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A multitrophic culture system for the production of black soldier fly larvae (*Hermetia illucens*)

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Abstract
Goals number 2, 11 and 12 of the 17 sustainable development goals, enacted by the United Nations as part of the 2030 Agenda for sustainable development, aim to end hunger as a priority, create sustainable cities and above all encourage responsible consumption and production. With increasing world population and higher demand for food, we need to find ways of producing cheap sources of protein and lipid that may in turn be used as animal or aquaculture feed. A multitrophic system involving mealworm larvae (MWL, *Tenebrio molitor*) and black soldier fly larvae (BSFL, *Hermetia illucens*) was developed to transform fruit and vegetable kitchen waste into usable biomass. MWL, fed mainly on kitchen waste, reached an average prepupal length of 2.4 cm, fresh weight of 0.12 g and dry matter protein and lipid contents of 44.2% and 16.5% respectively, with an average specific growth rate (SGR) of 2.2%/day and a feed conversion ratio (FCR) of 7.9. Conversely, BSFL fed on a variety of feeds, including MWL frass, kitchen waste and oats, had an average prepupal length of 1.3 cm, fresh weight of 0.16 g and dry matter protein and lipid contents of 41.4% and 26.3% respectively, with an average SGR and FCR of 4.3%/day and 8.9 respectively. The BSFL fed MWL frass obtained some of highest SGR and lowest FCR values, with one group achieving 7.5%/day and 2.9 respectively. This investigation has demonstrated the feasibility of a multi-trophic production system using kitchen waste to feed MWL whose frass was in turn used to feed BSFL thereby producing protein- and lipid-rich biomass that can serve as animal or aquaculture feed.

Keywords Multi-trophic · Insect meal · Frass · Sustainability

1 Introduction

Current livestock feed production often depends on ingredients obtained from the unsustainable exploitation of natural resources. The demand for meat and thereby livestock products has quadrupled in the last 50 years, presently reaching more than 340 million tons of meat yearly [1]. Good agricultural land and cultivated crops are sacrificed to feed all of this livestock, making current food production strategies unsustainable. Twenty million tons out of around 90 million tons of global fish catch are used for fishmeal and fish oil production, with about 90% of this fraction otherwise being suitable for direct human consumption [2]. However, aquaculture alone uses 70% of the available fish meal as well as fish oil. It is predicted that the world population could reach 9.6 billion by 2050 [3] and consequently a 76% increase in meat demands [4] for the growing population in addition to changing lifestyle where the consumption of 'quickly-accessible' meat is increasing in popularity. A higher demand for meat translates into an increase in the use of ingredients for feed production which in turn calls for more efficient and sustainable methods of meat production, whilst the prices are kept low.

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Insect meal is considered a viable alternative ingredient in livestock feeds. Some insects can be utilised to transform organic kitchen waste, into usable biomass. The three insects currently of most interest are *Tenebrio molitor* or yellow mealworm beetle larvae (MWL), *Hermetia illucens* or black soldier fly larvae (BSFL) and *Musca domestica* or domestic housefly larvae (DHL). Insects require modest energy inputs compared to livestock production, with limited adverse impacts on the environment [5]. Additionally, insects thrive better when kept in high population densities in a confined environment, eliminating the requirements of massive production areas [6]. For instance, to produce the same quantity of protein as poultry, mealworms require 2–3 times less land [7]. The water footprint is also lower [8], and insects can gain more weight in relation to the amount of CO_2 produced compared to livestock [9]. Large-scale insect meal production is a relatively new industry, and the industry is still learning how to tackle the problems and constraints within the production process as it determines the best culture conditions and feed sources needed to optimise production efficiency and maximise its sustainability.

When produced commercially, MWL are fed on cereals as well as fruits and vegetables to provide additional nutrients and water. Different fruits and vegetables have different nutrient and water compositions that result in significantly different prepupae weights, whilst not necessarily influencing the growth performance of the mealworms in a significant way [10]. Optimizing the use of a combination of cereals and organic kitchen waste, increases survival rates and decreases the production time. Though a calorie-rich feed helps to boost MWL growth rate and pupal mass, it may result in higher mortality [11]. Additionally, a diet with an abundance of calories may result in reduced diseases resistance. Shapiro-Ilan [12] found that a diet heavy on lipids increased MWL susceptibility to nematodes [12].

Conversely, for the rearing of BSFL, the most popular feeding substrate utilised on a commercial scale is organic waste. Many studies have shown that BSFL growth is greatly influenced by the type of feed provided [13]. A high-protein-content feed averaging 21% protein [11], results in the best growth rate of the BSFL since they are not so efficient in breaking down low-protein (and consequently high-carbohydrate, high-fibre) feeds. They tend to develop fastest when reared on a high protein diet, with a significantly shorter developmental time, in contrast to the growth obtained with a poor protein diet [11]. Studies have shown that the rearing of BSFL and other insects produces significantly less ammonia emissions compared to production of other animals [9].

MWL frass is a potentially nutritious feed for BSFL. The nutrient composition of frass depends on what the MWL larvae have been fed upon. Gobbi et al. [14] showed that the diet provided to the BSFL greatly influenced the morphology of the adults and survivability of the larvae and the adults [14]. Thus, it is of utmost importance to identify the ideal feeding regime in order to improve mass-production.

To date, no studies involving BSFL fed on MWL frass have been published. The aim of this study was to investigate the potential of a multitrophic culture system utilising oats and organic kitchen waste as feed for MWL and in turn using their frass for the culture of black soldier fly larvae.

2 Materials and methods

2.1 *Tenebrio molitor* adults and larvae production

Tenebrio molitor adults obtained from a local supplier, were grown under natural light, were provided with oat flakes, which served as the laying substrate and further fed with organic kitchen waste predominantly consisting of melon, spinach and cabbage added ad libitum for additional moisture. The bottom of the breeding container was covered with 1 mm mesh, allowing eggs to fall into a bottom container also containing oat flakes. Humidity (measured with a Brannan In-door/Outdoor Thermohygrometer) for both adults and MWL, was kept within a range of 40–50%. Temperature was maintained with a heater in an insulated room, with an average temperature range of 25–29 °C. The MWL were additionally fed with fruit and vegetable kitchen waste ad libitum. Depending on the available kitchen waste at the time of feeding, the quantity provided depended on how long it took the MWL to consume that feed. Five batches of MWL (B1 to B5) averaging at 2000 larvae, were set up for the production of frass as consecutive runs, with each run using whatever larvae were available at the start of the experiment. The frass produced was collected throughout the MWL stages and stored in sealed containers at – 20 °C.

2.2 *Hermetia illucens* adults and larvae production

Adult *Hermetia illucens* were obtained from a local supplier and were kept indoors in a mesh cage measuring 200 × 200 × 200 cm, temperature was maintained at the 25–29 °C range with a heater in an insulated room, and room humidity kept in the 70–80% range using a shallow container filled with water. Lighting was provided by a broad -spectrum light bulb, with an L: D photoperiod of 9:15. Corrugated cardboard was used as the egg laying substrate suspended above moistened chicken feed as an attractant. On every third day, the cardboard containing the eggs were placed above the particular feeding substrates to be tested.

The feeds tested were the mealworm frass only (FR), frass and oats in a 1:1 ratio (FROT), commercial chicken feed (CF), oats (OT), kitchen waste (KW) consisting of vegetable and fruit rinds and a mix of bird seeds (SE). Depending on BSFL availability, for FR, CF and KW, two separate runs were carried out (FR1, 2; CF1, 2 and KW1, 2), whereas for FROT, OT and SE, a single run was carried out. To assess feed requirements, the larvae were emptied into a separate container, and any remaining feed noted. If there were no remains, it was concluded that all the feed had been consumed. This process was normally carried out late in the day, allowing sufficient time for the larvae to fully consume the provided feed. Thus, the larvae were provided with feed whenever the previous provision was fully consumed, in a feed to water ratio of 1:2. To follow the growth of the BSFL larvae, irrespective of the starting batch number, one hundred larvae were randomly taken from each batch to be weighed and measured at frequent intervals until the prepupal stage was reached. Experimental sampling was carried out and measurements were started when the larvae reached 0.06 g. Some of the prepupae were set aside to ensure a continuous source of reproducing adults whilst the majority were starved for 2 days and then killed by freezing at – 20 °C for subsequent nutrient analysis.

2.3 Nutrient analysis of MWL and BSFL

Moisture content of BSFL prepupae was determined by oven drying to constant weight at 100 °C, crude protein was analysed using the Kjeldahl method [15], crude lipid content was measured using the Randall method and crude ash by using a muffle incineration furnace at 500 °C.

2.4 Calculations and statistical analysis

Growth performance and feed utilisation were assessed using standard growth indicators, namely: percentage weight gain (%WG), percentage length gain (%LG), feed conversion ratio (FCR) and specific growth rate (SGR) according to Kolsater [16] for the MWL and according to Liland et al. [17] for the BSFL. The equations vary as for the MWL the initial weight was not known. Degradation (D), was calculated to find waste reduction index (WRI), using the equations used by Diener et al. [18]. The calculations are shown below:

Percentage daily wet weight gain; WG (%)/day:

$$\%\text{WG/day} = \frac{(\text{Final larvae mass(g)- Initial larvae mass(g)})}{\text{Initial larvae mass (g) x Time (days)}} \text{x } 100$$

Percentage daily length gain; LG (%)/day:

$$\%\text{ LG/day} = \frac{(\text{Final larvae length(cm)-Initial larvae length(cm)})}{\text{Initial larvae length (cm) x Time (days)}} \text{x } 100$$

Wet Feed conversion ratio; FCR:

$$\text{FCR} = \frac{\text{Total feed input (g)}}{\text{Final larval mass (g) - Initial larval mass (g)}}$$

Specific growth rate; SGR (%)/day: for the MWL (Calculated on a wet weight basis): [16]:

$$SGR = \frac{\text{Daily \% feed given}}{\text{FCR}}$$

For the BSFL [17, pg 746]:

$$SGR = \frac{(\text{lnFinal weight (g)- ln Inital wieght (g)) x 100}}{\text{Time (days)}}$$

Degradation (D) [18]:

$$D = \frac{\text{Total amount of feed (g) - Residue/frass (g)}}{\text{Total amount of feed (g)}}$$

Waste reduction index (WRI) [18]:

$$WRI = \frac{\text{Degradation}}{\text{Time (days)}} \text{x 100}$$

Efficiency of Conversion of Digested Food (ECD) [18]:

$$ECD = \frac{\text{Prepupal biomass (g)}}{\text{Total feed offered (g) - Residue/frass (g)}}$$

Statistical analysis of the results obtained with duplicate BSFL treatments was carried out using SPSS (Version 27, IBM). The statistical tests used were the Levene's test for homogeneity across the groups, Shapiro–Wilk test to test for normality and if they were normally distributed, ANOVA was applied to determine if there are any significant differences between the groups. If the values were not normally distributed, the Kruskall-Wallis test was used instead.

3 Results

3.1 *Tenebrio molitor* growth performance and nutrient analysis

Table 1 presents the performance parameters of the different batches (B1-B5) of MWL as well as frass outputs. The number of larvae in each run was the most abundant for B2, followed by B1, B4, B3 and B5, with an average population size of around 2000 MWL, which accounts for the different larval densities. The final weights achieved by the five batches were similar, even though the time taken for them to reach the prepupal stage varied considerably from 60 to 142 days. The mass of the larvae averaged around 0.12 g, with a 0.9–0.15 g low to high extremes for B5 and B3 respectively. The

Table 1 Performance parameters of the different batches of MWL

MWL batch	B1	B2	B3	B4	B5	Av	SD
Days to reach prepupal stage	60	90	142	109	88	97.8	± 30.27
Number of larvae	2077	3967	1576	1651	1198	2094	± 1092.43
Larval density[1]	3.15	6.01	2.39	2.50	1.82	3.17	± 1.65
Final total fresh larvae mass (g)	249.30	436.40	234.70	200.600	102.80	244.76	± 121.40
Final unit mass (g)/larva	0.12	0.11	0.15	0.12	0.09	0.12	± 0.02
Wet SGR (%/day)	3.33	2.22	1.41	1.83	2.27	2.21	± 0.72
Total feed given (g)	1464.40	2760.30	2329.80	1171.60	1188.00	1782.82	± 721.71
Average available feed/larvae/day (g)	0.01	0.01	0.01	0.01	0.01	0.01	± 0.002
Wet FCR	5.87	6.33	9.93	5.84	11.56	7.90	± 2.66
Average frass (g) /larva	0.15	0.14	0.23	0.12	0.08	0.14	± 0.06
% frass /total feed	20.69	20.82	15.68	16.40	8.16	16.40	± 5.15
D	0.79	0.79	0.84	0.84	0.92	0.84	± 0.05
WRI	1.32	0.88	0.59	0.77	1.04	0.92	± 0.28
ECD	0.21	0.20	0.12	0.20	0.09	0.17	± 0.06

[1]Expressed as number of larvae occupying 1cm^2 of the culture container

SGR was the highest for B1, followed by B5 and B2 and the lowest for B4 and B3. Although average feed available was the same for all batches, at 0.01 g per larvae per day, the FCRs varied considerably, with the lowest for B4 and B1 and the highest for B5, B3 and B2 respectively. The amount of frass produced per larvae, differed from one replicate to another, varying from 0.08 g for B5, to 0.23 g for B3, with an overall average of 0.14 g per larvae. Waste reduction index (WRI), was the highest for B1 and the lowest for B3. The efficiency of conversion of digested food (ECD) was the highest for B1, B2 and B4 whilst the lowest was for B3 and B5.

3.2 ***Hermetia illucens*** **growth performance and nutrient analysis.**

Table 2 shows production and performance parameters of the individual batches of BSFL provided with different feeds. The starting number of BSFL, which depended on what was available from the breeder, was the most abundant for OT, followed by KW2, FROT, CF1, KW1, CF2, FR2 and FR1, consequently resulting in different larval densities. The final weights achieved by the nine BSFL batches were similar, even though the time taken for them to reach the prepupal stage varied between 11 and 42 days. The average unit mass of the larvae fluctuated from 0.10 g for the FROT and KW1 batches to 0.29 g for the FR1 batch. The wet SGR was the highest for FR2, followed by OT and FR1 and the lowest for KW2, CF2 and KW1. The feeding rate differed from one feed type to another, with KW1, KW2 and OT having the highest feeding rates, FR1, FR2 and SE having the lowest feeding rates and FROT, CF1 and CF2 an intermediate feeding rate. These are reflected in the amount of feed consumed per batch. Consequently, the wet FCRs varied considerably, being lowest for OT and FR2 and the highest for KW 1 and KW2 treatments.

Table 3 shows the comparative data for the treatments carried out in duplicate, namely FR, CF and KW. The CF and FR treatments took the shortest period to reach prepupal stage compared to the FR treatment. The number of larvae was the highest for the KW treatment reflected as the highest final population larval biomass. However, the weight gain per larvae, was the highest for the FR feed. As a result, the FR treatment gave the highest %WG, %LG and wet SGR and consequently, the lowest wet FCR. Out of the three treatments, KW had the lowest SGR and the highest FCR. It also exhibited much lower percentage weight gain (%WG) and percentage length gain (%LG) compared to the FR and CF treatments. