Research Article

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Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates

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Abstract

BACKGROUND: Black soldier fly larvae are converters of organic waste into edible biomass, of which the composition may depend on the substrate. In this study, larvae were grown on four substrates: chicken feed, vegetable waste, biogas digestate, and restaurant waste. Samples of prepupae and substrates were freeze-dried and proximate, amino acid, fatty acid and mineral analyses were performed.

RESULTS: Protein content of prepupae varied between 399 and 431 g kg^{-1} dry matter (DM) among treatments. Differences in amino acid profile of prepupae were small. On the other hand, the ether extract (EE) and ash contents differed substantially. Prepupae reared on digestate were low in EE and high in ash (218 and 197 g kg^{-1} DM, respectively) compared to those reared on vegetable waste (371 and 96 g kg^{-1} DM, respectively), chicken feed (336 and 100 g kg^{-1} DM, respectively) and restaurant waste (386 and 27 g kg^{-1} DM, respectively). Prepupal fatty acid profiles were characterised by high levels of C12:0 in all treatments.

CONCLUSION: Since protein content and quality were high and comparable for prepupae reared on different substrates, black soldier fly could be an interesting protein source for animal feeds. However, differences in EE and ash content as a function of substrate should be considered.

Keywords: black soldier fly; vegetable waste processing; feed; protein; fatty acid, amino acid

INTRODUCTION

European livestock production greatly depends on the import of protein resources. The most important protein-rich ingredient for terrestrial animal feeds is soybean meal.[1] However, cultivation of crops allocated to livestock, like soybean, puts pressure on land availability, particularly in tropical areas. Consequently, these areas are subjected to deforestation, threatening tropical forests that are reservoirs of biodiversity and provide key ecosystem services.[2] Besides this negative environmental impact, conventional protein resources are also becoming less favourable from an economic point of view. The growing demand for these ingredients has led to increased market prices over the last 5 years. Moreover, feed costs represent 60–70% of total production costs.[3] Therefore, the need for alternative protein sources for livestock is becoming increasingly urgent. Such an alternative protein source can be provided by insects. Moreover, some insects can be reared on organic waste streams and might have a favourable feed conversion efficiency because they are cold-blooded.[4]

Insects contain high amounts of energy, protein and essential amino acids, fatty acids and micronutrients (e.g. copper, iron, zinc).[3,5,6] The majority of insects has a crude protein content higher than 30% on a dry matter basis.[4,5,7] Makkar *et al.*[7] stated that insects might have an essential amino acid profile that matches with the required amino acid profiles of growing pigs and broiler chickens. In general, insects contain high amounts of lysine, threonine and methionine, which are major limiting essential amino

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acids in low-protein cereal- and legume-based diets for pigs and poultry.[3,5] Moreover, a recent study conducted by De Marco *et al.*[8] showed that insect meals derived from *Tenebrio molitor* and *Hermetia illucens* are valuable sources of apparent metabolisable energy and digestible amino acids for broiler chickens. Although insects are seen as an alternative protein source, they are also high in fat.[5,7] Several studies indicate that traditional protein and fat sources commonly used in feed formulation can be replaced by insects without adverse effects on animal performance and product quality.[9–12] The insect species with the highest potential for large-scale production are the black soldier fly (BSF) (*Hermetia illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*). These species can potentially be used to upgrade low value organic waste streams, of which globally an approximate amount of 1.3 billion tons per year are produced, into high-value protein.[13]

BSF larvae have already been formulated as a component of complete diets for poultry,[14] swine,[10] and for several commercial fish species.[15,16] They were found to support good growth and, therefore, it was generally concluded that BSF larvae can be a suitable protein source for animal feed. After completing its larval development, the insect enters the prepupal stage. In this stage, the larva stops feeding and empties its digestive tract. Then, the prepupae migrate in search of a dry and protected site in preparation for metamorphosis. The adults do not need to feed and rely on the nutrients stored from the larval stage.[17] In the present study, the nutritional quality of prepupae was determined. As compared to the larval stages, the prepupae might offer two advantages: (1) the prepupa empties its digestive tract, reducing the risk to carry pathogenic microorganisms; and (2) the prepupal migrating behaviour offers opportunities for harvesting in a scaled-up rearing system. Previously, it was demonstrated that the fat and ash content of BSF larvae were extremely variable depending on the type of manure they were fed on.[10,15,18] For proper application in least-cost formulation software, it is of paramount importance to understand the factors that contribute to the variation in nutritional value. So far, an in-depth analysis of the nutritional value of BSF larvae reared on commercially available vegetable waste streams has not been reported.

In this study, larvae of *H. illucens* were grown on three different vegetable waste substrates and a chicken feed diet, as a high-quality reference substrate. In order to evaluate the nutritional value for monogastric farm animals, the proximate and nutrient composition of the prepupae were investigated and relations with substrate composition were established.

MATERIAL AND METHODS

Rearing and harvesting

First instar *H. illucens* larvae were taken from a stock colony of the Department of Crop Protection, Faculty of Bioscience Engineering, Ghent, Belgium. Four different substrates were tested for their effects on the growth performance and composition of BSF larvae: chicken feed, vegetable waste, biogas digestate and restaurant waste. The chicken feed was a layer hen feed containing 155 g kg^{-1} crude protein on 'as is' basis (Legkorrel TOTAL 77; Aveve Veevoeding, Merksem, Belgium). Fresh vegetable waste was obtained from Ardo NV (Ardooie, Belgium), a company processing fruit and vegetables. The vegetable waste was a mixture of carrots, peas, salsify and celery. This waste is anaerobically fermented for energy production on the same site by Trevi NV (Belgium). The digestate resulting from this biogas fermentation was centrifuged into a liquid and a solid fraction; the latter was used as a substrate in this experiment. Restaurant waste was obtained from a student restaurant at Ghent University and contained potatoes, rice, pasta and vegetables. In accordance with EC Regulation No 1069/09,[19] none of the substrates contained animal products. Water was added to the chicken feed (70 mL 100 g^{-1} of substrate) in order to guarantee an optimal moisture content for growth of the larvae. This was not necessary for the other substrates, since their moisture content was deemed sufficient for optimal larval growth.

About 1000 of 6- to 8-day-old BSF larvae were placed in quadruplicate onto 600 g fresh (i.e. wet) material of each substrate in plastic buckets. The buckets (5.5 L) were screened with fine-mesh cotton gauze and covered with a lid provided with a single ventilation hole. The buckets were placed in a climatic chamber set at a temperature of 27 ± 1 °C and a relative humidity of 65 ± 5%. The larvae were subjected to a feeding regime of 600 g fresh material every 3 days, until they reached the prepupal stage. All larvae and prepupae were harvested 6 days after the first prepupae had appeared, which was about 2–3 weeks after the start of the experiment. After sieving the substrate, prepupae were collected manually using forceps. The remaining larvae were placed back in the same bucket and provided with fresh substrate. All prepupae were collected in three or four times with 6-day intervals following the above procedure. The collected prepupae were washed with tap water and frozen at −20 °C overnight. Then, they were vacuum packed and stored at −20 °C. Samples of fresh substrate were also collected, vacuum packed and stored at −20 °C pending chemical analysis. Total prepupal biomass was recorded per bucket and single harvest. Prior to analysis, prepupae and substrate material were freeze-dried until constant weight. While freeze-drying may result in less complete moisture removal compared to oven drying, this difference is minimal and freeze-drying guarantees a better preservation of nutrients. Since no significant differences were observed in prepupal yield (wet weight) and dry matter content between the buckets of a particular substrate, the freeze-dried prepupal biomass of the buckets was pooled per substrate. This pooling was done to obtain a sufficient amount of material to perform the numerous analyses.

Proximate analyses

The proximate analyses consisted of analytical determinations of water (moisture), crude protein, crude fat (ether extract), crude ash and crude fibre.[20] Moisture content was determined by difference after freeze-drying. Crude ash was determined by incineration at 550 °C for 4 h in a combustion oven.[21] Total nitrogen content was determined by the Dumas method.[22] Crude protein content in substrates and prepupae were calculated by multiplying total N by 6.25. According to Finke[23] this factor is acceptable for estimating the true protein content of most insect species. However, since the exoskeleton of insects contains chitin, a nitrogen-containing polysaccharide, this may lead to an over-estimation of the crude protein content.[24] Therefore, the chitin content was analysed using the procedure described in Liu *et al.*[25] In addition, the nitrogen content of the chitin fraction was determined and by subtracting the chitin nitrogen from the total nitrogen in the prepupae and multiplying this value by 6.25, protein values were corrected. Ether extract (EE), a measure for crude fat, was analysed gravimetrically after extraction with diethyl ether with a Soxhlet system.[26] Before Soxhlet extraction, hydrolysis with 3 mol L^{-1} HCl at 100 °C for 60 min was performed. All these analyses were done in duplicate.

The content of soluble, insoluble and total dietary fibre in the substrates was determined using the Megazyme total dietary fibre assay procedure k-tdfr 05/12.[27] This method is a simplified modification of the AACC total dietary fibre (TDF) method, 32–05.01, and the AACC soluble/insoluble dietary fibre method (for oat products), 32–21.0. These analyses were performed singly.

The non-fibre carbohydrate fraction was estimated by subtracting the sum of the other components from 100.

Amino acid, fatty acid and mineral composition

Amino acid, fatty acid and mineral composition was determined on freeze-dried prepupae and substrate material (single analyses). Amino acid composition of protein bound amino acids was determined by HPLC performed on oxidised and hydrolysed samples, following the procedure in 2009/152/EC. In addition, tryptophan was determined separately, since this amino acid is destroyed during acid hydrolysis.[28] Fatty acid composition was assessed by gas chromatography (GC) following the procedure described by Raes *et al.*[29] on the Soxhlet extracted fraction. The mineral composition was determined by ICP-OES.[30] The preparation included incineration at 450 °C until the ash was grey to red–brown followed by dissolving the ash in diluted nitric acid (7 mol L^{-1}).[31] The extraction of iron was performed separately using aqua regia.[32]

Statistical analysis

All data were analysed using SPSS 22.0 (2009) (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyse the data of the harvested prepupal biomass (wet weight) and moisture content. A post hoc Tukey (homoscedasticity) or Tamhane test (heteroscedasticity), based on the outcome of a Levene test, was performed to separate the means. To compare the mean development periods from the inoculation of the buckets until emergence of the first prepupae, a non-parametric Kruskal–Wallis test was used. When significant differences were detected, means were compared using Mann–Whitney *U* tests. Correlations of the composition of the substrates with that of the prepupae were tested using regression analysis. *P*-values below 0.05 were considered statistically significant.

RESULTS

Larval development and prepupal yield

Larvae reared on chicken feed developed at the highest rate (Table 1). After 12 days the first prepupae were observed in buckets containing chicken feed, whereas on vegetable waste and biogas digestate it took 15 days for the first prepupae to emerge. Larvae reared on restaurant waste developed the slowest with prepupae emerging not before 18 days. In addition, on the latter substrate it took approximately 4 weeks for all larvae to develop into prepupae, which was about 1 week longer than on the other substrates. The amount of substrate [on a dry matter (DM) basis] supplied to the larvae per bucket prior to the first harvest was 930 g, 1259 g, 534 g and 1019 g for chicken feed, restaurant waste, vegetable waste and digestate, respectively.

Chemical composition of prepupae and substrates

The DM content of the offered substrates was comparable for three of the four substrates with values between 243 and 262 g kg^{-1} (Table 2). Only the vegetable waste was substantially higher in moisture with a DM value of 127 g kg^{-1}. However, contents of protein, ash and fibre were highly variable among the substrates. The EE contents of the substrates were rather low (21–62 g kg^{-1} DM) with the exception of restaurant waste (139 g kg^{-1} DM). The chicken feed, vegetable waste and restaurant waste contained high amounts of non-fibre carbohydrates (425, 449 and 618 g kg^{-1} DM, respectively), whereas the digestate was almost completely deprived of non-fibre carbohydrates.

