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Bioconversion of Poultry and Fish Waste by *Lucilia Sericata* and *Sarcophaga Carnaria* Larvae

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Abstract: Agricultural, industrial, and household waste contaminates the environment, disrupts the food chain, and spreads infectious diseases. Fly larvae digest animal waste, and in so doing significantly reduce their volume and convert the waste to materials that can be used as food additives and fertilizers for organic farming. Larvae of *L. sericata* and *S. carnaria* were efficient in reducing the mass of poultry and fish waste by 2.5–5.5 fold. The average yield of larvae reared on fish and poultry waste was approximately 304 g, while the bioconversion rate ranged between 16.6 and 39.6%. Water and undigested waste accounted for 60%–77% of the larvae and pupae body mass, while protein followed by fat and carbohydrate were the most important components. Representatives of Enterobacteriaceae, Pseudomonas, Aeromonas, Micrococcus and sulfite-reducing Clostridia species were isolated from the waste products as well as from the larvae and pupae of both flies used. The weights of striped bass fed with standard fish food supplemented with larvae increased slightly, when compared to those fed on standard food alone. The species of fly, the number of eggs, the type of waste material used and its chemical composition affected the bioconversion rate, the yield of fly larvae, and the waste mass reduction. Fly larvae and their byproducts could be used beneficially as a supplement for animal food for the poultry and fish industry, as well as reducing the quantity of waste.

Key words: *Lucilia sericata*, *Sarcophaga carnaria*, bioconversion, poultry and fish waste, Israel.

Introduction

Waste management is one of the big challenges facing human society today. Agricultural, industrial and household wastes can contaminate the environment, disrupt the food chain, and spread infectious diseases. The problem of processing organic wastes is especially important in countries with high population densities. At present there is no evidence that a practical facility for processing the poultry and fish wastes generated by the food industry could be operated profitably.

Fly larvae can digest animal waste, thereby they significantly reduce its volume, and also convert the waste to material that can be used as fertilizers for organic farming (Newton et al., 1977; Covarrubias et al., 1994; Sheppard et al., 1994, 2002; Fontenot, 1999).

Fly larvae are a cheap source of protein, fat and enzymes (Espinosa-Fuetes and Terra, 1987), and can be used as unconventional food additives in poultry and fish breeding (Akpodiete et al., 1997; Knan et al., 1999). Promising results were reported for the fish Nile tilapia (Chrappa and Sabo, 1998) and salmon (Spinelli et al.,

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1979). Sheppard et al. (1994) used black soldier-fly larvae as chicken feed. Pro et al. (1997) and Inaoka et al. (1999) demonstrated that chicken food supplemented with *Musca domestica* larvae was nutritionally equivalent to more expensive commercial diets.

The use of wastes from the animal food processing industry as substrates for breeding blow fly (*Lucilia sericata*) and flesh fly (*Sarcophaga carnaria*) larvae is proposed as an innovative low-cost, low-maintenance approach to solving the ecological problems of processing and disposing of poultry and fish waste. The use of such waste is free from Bovine Spongiform Encephalopathy infection, and is in accordance with the European Union's Common Position (No. 12/2002).

The aim of the present study was to study the ability of *L. sericata* and *S. carnaria* larvae to biodegrade poultry and fish waste and the use of such larvae as high-value food additives in the fish industry.

Materials And Methods

Poultry and Fish Wastes

The following waste products were used for larval feeding:

(a) Poultry waste from the Off-HaGalil (Kiryat Shmona) poultry processing plant, which comprised skin, internal organs and meat remains, and which was used for the production of dog food (referred as PW I).

(b) Poultry waste from the Off-HaGalil poultry processing plant, which comprised ground bones, skin, internal organs, and meat remains (referred as PW II).

(c) Wastes from fish (herring, mackerel, sprat, tuna, capelin, Nile perch, hake, Alaska Pollack and salmon), which were obtained from the Yona Company (Tirat HaCarmel) and from the Fish Product Laboratory of the Kimron Veterinary Institute in Bet Dagan.

Flies

L. sericata and *S. carnaria* were reared at 80% relative humidity, 26ºC–28ºC, 12/12 hour light/darkness. Caged adults were fed with granular sugar and water. Oviposition was induced with beef liver. The eggs were transferred to aluminum foil trays loaded with the experimental wastes. The trays were set over a larger plastic bin that one third of it was filled with saw dust. Mature satiated larvae crawled over the wall of the aluminum tray and dropped into the saw dust to pupate. The plastic bin was covered with a netted lid to prevent larvae from escaping.

Preliminary studies using various numbers of fly eggs showed that larvae from *L. sericata* hatched from 15,000–20,000 eggs and larvae from *S. carnaria* hatched from 5,000 eggs, liquefied the medium towards the end of their development and produced the best bioconversion rates. Therefore, these numbers of eggs were used for the experiments.

