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Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation

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Abstract

OBJECTIVES To determine the capacity of black soldier fly larvae (BSFL) (*Hermetia illucens*) to convert fresh human faeces into larval biomass under different feeding regimes, and to determine how effective BSFL are as a means of human faecal waste management.

METHODS Black soldier fly larvae were fed fresh human faeces. The frequency of feeding, number of larvae and feeding ratio were altered to determine their effects on larval growth, prepupal weight, waste reduction, bioconversion and feed conversion rate (FCR).

RESULTS The larvae that were fed a single lump amount of faeces developed into significantly larger larvae and prepupae than those fed incrementally every 2 days; however, the development into prepupae took longer. The highest waste reduction was found in the group containing the most larvae, with no difference between feeding regimes. At an estimated 90% pupation rate, the highest bioconversion (16–22%) and lowest, most efficient FCR (2.0–3.3) occurred in groups that contained 10 and 100 larvae, when fed both the lump amount and incremental regime.

CONCLUSION The prepupal weight, bioconversion and FCR results surpass those from previous studies into BSFL management of swine, chicken manure and municipal organic waste. This suggests that the use of BSFL could provide a solution to the health problems associated with poor sanitation and inadequate human waste management in developing countries.

keywords *Hermetia illucens*, sanitation, prepupal yield, biomass, feed conversion rates

Introduction

Providing hygienic, affordable and manageable sanitation is vital to the improvement in public health in both developed and developing countries. 2.6 billion people in developing regions have no access to improved sanitation (WHO/UNICEF 2010). With 44% of these people practicing open defecation, there are serious risks to public health that can lead to an increase in disease spread (Esrey *et al.* 1991). Strong evidence suggests that improved sanitation has a significant effect on health in developing regions (Esrey *et al.* 1991).

On-site improved sanitation includes pit latrines with slabs, ventilated improved pit latrines (VIP), pour-flush pit latrines and composting toilets (WHO/UNICEF 2010). 1.7 billion people in low- and middle-income communities around the world use these forms of improved sanitation (WHO/UNICEF 2010). However, it has been reported in Vietnam (Biran 2010a) and Tanzania (Biran 2010b) that the biggest problem faced by pit latrine owners is the disposal of pit latrine waste. Adequate pit latrine emptying services are not available in many areas in developing countries and can be expensive (Still 2002). The emptying process can also be inconvenient for the latrine owner and cause bad smells in the surrounding area (Biran 2010a,b). Digging a new pit is an alternative, but too expensive for many. Also, it may be impossible in areas which lack space, such as emergency camps and unplanned settlements (Patinet 2010).

Effective treatment and management of human faecal waste is vital to prevent adverse health and environmental effects (WHO/UNEP 2006). The method of waste treatment must be considered, particularly in low-income countries with insufficient piped sewerage systems. It is possible to remove pathogens while transporting faecal sludge to a wastewater treatment plant, but in practice, unregulated services and the prohibitive cost, lack of infrastructure and resources render this method of waste treatment in developing countries unfeasible (Helmer & Hespanhol 1997; Kariuki *et al.* 2003; WHO/UNEP 2006). Composting can be used to remove pathogens in sewage sludge if maintained correctly (USEPA 2003). But

pathogens are not always inactivated throughout the entire compost mass (Droffner & Brinton 1995; Hutchison *et al.* 2005). Biogas systems combine human excreta with animal waste, agricultural waste and water (NNFCC 2011), but only remove some of the pathogenic organisms. With a large increase in the number of pit latrines being built in developing countries, more consideration needs to be given to improving methods of pit emptying and safe waste processing and disposal.

One prospective solution for waste processing is the larvae of *Hermetia illucens* (L.), commonly known as the black soldier fly larvae (BSFL). The adult flies are neither a nuisance species nor a mechanical vector of disease, as they do not need to feed, surviving on fat stores from their larval stage (Furman *et al.* 1959). As the females oviposit around the edges of larval food sources (Copello 1926), they do not transmit pathogens from faecal waste to human food unlike filth flies such as *Musca domestica*. Although there have been rare cases of accidental myiasis caused by the consumption of ripe, unwashed fruit (Calderón-Arguedas *et al.* 2005; Fuentes Gonzalez & Risco Oliva 2009), but given their worldwide distribution (Leclercq 1969), such cases represent negligent risks to humans. Unlike the adults, the larvae are detritivores feeding on human cadavers (Dunn 1916), decaying vegetables (Malloch 1917), human pit latrine waste (Bradley 1930) and animal manure (Tingle *et al.* 1975; Booram *et al.* 1977; Newton *et al.* 2005). The final larval stage (prepupal) is indicated by a change in colour from white to dark brown (May 1961). The prepupae crawl out of the feeding material to pupate, climbing slopes of 40° when dry, making them easy to direct for harvesting (Sheppard *et al.* 1994). The prepupal stage contains high protein and fat levels, 42–45% and 31–35%, respectively (Hale 1973; Newton *et al.* 1977). These nutritional qualities give the prepupae value, as they can be converted into beneficial end products (Sheppard *et al.* 1994). They can provide a suitable replacement for conventional fat and protein sources and can be fed to animals such as cockerels (Hale 1973), pigs (Newton *et al.* 1977), catfish and tilapia (Bondari & Sheppard 1987), and rainbow trout (St-Hilaire *et al.* 2007). The prepupae can also be fractionated into their component parts; protein separated for animal feeds and fats converted into biodiesel (Li *et al.* 2011a,b). BSFL are also known to reduce oviposition of the disease-spreading house fly, *M. domestica* (Sheppard 1983). The quantities of organic material consumed by BSFL can significantly reduce swine, chicken and cattle manure in the animal husbandry industry (Sheppard *et al.* 1994; Newton *et al.* 2005). BSFL can also reduce *Escherichia coli* and *Salmonella enterica* pathogen loads in chicken and cattle manure (Erickson *et al.* 2004; Liu *et al.* 2008), and human faeces (Lalander *et al.* 2013).

Although there has been much research focusing on the use of BSFL to manage swine, chicken and cattle manure (Sheppard *et al.* 1994; Newton *et al.* 2005), as well as municipal organic waste (Diener *et al.* 2009, 2011), few studies investigated their consumption of human faecal waste (Dang 2010; Lalander *et al.* 2013). This study aims to determine the efficiency of BSFL at consuming fresh human faeces, under different feeding conditions and feeding rates. Efficiency is determined by calculating waste reduction, bioconversion and feed conversion rates (FCR). The results will help optimise the way in which BSFL are fed human faeces, increasing waste reduction and prepupal biomass generation. The value of the various components of the prepupae could provide a source of income; the economic benefits through selling BSF products could be an incentive to communities, entrepreneurs, non-government organisations and governments to improve faecal sludge management.

Materials and methods

Black soldier fly larvae

The experiments were carried out at the London School of Hygiene and Tropical Medicine, UK. The BSFL used in the study were 18 days old, extra small Phoenix Worms™ (ISR, Georgia, USA). The larvae were kept in an inert material provided by the supplier that prevented the larvae from gaining weight. The BSFL were stored at 20 °C in an insectary at LSHTM until they were needed for the experiments.

Faecal sample collection

Ethical approval for sampling human tissue was obtained from the LSHTM Ethics Committee, and all experiments complied with current laws. Volunteers for the study were recruited from university staff and the general public. The project was explained to all volunteers by the author who conducted the experiment; and the volunteers signed an informed consent form. A collection kit, consisting of a sealable faecal collection pot, a pair of purple nitrile gloves, a ziplock bag and a large padded envelope, was given to volunteers at least 1 day prior to the sample being produced. The volunteers produced a faecal sample and contacted the author on the same day for collection. Samples were collected every 2 days from different volunteers throughout the experiment and stored in a refrigerator at 5 °C for a maximum of 48 h, until required.

Experimental design

The experiment used two feeding regimes: Feeding Regime 1 (FR-1) and Feeding Regime 2 (FR-2). In FR-1, the larvae were provided with fresh faeces every 2 days (incremental feeding) for 12 days. In FR-2, the larvae were only provided with one sample of faeces at the beginning of the experiment (lump amount feeding). The quantity of faeces added was calculated according to an optimal feeding ratio of 100 mg/food (faeces) larva/day, as determined by a study that fed BSFL on municipal organic waste (Diener *et al.* 2009, 2011), or an excess feeding ratio of 1000 mg/faeces larva/day. The feeding regimes were divided into three groups (A, B and C), which differed in larval density (1, 10 or 100 larvae per treatment) and feeding ratio (Table 1). Equal quantities of faeces, without larvae, served as controls for all groups within both feeding regimes.

Faecal and larval weights (Day 0)

Sterilised 50-ml falcon tubes (VWR International Ltd, Leicestershire, UK) were weighed and labelled for Groups A and B. Larger containers for Group C, autoclaved 324-ml glass jars (Jam Jar Shop, Telford, UK), were weighed and labelled to include Feeding Regime, Group and replicate number. Prior to distribution, the samples of human faeces were combined and mixed thoroughly in a large bowl to remove variation between samples. The mixed faeces was weighed (Oertling RB153, Birmingham, UK) and divided between treatment and control replicates. Larvae were counted and weighed on an analytical balance (Oertling NA114, Birmingham, UK) in groups for each treatment before adding to the faecal sample. Parafilm (Bemis Flexible Packaging, Oshkosh, USA) was stretched over the top of the containers to prevent the larvae from escaping and then perforated with a needle to allow the larvae to breathe. Treatment and control replicates were stored at 27 °C and 67% relative humidity (Diener *et al.* 2009, 2011) for 2 days in an incubator (GenLab, Widnes, England).

Faecal and larval weights (Day 2–12)

New falcon tubes and glass jars were weighed and labelled using the FR-1 nomenclature. Fresh faecal samples were mixed in a large bowl to remove variation between samples. The faeces were divided between the new treatment and control replicates, and the containers and faeces were weighed.

All FR-1 and FR-2 replicates were taken out of the incubator. The larvae were removed from the FR-1 treatment replicates, weighed and then placed in a new FR-1 treatment replicate, with a fresh faecal sample and the same identification number. Sample of larvae was removed from the FR-2 treatment replicates, counted, weighed and returned to their treatment replicates. The FR-2 control replicates were weighed. Parafilm was replaced over the top of the new FR-1 and original FR-2 treatment and control replicates and perforated with a needle. All treatment and control replicates were returned to an incubator at 27 °C and 67% relative humidity.

It was not necessary to wash the larvae before weighing, as it was shown in a preliminary experiment that washing did not significantly alter larval weight (Banks 2010). This process was repeated every 2 days for the 12 days of the experiment. Once the experiment ended, the larvae were removed from the treatment replicates. Once larvae developed into prepupae, indicated by a change in colour from white to dark brown (May 1961), they were removed from their treatments and weighed. The prepupae were then freeze-dried (Edwards Modulyo, West Technology, Bristol, England) until a constant weight was reached.

Table 1 Description of *Hermetia illucens* Feeding Regime 1 (incremental feeding), and Feeding Regime 2 (lump sum feeding) used in the experiment, includes group allocation, feeding ratio, number of larvae, quantity of faeces added, feeding occasions, total feed added and number of treatment and control replicates

	Incremental feeding (FR-1)			Lump sum feeding (FR-2)		
Group	A	B	C	A	B	C
Feeding ratio (mg/larvae/day)	1000	100	100	1000	100	100
Number of larvae (*n*)	1	10	100	1	10	100
Quantity feed added (g)	2	2	20	12	12	120
Feeding occasions (*n*)	6 (every 2 days)			1 (at beginning)		
Total feed added (g)	12	12	120	12	12	120
Treatment replicates (*n*)	40	40	6	40	40	6
Control replicates (*n*)	10	10	3	10	10	3

Statistical analysis

The data were analysed using Intercooled Stata 12.0 for Windows (StataCorp LP, TX, USA). Data were visualised using box-plot graphs, tested for normality using the Shapiro–Wilk test and, if necessary, and log-transformed. Percentage data were transformed, depending on range (Parsad 2005).

Analysis of variance (ANOVA) was used to determine significant differences between feeding regimes, and between groups within the same feeding regime. The variables comprised total feed added, total residue, waste reduction, larval mean weight, prepupal mean weight, percentage pupation and prepupal yield. The bioconversion and FCR were calculated for actual yield and an estimated 90% harvest.

Results

Larval development

Mean larval weight data were normal (Shapiro–Wilk $P > 0.05$) after the removal of outlying data points that were the result of a single larva that failed to thrive (E. Pieterse, personal communication). There were significant differences between mean larval weights in all three groups of the different feeding regimes (Figure 1). In Groups A and B, by day 6, the larvae in Feeding Regime 2 (FR-2) were highly significantly larger than in Feeding Regime 1 (FR-1) (Group A – $P < 0.0001$, $F = 23.69$, df = 1,45; Group B – $P < 0.0001$, $F = 117.04$, df = 1,48). In Group C, the larvae in FR-2 were significantly larger ($P = 0.0254$, $F = 8.00$, df = 1,7) by day 6, and highly significantly larger ($P < 0.0001$, $F = 98.15$, df = 1,7) by day 8. In Groups A and B, the majority of the FR-1 larvae had developed into prepupae by day 8. In Group A, it took further 4 days for the larvae in FR-2 to reach the same stage. In Group B, it took further 2 days for the larvae in FR-2 to reach the same stage. In Group C, the majority of prepupae in FR-1 had developed by day 10. Only 8.2% (Table 3) of the larvae in FR-2 reached the prepupal stage by day 12.

Waste reduction

Waste Reduction data were between 0 and 100%. The data were arcsine-transformed before an ANOVA was performed. All treatment groups had significantly higher waste reduction ($P < 0.0001$) than control groups, in both feeding regimes. The lowest treatment waste reduction (Table 2) was found in Group A; FR-2 (25.2% ± 0.80 SE). This was significantly lower ($P < 0.0001$, $F = 26.15$, df = 1,78) than FR-1 (33.4% ± 1.44 SE). In Group B, there was a significant difference between the feeding regimes ($P = 0.0032$, $F = 9.24$, $df = 1,78$), with FR-1 having higher waste reduction than FR-2; 49.7% ± 1.03 SE and 45.8% ± 0.73 SE, respectively. The highest waste reduction (FR-1 = 54.2% ± 0.86 SE, FR-2 = 54.6% ± 2.20 SE) was found in Group C, with no significant difference ($P = 0.8633$, $F = 0.03$, df = 1,10) between the feeding regimes.

Prepupal yield, bioconversion and feed conversion rate

Pupation data were between 0 and 100% and therefore arcsine-transformed before an ANOVA was performed. The

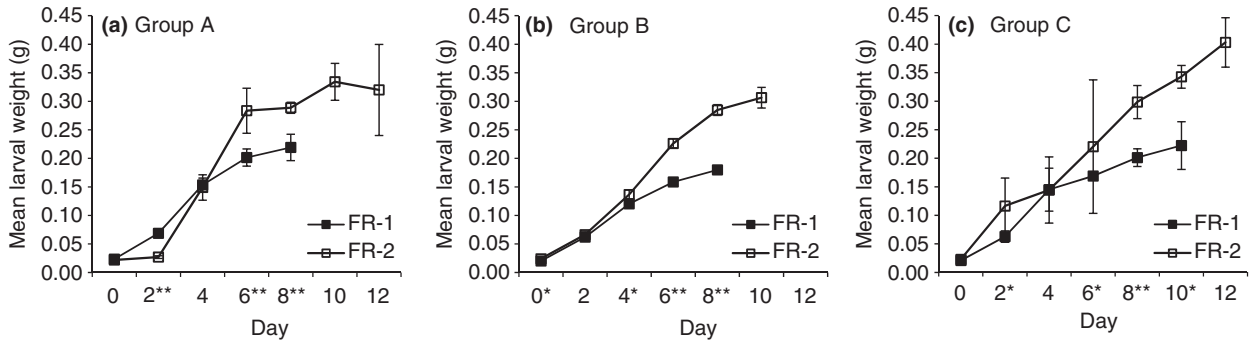

Figure 1 *Hermetia illucens* larval wet weight in grams (arithmetic mean ± 95% CI), over 12 days, for two different feeding regimes, FR-1 (filled squares), were fed fresh faeces every 2 days, and FR-2 (empty squares) were fed a large lump sum of faeces at the start of the experiment. Panel a contains Group A data from replicates ($n = 40$) of a single larva fed 1000 mg/faeces larvae/day. Panel b contains Group B data from replicates ($n = 40$) of 10 larvae fed 100 mg/faeces larvae/day. Panel c contains Group C data from replicates ($n = 6$) of 100 larvae fed 100 mg/faeces larvae/day. Day numbers followed by a * indicate significant difference ($P \leq 0.05$), and ** indicates highly significant different ($P \leq 0.0001$).