The DM content of prepupal biomass was comparable among the treatments, ranging between 381 and 410 g kg^{-1}. This was also the case for the protein content (399–431 g kg^{-1} DM) (Table 1). In contrast, EE and ash contents were significantly affected by the rearing substrate. Prepupae reared on digestate were low in EE (218 g kg^{-1} DM) and high in ash (197 g kg^{-1} DM) compared to those reared on the unfermented vegetable waste (371 g kg^{-1} DM EE and 96 g kg^{-1} DM ash). The chitin content was comparable for prepupae among the substrates, ranging between 56 and 67 g kg^{-1} DM. The chitin extracts contained between 60 and 62 g N kg^{-1}, which is comparable with commercial chitin used as a standard by Liu *et al.*[25] but lower than the content in pure chitin.[24] The corrected protein values were between 377 and 407 g kg^{-1} DM.

Table 1. Development time (starting from feeding organic waste, days), yield (g wet weight) and proximate composition of black soldier fly prepupae reared on different substrates

Parameter	Chicken feed	Digestate	Vegetable waste	Restaurant waste
Development time*	12.3 ± 0.5^a	15.0 ± 0.0^b	15.5 ± 1.0^b	19.0 ± 0.8^c
Yield*	219.8 ± 7.8^a	90.8 ± 3.6^c	140.3 ± 4.4^b	154.1 ± 5.1^b
Moisture*†	613 ± 8^a	614 ± 29^a	590 ± 10^a	619 ± 9^a
Crude protein‡	412 (0.6)	422 (1.4)	399 (0.2)	431 (0.6)
Chitin‡	62 (2.8)	56 (1.5)	57 (1.8)	67 (1.3)
Chitin corrected protein‡	388	401	377	407
Ether extract‡	336 (0.4)	218 (0.5)	371 (1.1)	386 (2.3)
Crude ash‡	100 (1.0)	197 (0.3)	96 (0.7)	27 (0.3)

*Means ± standard deviation within a row followed by different letters are significantly different ($P < 0.05$).
†g kg^{-1}, as is.
‡Means (and coefficients of variation) in g kg^{-1} dry matter.

Table 2. Proximate composition of the substrates used to rear black soldier fly larvae

Parameter	Chicken feed	Digestate	Vegetable waste	Restaurant waste
Moisture*	742 (0.0)	757 (0.2)	873 (0.3)	738 (0.7)
Crude protein†	175 (1.0)	246 (0.3)	86 (0.9)	157 (6.1)
Ether extract†	53 (0.8)	62 (0.5)	21 (13.5)	139 (0.5)
Crude ash†	115 (0.2)	299 (0.3)	108 (2.3)	45 (0.9)
Soluble fibre‡	57	5	5	0
Insoluble fibre‡	175	381	331	41
Total dietary fibre‡	232	386	336	41
Non-fibre carbohydrates	425	7	449	618

*Means (and coefficients of variation) in g kg^{-1}, as is.
†Means (and coefficients of variation) in g kg^{-1} dry matter.
‡Single analyses in g kg^{-1} dry matter.

Table 3. Amino acid profile of the tested substrates (Subst.) and black soldier fly prepupae (Prep.) ($g\ kg^{-1}$ dry matter)

	Chicken feed		Digestate		Vegetable waste		Restaurant waste	
Amino acid	Subst.	Prep.	Subst.	Prep.	Subst.	Prep.	Subst.	Prep.
Alanine	8.6	25.2	12.5	24.3	3.7	24.2	6.6	27.8
Arginine	10.6	20.3	9.6	20.3	5.0	20.0	7.3	19.9
Aspartate	14.4	37.8	21.7	33.6	15.6	35.9	14.5	36.9
Cystine	2.8	2.5	2.2	2.4	0.6	2.1	2.0	2.2
Glutamic acid	33.0	41.9	27.0	39.8	7.8	41.3	33.2	45.8
Glycine	7.7	22.6	10.6	22.6	3.0	22.2	6.0	25.2
Histidine	4.1	13.6	3.6	13.5	1.4	12.4	3.6	13.8
Iso-leucine	6.5	17.2	9.8	18.4	2.8	17.3	6.0	19.1
Leucine	13.8	28.6	15.5	29.5	4.6	28.0	11.1	30.6
Lysine	7.1	23.4	10.3	25.7	3.8	22.6	6.9	23.0
Methionine	3.1	7.6	4.1	8.7	1.0	7.6	2.8	7.1
Phenylalanine	8.2	17.0	9.7	18.7	2.9	16.3	6.8	16.4
Proline	10.4	22.5	8.3	22.1	2.9	21.4	10.9	25.1
Serine	7.7	16.6	8.0	15.5	2.8	15.0	6.9	15.9
Threonine	6.4	16.4	9.5	16.8	2.8	15.4	5.5	16.2
Tryptophan	1.5	6.7	1.6	6.2	0.9	5.8	1.8	5.4
Valine	7.9	24.1	11.7	24.9	3.4	24.8	7.3	28.2

The difference in proximate composition between prepupae reared on restaurant waste and those reared on vegetable waste was substantially smaller than the difference between the respective substrates. Moreover, the development of BSF larvae on vegetable waste was faster than on restaurant waste and less substrate had to be fed (on a DM basis). However, the final yield of prepupal biomass was higher for insects reared on restaurant waste.

There was no significant correlation between protein and EE contents of the substrate and those of the prepupae ($R^2 = 0.349$; $P = 0.409$ for protein and $R^2 = 0.054$; $P = 0.768$ for EE). However, a high correlation was observed between the ash contents of substrates and prepupae ($R^2 = 0.954$; $P = 0.023$). A high correlation was also found between the EE content of the prepupae and the non-fibre carbohydrate content of the substrate ($R^2 = 0.942$; $P = 0.030$).

Amino acid, fatty acid and mineral composition

The most prevalent essential amino acids in the prepupal biomass were lysine, valine and arginine, with levels between 20 and 30 $g\ kg^{-1}$ DM (Table 3). Despite substantial differences in amino acid composition of the substrates, differences in amino acid content of prepupae reared on different substrates were small. Lysine levels were between 23.4 and 25.7 $g\ kg^{-1}$ DM and all prepupae contained between 15.4 and 16.8 $g\ kg^{-1}$ DM of threonine. The contents of isoleucine and valine ranged from 17.2 to 19.1 $g\ kg^{-1}$ DM and from 24.1 to 28.2 $g\ kg^{-1}$ DM, respectively. Levels of other (semi) essential amino acids were 7.1 to 8.6 $g\ kg^{-1}$ DM for methionine, 5.4 to 6.7 $g\ kg^{-1}$ DM for tryptophan and 19.9 to 20.3 $g\ kg^{-1}$ DM for arginine.

The fatty acid composition of the prepupae was largely composed of saturated fatty acids [648–828 $g\ kg^{-1}$ fatty acid methyl esters (FAME)] (Table 4). Whereas prepupae fed digestate contained only 648 $g\ kg^{-1}$ FAME saturated fatty acids, those reared on the other substrates contained 774–828 $g\ kg^{-1}$ FAME. The EE of the former group was also rich in monounsaturated fatty acids as compared to the other prepupae (191 vs 95–120 $g\ kg^{-1}$ FAME). The levels of *n*-6 polyunsaturated fatty acids (PUFA) of the prepupae ranged between 46 and 120 $g\ kg^{-1}$ FAME, whereas the levels of *n*-3 PUFA were rather low, ranging from 9 to 23 $g\ kg^{-1}$ FAME. The fatty acid profile of the prepupae was characterised by high levels of C12:0. The EE of prepupae reared on chicken feed, vegetable waste and restaurant waste contained at least 573 g C12:0 kg^{-1} FAME, whereas prepupae fed digestate contained only 437 g C12:0 kg^{-1} FAME. Prepupae reared on digestate contained a significant amount of branched chain fatty acids.

Calcium levels were very variable ranging between 66 $g\ kg^{-1}$ DM for prepupae reared on digestate and 1 $g\ kg^{-1}$ DM for those fed on restaurant waste (Table 5). Comparable differences were also observed in the respective substrates (16 vs. 1 $g\ kg^{-1}$ DM). However, the calcium content of the prepupae was not always linked to that of their respective substrates ($R^2 = 0.179$; $P = 0.581$). For example, the calcium content of prepupae fed chicken feed was equal to that of prepupae reared on vegetable waste (29 $g\ kg^{-1}$ DM), while the calcium levels of the respective substrates were markedly different (29 vs. 7 $g\ kg^{-1}$ DM, respectively). The contents of the other minerals were all within a small range. Phosphorus levels ranged between 4.0 and 5.0 $g\ kg^{-1}$ DM while the potassium contents were between 5.9 and 6.8 $g\ kg^{-1}$ DM. The iron content of the prepupae was variable but was not related to that in the substrate ($R^2 = 0.511$; $P = 0.285$).

DISCUSSION

In this study, the influence of the proximate and nutrient composition of the rearing substrate on that of BSF prepupae was investigated. The substrates used were commonly available vegetable waste streams and a high-quality chicken feed as a reference. The total biomass of the harvested prepupae differed substantially among the four tested substrates. The total prepupal biomass was highest for chicken feed, and lowest for biogas fermentation digestate. Our results on digestate are comparable with those obtained by Li *et al.*[33] for prepupae reared on dairy manure. The 1200 larvae inoculated in their experiment yielded only 70.8 g prepupal

Table 4. Fatty acid composition of the tested substrates (Subst.) and black soldier fly prepupae (Prep.) (g kg^{-1} fatty acid methyl esters)

	Chicken feed		Digestate		Vegetable waste		Restaurant waste	
Fatty acid	Subst.	Prep.	Subst.	Prep.	Subst.	Prep.	Subst.	Prep.
C10:0	1.4	14.3	8.5	11.7	2.3	16.3	13.3	20.3
C12:0	14.5	573.5	97.5	436.5	21.3	608.9	154.9	575.6
C14:0	3.3	73.4	43.1	68.7	12.8	94.8	59.0	71.4
C16:0	160.0	96.5	236.3	101.2	305.2	87.0	231.2	102.9
C18:0	25.1	13.6	38.5	17.5	31.8	11.1	67.5	9.8
SFA	214.6	774.4	483.2	648.2	406.8	828.0	540.5	782.9
Iso- and ante-iso	0.5	1.0	80.3	64.6	4.6	7.1	6.0	2.9
C16:1	2.0	19.7	8.8	75.8	15.3	29.3	17.2	33.4
c9C18:1	239.6	75.4	119.3	79.3	66.0	56.6	251.3	79.7
c11C18:1	8.4	2.3	35.7	23.2	28.3	3.3	99.0	1.2
MUFA	255.3	100.1	189.8	190.8	119.6	95.4	289.4	119.9
C18:2*n*-6	499.9	115.5	163.5	79.0	312.2	45.2	138.3	78.3
n-6 PUFA	501.0	115.9	175.6	80.4	319.3	46.2	142.4	80.0
C18:3*n*-3	24.3	7.0	17.3	8.3	116.4	13.7	16.3	11.0
C18:4*n*-3	0.5	0.7	0.8	6.5	4.4	8.7	2.1	0.5
C20:5*n*-3	0.2	0.6	1.3	1.1	1.3	0.1	0.7	2.3
C22:6*n*-3	3.2	0.1	35.0	0.2	15.0	0.1	1.4	0.1
n-3 PUFA	28.5	8.6	71.1	16.0	149.7	23.3	21.8	14.3

Table 5. Mineral composition of the tested substrates (Subst.) and black soldier fly prepupae (Prep.) (g kg^{-1} dry matter)

	Chicken feed		Digestate		Vegetable waste		Restaurant waste	
Element	Subst.	Prep.	Subst.	Prep.	Subst.	Prep.	Subst.	Prep.
Ca	29.49	28.70	15.55	66.15	6.83	28.72	1.41	1.23
Cu	0.03	0.01	0.02	0.01	0.01	0.01	0.00	0.01
Fe	0.29	0.35	23.59	0.43	1.06	0.11	0.42	0.11
K	7.31	6.16	11.3	6.75	10.65	5.94	8.04	5.98
Mg	2.57	2.65	4.98	3.13	1.49	2.46	0.53	2.11
Mn	0.09	0.22	0.19	0.38	0.05	0.24	0.01	0.02
Na	1.62	0.67	6.32	0.89	8.39	0.60	8.12	0.68
P	5.56	4.99	15.35	4.44	2.39	4.04	2.37	4.08
S	0.73	0.20	4.54	0.31	1.11	0.18	0.51	0.11
Zn	0.12	0.16	0.10	0.05	0.07	0.07	0.02	0.07

biomass compared to the 90.8 g from the initial 1000 larvae in our study. On the other hand, the amount of dairy manure fed to the BSF larvae by Li *et al.*[33] was substantially lower than the amount of digestate used in our study (582 vs. 1019 g DM). Fresh vegetable waste showed to be the most favorable substrate in terms of substrate biomass fed (533.8 g DM) versus prepupal biomass yield (140.3 g DM). Since natural populations of *H. illucens* are adapted to decompose decaying organic materials (food waste, rotting fruit, plant litter, manure, etc.), this material is probably preferred. The slow development of larvae reared on restaurant waste may be due to the high amount of grease in the substrate, which could be observed on top of the substrates in the rearing buckets. According to Barry[34] grease is difficult to process for BSF larvae, leading to a prolongation of their developmental time.

The values for the protein content of the BSF prepupae are within the range of those reported in the literature (400–440 g kg^{-1} DM), whereas the variability in EE and ash contents could also be anticipated based on earlier reports.[7] However, the protein content may have been over-estimated by about 20–25 g kg^{-1} because of the presence of chitin in the prepupae. The chitin contents of the prepupae in this study are slightly lower than the contents reported in the literature, ranging from 75 g kg^{-1} DM (Finke[35]) up to 87 g kg^{-1} DM (Diener *et al.*[24]). This might be due to differences in the applied methodology. The presence of chitin in a commercial BSF meal may be of interest since chitin has been reported to negatively influence nutrient digestibility even at low inclusion levels in some fish species[36] and in poultry.[8] The differences in EE content can likely be explained by a higher synthesis of fatty acids, mainly C12:0, in larvae reared on energy dense substrates. Chicken feed and vegetable waste contained high levels of non-fibre carbohydrates whereas restaurant waste was rich in both non-fibre carbohydrates and EE. On the contrary, almost no non-fibre carbohydrates were present in the biogas fermentation digestate. This could be anticipated since most of the carbohydrates were likely used by microorganisms transforming them into methane during the fermentation process.

The amino acid composition of the BSF prepupae is similar to that reported in the literature for most amino acids, including those with relevance for animal feed, like lysine, isoleucine, threonine, valine and methionine.[10,12,15,16] Moreover, the levels of these essential amino acids in BSF prepupae appeared to be sufficient to comply with requirements for pigs[37] and poultry.[38] The possible deficiency in methionine + cystine and threonine reported by Makkar *et al.*[7] was not reflected in the amino acid profiles of the prepupae in our study. For tryptophan, another essential amino acid for pigs and poultry, only few data are available in the literature. Newton *et al.*[10,15] suggested a substantial variability in levels of tryptophan (2.0–5.9 g kg^{-1} DM) in BSF prepupae reared on different substrates. However, such a variation was much less pronounced in the prepupae harvested in the present study, containing between 5.4 and 6.7 g tryptophan kg^{-1} DM. In addition, this small range was observed for all amino acids of all prepupae in our study, suggesting that rearing substrate had no substantial influence on the amino acid composition of the prepupae. When the values of essential amino acids in BSF prepupae are compared with those of soybean meal with a similar crude protein content (440 g kg^{-1} DM), the profiles appear to be largely consistent.[37] Moreover, if BSF prepupae were to be defatted, which is the case for soybean meal, crude protein levels of over 60% could be reached. Consequently, such defatted prepupal meal would have an amino acid composition superior to that of soybean meal.

The fatty acid profiles of the prepupae are in line with those reported by several authors.[12,16,35] When comparing the fatty acid profile of the prepupae with that of the respective substrates they had developed in, it appears that the substrate only partially affects the fatty acid profile of the prepupae. Interestingly, lipids of the harvested prepupae were mainly composed of C12:0, even when the substrate contained this fatty acid only in trace amounts. This suggests that C12:0 in BSF was synthesised from other nutrients present in the substrate, such as carbohydrates (starch and sugars). This conversion of carbohydrates, which are a major component in the diet of various insect species, into lipids stored in their fat body, has been well documented.[39–41] Interestingly, appreciable amounts of branched chain fatty acids were found in prepupae reared on digestate. These fatty acids are mainly synthesised by bacteria and fungi,[42,43] suggesting that they originated from the anaerobic bacteria from the biogas fermentation.

A further element that can be mentioned in favour of the inclusion of BSF prepupae in poultry and pig feed, given their richness in C12:0, is the faster and more efficient absorption and metabolism of medium chain fatty acids (MCFA) compared to long chain fatty acids (LCFA) and their nutraceutical potential.[44] Skrivanova *et al.*[45] showed that C12:0 had the highest activity against *Clostridium perfringens* as compared to other MCFA. Furthermore, it had the lowest impact on the beneficial Lactobacilli. This mechanism could optimise performance and health of pigs and poultry by management of the microbiota in the upper part of the small intestine, which is dominated by Gram-positive bacteria. Even though these results have been obtained with specific fatty acid supplementation, the proposed effects can be mimicked with natural sources of these fatty acids such as insect meals. As in-feed antibiotics are banned in the EU since January 2006 (regulation EC/1831/2003) there is an increasing need for reliable in-feed antibiotic alternatives.[46] On the other hand, the high fat content of the prepupae could limit their application as a feed ingredient. Therefore, it could be interesting to partially extract the fat from the prepupal meal. Thus, a sufficient amount of C12:0 rich fat could be kept in the feed creating added value compared to soybean meal, while the extracted part could be useful as a high quality oil product, for example for the production of biodiesel.[33]

Calcium levels in BSF prepupae from our experiments were rather low compared to the levels reported by Makkar *et al.*[7] Only prepupae reared on digestate contained a value situated between the range reported in the latter study (50–86 g kg^{-1} DM). The high ash content of prepupae reared on the digestate compared to those reared on restaurant waste was mainly due to a much higher level of calcium in the prepupae. However, Finke[35] reported a more similar value to that of prepupae reared on restaurant waste (6 g kg^{-1} DM). The prepupal contents of other minerals with importance for animal feed, such as phosphorus, potassium and magnesium, appear to be unaffected by the rearing substrate. Moreover, phosphorus levels are in compliance with the requirements of pigs[37] and poultry.[38] On the other hand, a high ash content could also be undesirable for the use of BSF prepupae as an ingredient in a feed formulation. Since calcium levels of prepupae reared on digestate are well above the recommendations for pigs[37] and even layer hens,[38] using these prepupae could have certain drawbacks. This may be a concern especially in feed formulations for young animals like piglets. High feed calcium levels may increase the stomach pH, increasing the risk of bacterial infection.[47] Moreover, extracting fat from the prepupae, as suggested above, would raise their mineral content even more. However, prepupae reared on energy rich substrates with a low content of ash and fibre, like the restaurant waste, appear to have a very low ash content making them more suitable as a feed ingredient.

CONCLUSION

Our findings indicate that a rearing system of BSF larvae on vegetable waste streams could deliver a high quality insect resource with potential for being incorporated in animal feed. The quality of this resource would be constant in terms of crude protein content and amino acid profile, irrespective of the type of waste material the larvae were offered. However, fat and ash contents appear to be dependent on the rearing substrate. Larvae reared on energy dense substrates turn into prepupae with a high fat content, which is most rich in MCFA. This fat could provide an added value to the BSF prepupae in comparison to conventional feed resources. Future research should focus on how these prepupae can be incorporated in a feed formulation.

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