Biodegradation

Approximately 5,000 to 20,000 eggs were placed on 1 kg of poultry or fish waste for a period of 3–4 days. The weight of the grown larvae and the weight of the remaining undigested waste residues were recorded. The mass reduction factor was calculated according to the following formula:

$$\frac{\text{Weight of undigested waste residues} + \text{Weight of the larvae}}{\text{Weight of fresh waste}}$$

The bioconversion rate was calculated as: weight of the larvae/weight of the initial waste.

Body Measurements

Larvae and pupae were anesthetized with CO_2 and measured under a stereo-microscope. For each species 10 specimens of the third larval stage were weighed and measured.

Chemical Analyses

The chemical composition of the waste material was studied, both before the introduction and after the removal of the larvae. Proteins, lipids, minerals, water and dry matter content were determined as specified by the Association of Official Analytical Chemists (Cunniff, 1995).

Bacteriological Analysis

Bacteria were isolated from the original waste, waste-residues, fed larvae and pupae. A 10-g aliquot of relevant material was mixed with 90 ml of sterile peptone in a Model BA6021 Stomacher 400 (Seward Laboratory, UAC House, UK) for 2 min. Serial tenfold dilutions of the homogenized mixture were plated on Plate Counting Agar (PCA) (Difco, Detroit, MI, USA), and bacterial species were identified by culturing in selective media (Difco): MacConkey agar for Enterobacteriaceae; Baird-Parker agar for Staphylococci; Cetrimide agar for Pseudomonas; Oxford Listeria agar for Listeria; and TSC agar for Clostridia. Final biochemical identification was carried out with API NE, API 20E and API Staph (BioMerieux Vitek, Inc., Marcy-I'Etoile, France).

Fish Breeding

Three groups of 45 striped bass (*Morone saxatilis*) (average weight 125.8 g) each were placed into a 1 m^3 plastic tank of water at 24ºC–25°C. The water was replaced every 8 hrs. The fish group 1 was fed with 6.3 g of standard food daily, group 2 with the usual portion of standard food supplemented with 10% larvae of *L. sericata*, and group 3 with 10% of the usual portion of standard food replaced with larvae of the same species. The standard food was produced by the Zemakh Compound Institute (Jordan Valley, Israel) for premix fish feed and contained 45% protein, 9% fat, 32% carbohydrates, and vitamin supplements. *L. sericata* larvae, which were used for feeding fish, contained 14.5% protein, 5.5% fat and 3% carbohydrates. The fish were weighed at the beginning of the study and subsequently every 7–10 days for 47 days.

Statistical Evaluation

Averages and standard deviations were calculated with MS-Excel software.

Results

L. sericata larvae hatched from approximately 15,000 and 20,000 eggs placed on one kg of poultry waste (PW I) resulted in a weight reduction factor of 2.49 ± 0.38 and 5.53 ± 1.43, respectively, an average larval yield of 166.4 ± 12.6 and 348.9 ± 63.7 g, respectively and a bioconversion rate of 16.6 ± 1.3 and 34.9 ± 6.4%, respectively. *L. sericata* larvae hatched from approximately 15,000 and 20,000 eggs placed on one kg of poultry waste with bone residues (PW II) resulted in a weight reduction factor of 4.11 ± 0.3 and 5.53 ± 1.43, respectively, a larval yield of 307.1 ± 24.1 and 348.92 ± 63.7 g, respectively and a bioconversion rate of 30.7 ± 2.6 and 34.9 ± 6.5%, respectively. *S. carnaria* larvae hatched from approximately 5,000 eggs placed on one kg of Nile perch waste resulted in a weight reduction factor of 4.85 ± 0.25, a larval yield of 395.7 ± 4.8 g and a bioconversion rate of 39.6 ± 0.5%. (Table 1).

Fed on different fish wastes, *L. sericata* larvae achieved a bioconversion rate, which varied from 11.8% (capelin wastes) to 41.3% (tuna wastes), the waste weight reduction factors ranged from 2.8 (capelin wastes) to 7.1 (herring wastes) and the total larval yield ranged from 118.3 g (capelin wastes) to 412.8 g (tuna wastes). The undigested residues for herring accounted for 150.2 and for capelin 440.3 g, respectively of the initial mass (Table 2).

The average weight for *S. carnaria* larvae was 0.18 ± 0.01 g, the average length was 16.5 ± 0.6 mm and average width 4.3 ± 0.3 mm. For *L. sericata* larvae the average weight was 0.03 ± 0.01 g, the length 8.4 ± 0.7 mm and the width 2.5 ± 0.4 mm. For *S. carnaria* pupae the weight was 0.13 ± 0.02 g, the length 11.3 ± 0.3 mm and the width 4.8 ± 0.2 mm. For *L. sericata* pupae the weight was 0.5 ± 0.04 g, the length 8.5 ± 7.5 mm and the width 2.5 ± 2.0 mm.

The composition of *L. sericata* and *S. carnaria* larvae and pupae and of the undigested residues of their food is shown in Table 3. Accordingly, water accounted for 60%–77% of the body mass, while protein followed by fat and carbohydrate were the most important components.

The bacteriological composition of poultry and fish wastes and their residues as well as those of *L. sericata* larvae and *S. carnaria* pupae developed on these media is shown in Table 4. The total CFU g^{-1}counts in the various wastes, as well as in larvae and pupae ranged

Table 1: Efficacy of bioconversion by breeding *L. sericata* and *S. carnaria* larvae hatched from approximately 5,000-20,000 eggs on 1 kg of poultry or fish waste

Species of fly	*Number of eggs*	*Medium*	*Number of experiments*	*Average final weight (g)(±SD)*	*Average weight reduction factor (±SD)*	*Average total weight of larvae (g) (±SD)*	*Average bioconversion rate (%) (±SD)*
L. sericata	15,000	PW 1	11	408.6 (40.7)	2.49 (0.38)	166.4 (12.6)	16.6 (1.3)
L. sericata	20,000	PW 1	11	192.1 (49.2)	5.53 (1.43)	348.9 (63.7)	34.9 (6.4)
L. sericata	15,000	PW II	7	242.5 (17.8)	4.11 (0.3)	307.1 (24.1)	30.7 (2.6)
S. carnaria	5,000	Nile perch	4	206.5 (11.0)	4.85 (025)	395.7 (4.8)	39.6 (0.5)

Table 2: Efficacy of bioconversion by breeding *L. sericata* larvae hatched from approximately 20,000 eggs on 1 kg of fish waste

Fish waste	*Weight reduction factor*	*Total weight of larvae (g)*	*Bioconversion rate (%)*	*Undigested residues (g)*
Herring	7.12	321.1	32.1	150.2
Capelin	2.27	118.3	11.8	440.3
Nile perch	5.61	282.4	28.2	153.7
Sprat	2.39	218.3	21.8	418.5
Tuna	2.37	412.8	41.3	321.7
Mackerel	2.94	311.9	31.2	363.9
Salmon	2.86	350.0	35.0	350.0

Table 3: Chemical composition of *L. sericata* and *S. carnaria* larvae and pupae, as well as of the undigested residues

Sample	*Dried material (%)*	*Water (%)*	*Ash (%)*	*Carbohydrate (%)*	*Fat (%)*	*Protein (%)*
L. sericata larvae	28.0	60.0	1.3	3.0	9.1	14.5
L. sericata pupae	23.3	77.0	1.8	2.6	5.5	13.4
Undigested residues (PW 1)	37.0	63.0	2.0	0	11.3	23.7
S. carnaria larvae	24.9	75.1	1.1	2.9	5.5	15.4
S. carnaria pupae	35.3	64.7	1.3	4.7	6.3	23.0
Undigested residues (Nile perch)	37.7	67.8	3.0	0	8.0	26.7

Table 4: Bacterial flora composition of poultry and fish wastes and their residues as well as the bacterial flora of *L. sericata* larvae and *S. carnaria* pupae developed on these media

Sample	*Total counts-CFU g^{-1}*	*Bacterium*
Poultry waste (PW 1)	1.0×10^5	*Micrococcus* spp. *Pseudomonas* spp. *Enterobacteriaceae* spp. *Staphylococcus* spp.
Fish waste (Nile perch)	2.6×10^5	*Micrococcus* spp. *Pseudomonas* spp. *Aeromonas hydrophila* *Enterobacteriaceae* spp. *Proteus vulgaris*
Larvae of *L. sericata*	3.5×10^6	*Enterobacter cloacae* *Citrobacter* spp. *Micrococcus* spp. *Pseudomonas* spp. Sulfite-reducing Clostridia
Pupae of *S. carnaria*	4.3×10^7	*Proteus vulgaris* *Enterobacter cloacae* *Citrobacter* spp. *Micrococcus* spp. *Pseudomonas* spp. Sulfite-reducing Clostridia
Undigested residues (PW 1)	2.6×10^9	*Proteus vulgaris* *Enterobacter cloacae* *Citrobacter* spp. *Micrococcus* spp. *Pseudomonas* spp. Sulfite-reducing Clostridia *Shewanella putrefaciens*

from 10^5 to 10^9. Representatives of Enterobacteriaceae, Pseudomonas, Aeromonas, Micrococcus and sulfite-reducing Clostridia species were isolated.

Table 5 shows the weights of striped bass fed with standard fish food supplemented with larvae of *L. sericata*. It can be seen that the weight increase was similar in all three groups, although those in the group that received standard feed supplemented with 10% of larvae were slightly heavier.

Discussion

The utility and economic advantages of using organic wastes as a substrate for breeding fly larvae have been documented in many studies. However, most of these studies were carried out on such wastes as poultry or swine manure, or animal and human organic wastes, and they used the house fly, *Musca domestica* (Covarrubias et al., 1994; Barnard et al., 1998; Watson et al., 1998) or the black-soldier-fly, *Hermetia illucens* (Newton et al., 1977; Sheppard et al., 1994, 2002) larvae.

Our results show that larvae of *L. sericata* and *S. carnaria* reduced the mass of poultry and fish waste by factors of approximately 2.5–5.5. The average yield of larvae reared on fish and poultry wastes was approximately 304 g, while the bioconversion rate ranged between 16.6% and 39.6%.

The bioconversion rate with *L. sericata* was higher with 20,000 eggs compared with 15,000, i.e., 34.9% vs. 16.6%. The bioconversion rate with 20,000 eggs of *L. sericata* was highest with tuna (41.3), followed by salmon (35.5), herring (32.1) mackerel (31.2), Nile perch (25.2), and capelin (11.8). As expected, different fish or poultry waste produced different bioconversion rates and total larval yields. The larger *S. carnaria* demonstrated a higher bioconversion rate than *L. sericata.*

The larvae of *L. sericata* and *S. carnaria* contain 14%–15% protein and 8%–9% fat. The dried black soldier fly (*H. illucens*) prepupae contain 42% protein and 35% fat (Newton et al., 1977) and it supports good growth of chicks (Hale, 1973), swine (Newton, 1977) and rainbow trout (St-Hilaire et al., 2007). It was shown that a prepupae meal can replace at least 25% of the fish meal in a diet with no reduction in gain or feed conversion ratio in rainbow trout (St-Hilaire et al., 2007).

The results obtained here, using *L. sericata* larvae, revealed that the greatest output of the maggots and the smallest quantity of undigested residues were obtained from the wastes that contained high proportions of protein and/or fat. The highest yield (41.3%) of *L. sericata* larvae was obtained from tuna wastes, probably because of the high protein content in tuna.

Some opportunistic and pathogenic bacteria were isolated from the surface of maggots and the digested waste. These bacteria are likely to be also responsible for the liquidification/digestion of the medium.

Larvae of *L. sericata* produce and excrete different antibacterial substances, which diminishes the bacterial load on chronic wounds (Mumcuoglu et al., 2001; Huberman et al., 2007). Food safety standards and bacteriological considerations using manure-fed Hermetia prepupae were acceptable. Hermetia larval activity significantly reduced *Escherichia coli* and *Salmonella enterica* in hen manure (Erickson et al., 2004). Studies in China, the USSR, the USA, Mexico, and in Eastern Europe where larvae were fed to poultry, swine, shrimp, several species of fish, turtles, and frogs did not cause any health problems. It was reported that anti-microbial factors in the house fly larvae reduce the chance of the feedstuff transmitting pathogens, and actually improve animal health, while reducing pathogen content in the digested manure that are used to fertilize food crops.

In conclusion, it can be seen that the species of fly, the number of eggs, the waste material used, and its chemical composition affected the bioconversion rate,

Table 5: Changes in striped bass weights (g) fed on various types of food

Weighing on days	*Standard food*		*Standard food + 10 % larvae*		*90% standard food + 10 % larvae*	
	Exp. 1	*Exp. 2*	*Exp. 1*	*Exp. 2*	*Exp. 1*	*Exp. 2*
0	119.0	125.0	128.0	131.0	131.0	121.0
9	131.6	136.8	150.0	150.0	140.0	138.6
17	144.0	145.2	151.8	145.4	180.0	138.0
29	165.0	170.0	178.0	171.0	179.0	173.0
40	194.0	202.0	206.0	211.6	204.3	205.0
47	198.8	197.6	220.7	227.0	204.9	210.7

the yield of fly larvae and waste mass reduction. Fly larvae and their byproducts could be used advantageously as food for the poultry and fish-growing industries. This approach to the solution of an ecological problem, by utilization of biodegradation of wastes could be relatively simple and inexpensive, and could be mechanized on condition of adherence to food safety regulations.

Larval digestion could reduce the noxious odours from waste (Lorimor et al., 2001) and the undigested residues could be used as fertilizers for organic farming, and might generate additional benefits.

Isolation of new antibacterial and antifungal substances for novel applications could also be profitable. Blow fly larval hemolymph obtained in our experiments was subsequently used as a source for isolation of a new antimicrobial substance that has a wide antimicrobial spectrum including Gram-positive and Gram-negative bacteria, as well as certain fungi (Huberman et al., 2007).

One of the advantages of using fly species such as *L. sericata* and *S. carnaria* derives from the fact that these flies are not very anthropophilic, they do not bite, bother, or pester humans in any way.

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