n6	0.04 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.073
20:5n3; EPA	0.03 ± 0.01	1.76 ± 0.07	1.63 ± 0.05	1.66 ± 0.05	1.43 ± 0.04	0.034
22:5n3	0	0.1 ± 0.003	0.11 ± 0.01	0.14 ± 0.01	0.53 ± 0.04	0.017
22:6n3; DHA	0.006 ± 0.003	0.41 ± 0.01	0.43 ± 0.06	0.59 ± 0.04	1.66 ± 0.01	0.019
Total ALA, EPA, and DHA	0.23 ± 0.02	2.91 ± 0.11	2.76 ± 0.13	2.99 ± 0.11	3.96 ± 0.05	0.034

ALA = α-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

or swine manure. It required almost 2 mo for eggs to develop into prepupae in this study, whereas other researchers report 4–6 wk when larvae are fed poultry manure (Tomberlin et al. 2002). It is possible that the lower temperature used to grow the larvae in this study (i.e., 22 C) contributed to the slower development of the larvae. It is also possible that the black soldier fly does not grow as well on cow manure compared to swine or poultry manures. This finding has been observed by several of the authors independently of this research (Newton et al. 2005).

The biodegradability of manure has been shown to be highly dependent on its lignin

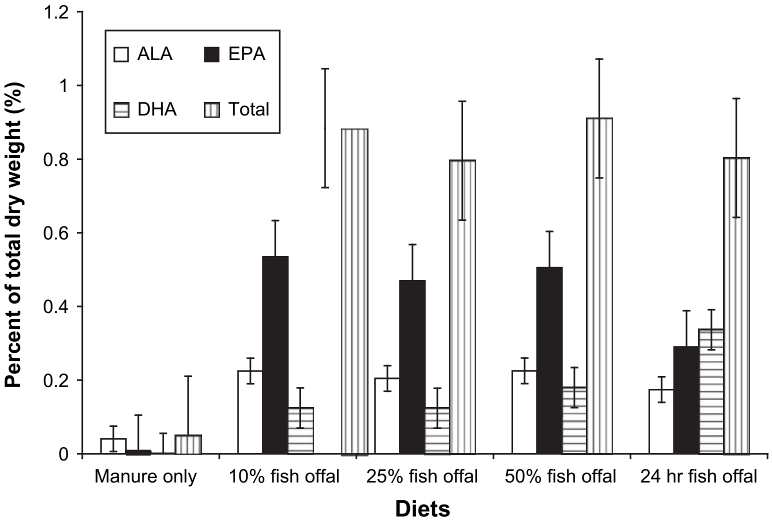

FIGURE 1. *Percent α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) on a total dry matter basis for prepupae fed diets containing different proportions of fish offal. Error bars indicate the pooled SE.*

content (Stanogias and Pearce 1987), which comes mainly from forage fibre in the diet. Because poultry and swine diets generally contain little forage, it would be expected that their feces would be more highly degradable than that of ruminants. The lower nutritional value of cow manure may explain why the larvae fed mixed diets had higher average weights compared to prepupae fed cow manure only (Table 1) and why it required more time to produce prepupae than previously reported. Analysis of cow manure is under way to further elucidate the reason for this apparent reduced growth.

The lipid content of black soldier fly prepupae can be increased and manipulated to include desirable fatty acids such as ALA, EPA, and DHA by feeding the larvae waste material from fish processing plants. Our findings indicate an increase (from 21 to 30%) in the lipid content of prepupae fed fish waste, and more importantly, substantial enrichment (2.5–3.8% of total lipid) of omega-3 fatty acids (Table 2). It may therefore be possible to utilize the black soldier fly, which is a nonpest species to reduce animal waste and recycle omega-3 fatty acids, while producing a high-quality animal-grade foodstuff that is a suitable replacement for fish meal and fish oil in animal diets.

Acknowledgments

This research was funded by Idaho State University (FRC grant #951). We thank W. Chalmers and T. G. Gaylord for their editorial comments.

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