Table 2 Total and geometric mean (±SE) wet weight of feed added and residue remaining, and mean (±SE) percentage waste reduction, by wet weight. *P* values indicate statistical differences in waste reduction between groups in different feeding regimes, significant effects are in bold

		Feed added (wet weight)		Residue (wet weight)		Waste reduction (wet weight)	
Group	Feeding regime	Total (g)	Mean (g)	Total (g)	Mean (g)	Mean (%)	*P*
A	FR-1	390.3	9.8 ± 0.23	260.2	6.5 ± 0.20	33.4 ± 1.44	**<0.0001**
	FR-2	481.5	12.0 ± 0.04	360.3	9.0 ± 0.10	25.2 ± 0.80	
B	FR-1	436.5	10.9 ± 0.08	219.8	5.5 ± 0.12	49.7 ± 1.03	**0.0032**
	FR-2	482.5	12.1 ± 0.04	261.4	6.5 ± 0.09	45.8 ± 0.73	
C	FR-1	658.1	109.7 ± 1.43	301.1	50.2 ± 0.81	54.2 ± 0.86	0.86
	FR-2	720.5	120.1 ± 0.08	327.1	54.5 ± 2.67	54.6 ± 2.20	

prepupal weight was significantly higher in FR-2 for all groups than in FR-1 (Table 3). Group A, FR-2 had the largest mean prepupal weight of 0.3151 g. All groups showed high levels of pupation, with the exception of Group C, FR-2. Prepupal mean weight was significantly affected by Feeding Regime ($P < 0.0001$, $F = 68.03$, df = 1,158) and Group ($P < 0.0001$, $F = 13.60$, df = 2,158), but not by an interaction between Feeding Regime and Group ($P = 0.0646$, $F = 2.79$, df = 2,158). When bioconversion and FCR are calculated (Table 4) for actual prepupal yield, Group B, FR-2, had the highest bioconversion (22.9%) and most efficient FCR value of 2.0. The lowest bioconversion and least efficient FCR was in Group C, FR-2 (1.6% and 33.9, respectively),

Table 3 *Hermetia illucens* prepupal geometric mean (±SE) wet weight, and percentage of larvae reaching prepupal stage. *P* values indicate statistical differences in prepupal weight and pupation between groups in different feeding regimes, significant effects are in bold

		Prepupal wet weight		Pupation	
Group	Feeding regime	Mean (g)	*P*	Percentage (%)	*P*
A	FR-1	0.2258 ± 0.0078	**<0.0001**	92.5	0.4624
	FR-2	0.3151 ± 0.0124		87.5	
B	FR-1	0.1936 ± 0.0026	**<0.0001**	82.8	**0.0001**
	FR-2	0.2986 ± 0.0039		92.5	
C	FR-1	0.1998 ± 0.0034	**0.0023**	85	**<0.0001**
	FR-2	0.2410 ± 0.0098		8.2	

Table 4 Bioconversion and feed conversion rate (FCR) of *Hermetia illucens* converting human faeces into prepupal biomass, for actual prepupal yield, and estimated 90% yield. The most efficient bioconversion and FCR for actual and estimated yield are in bold

		Actual prepupal yield					Estimated 90% yield				
Group	Feeding regime	Prepupal weight (g)	Feed added (g)	Bioconversion (%)	Feed consumed (g)	FCR	Prepupal weight (g)	Feed added (g)	Bioconversion (%)	Feed consumed (g)	FCR
A	FR-1	8.5	390	2.2	130.1	15.2	8.3	390	2.1	130.1	15.6
	FR-2	11.3	482	2.3	121.2	10.7	11.6	482	2.4	121.2	10.4
B	FR-1	65.3	437	14.9	216.7	3.3	69.9	437	16.0	216.7	3.1
	FR-2	110.7	483	**22.9**	221.1	**2.0**	107.9	483	**22.3**	221.1	**2.0**
C	FR-1	104.8	658	15.9	357.0	3.4	107.9	658	16.4	357.0	3.3
	FR-2	11.6	721	1.6	393.4	33.9	130.7	721	18.1	393.4	3.0

followed by Group A, FR-1 (2.2% and 15.2, respectively). When prepupal total weight was estimated using a 90% yield, Group B, FR-2 maintained the highest bioconversion (22.3%) and most efficient FCR value (2.0); however, Group C, FR-2 had improved bioconversion to 18.1% and FCR efficiency to 3.0. The lowest bioconversion (2.1%) and least efficient FCR (15.6) were found in Group A, FR-1.

Discussion

The BSFL fed fresh faeces every 2 days developed into smaller prepupae (Table 3) faster than the larvae fed once at the beginning of the experiment (Figure 1). Based on the slower development and larger prepupae of the larvae fed once, it is theorised that there was a nutritional imbalance in the lump amount diet that led to an increase in consumption to compensate for deficient nutrients (Raubenheimer & Simpson 1997; Bennett 2000; Wright *et al.* 2003). Both proteins and carbohydrates are critical in the development of insect larvae (Bennett 2000; Nijhout 2003; Lee *et al.* 2004; Simpson *et al.* 2006). However, there are few data regarding the protein and carbohydrate content of fresh and ageing faeces. If pit latrine material is used as a proxy, with the top layer being fresh material, and lower layers aged material, the protein content of the material drops rapidly within the first 20 cm (J. H. J. Ensink & B. Torondel, unpublished data). The increase in development time and larval size supports the hypothesis that reduced protein content in the lump sum diet causes a nutritional imbalance that leads to compensatory feeding. If the ageing material is losing nutritional value over time, and the feeding rate of insect larvae is highest in the later instars, it is likely that the older larvae will need to consume more low nutrition feed than larvae fed fresh, nutritionally balanced feed.

Growth rate plasticity (Metcalfe & Monaghan 2001; Tu & Tatar 2003; Wright *et al.* 2003; Dmitriew & Rowe 2005; Dmitriew 2011) means that larvae are capable of successfully developing on a range of resources that may be transient in nature. Insect herbivores are known to increase their consumption of plant tissue when feeding on low-quality plants (Kondoh & Williams 2001), which increases developmental time and leads to higher vulnerability to natural predators. A slow-growth, high-mortality hypothesis has been proposed in Lepidoptera (Benrey & Denno 1997; Fordyce & Shapiro 2003; Medina *et al.* 2005; Cornellisen & Stiling 2006) and Coleoptera (Häggström & Larsson 1995). Growth rate plasticity indicates that BSFL could be capable of consuming pit material with a range of nutritional contents and still be capable of developing into valuable prepupae.

Waste reduction, prepupal yield and feed conversion rates

The results from this study were calculated using wet weight, meaning results can only be compared to studies that calculate wet weight waste reduction. It can be seen that waste reduction levels in Groups B and C are comparable (Table 5) to those found when BSFL feed on chicken manure (Sheppard *et al.* 1994). It is possible that dry weight waste reduction could compare to that found with BSFL feeding on municipal waste (Diener *et al.* 2011); however, those data were not collected in this experiment. The waste reduction in Group A was far lower; however, this is to be expected with only one larva present for each replicate.

The percentage pupation ranged from 82.8 to 92.5% (Table 3), excluding Group C, Feeding Regime 2. The low figures of pupation in this group (8.2%) could be due to competition between the larvae combined with reducing quality of feed. However, a higher rate would have been recorded if the experiment had lasted longer. Therefore, a 90% yield of prepupae was calculated to compare the FCR against previous research (Table 5). The bioconversion and FCR of the single prepupae in Group A were comparable to the rates found in previous studies (Sheppard *et al.* 1994; Newton *et al.* 2005; Diener *et al.* 2011). However, Groups B and C have higher bioconversion rates and lower FCR values than reported in previous studies. The high bioconversion rates show that BSFL are effective at reducing human faeces, and a low FCR indicates that the larvae feeding on the lower feeding ratio of 100 mg/larva/day are more efficient at converting fresh human faeces into biomass than swine manure, chicken manure and municipal organic waste.

Based on a yield of 90% prepupae, the high waste reduction and effective FCR results support the use of BSFL in human waste management. The prepupae can be collected for their protein and fat, taking advantage of their self-harvesting behaviour of crawling out of their feeding medium. This behaviour removes issues that arise from separating them from remaining residue. However, it is unlikely that all of the prepupae will self-harvest, suggesting alternative methods of prepupal collection must be investigated for large-scale waste management solutions. Also, with further research, the waste reduction could be optimised, resulting in less remaining residue.

In summary, the study has demonstrated that BSFL feeding on fresh human faeces can develop successfully. The largest prepupae are produced when given a large quantity of feed, resulting in prepupae of a higher mass than previous studies. The larvae are effective at waste reduction and converting the waste into a valuable

Table 5 Effect of different feed sources on *Hermetia illucens* mean (±SE) prepupal weight, waste reduction capacity, prepupal yield, bioconversion and feed conversion rate (FCR). The most efficient bioconversion and FCR results are in bold. Other data are from previous studies into swine manure (Newton *et al.* 2005), chicken manure (Sheppard *et al.* 1994) and municipal organic waste (Diener *et al.* 2011)

Feed source	Mean Prepupal weight (g)	Feed added	Residue	Feed consumed	Waste reduction, %	Prepupal yield	Bioconversion (%)	FCR
Swine manure*	N/A	68 kg	42 kg	26 kg	≈ 39	≈ 2.7 kg	3.97	9.6
Chicken manure†	0.220 ± N/A	5240 kg	≈ 2620 kg	2620 kg	≈ 50	196 kg	3.74	13.4
Municipal organic waste*	0.220 ± 0.008	151 kg	48 kg	103 kg	68	17.8 kg	11.78	14.5
Human faeces†,‡								
Group	Feeding regime							
A	FR-1	0.2258 ± 0.0078	390 g	260 g	130 g	33	8 g	2.1	15.6
	FR-2	0.3151 ± 0.0124	482 g	360 g	121 g	25	12 g	2.4	10.4
B	FR-1	0.1936 ± 0.0026	437 g	220 g	217 g	50	70 g	16.0	3.1
	FR-2	0.2986 ± 0.0039	483 g	261 g	221 g	46	108 g	**22.3**	**2.0**
C	FR-1	0.1998 ± 0.0034	658 g	301 g	357 g	54	108 g	16.4	3.3
	FR-2	0.2410 ± 0.0098	721 g	327 g	393 g	55	131 g	18.1	3.0

*Dry weight.
†Wet weight.
‡Estimated 90% prepupal yield.

biomass. These results support the use of BSFL in a human waste management solution. However, a number of issues still need to be addressed. It has been shown that BSFL are capable of consuming fresh human waste on a small scale. However, upscaling of this experiment is needed to test whether BSFL are capable of developing into prepupae at high densities. To help develop the technology for use in developing countries, more research needs to be conducted on the ability of BSFL to consume pit latrine waste. Previous research shows how BSFL development time varies depending on diet, feeding rate, temperature and humidity (Tomberlin *et al.* 2002, 2009; Diener *et al.* 2009, 2011; Holmes *et al.* 2012). Therefore, further research is needed to assess the growth rate plasticity of BSFL on low-quality diets like pit latrine material. The BSFL will have to be tested on material from different latrine types, with different physical and chemical characteristics recorded to determine effects on waste reduction and prepupal yield. Also, considering that the prepupal biomass could be used to feed animals that are part of the human food chain, it is important to assess the potential risks regarding bioaccumulation of heavy metals and contamination by pathogens.

Ultimately, BSFL have the potential to improve sanitation in developing countries by providing a way to process dangerous human faecal waste, with the benefit of having the prepupae produced have a value that could provide a source of income for communities or local entrepreneurs, while the remaining residue, if safe, may be used as a fertiliser or soil conditioner.

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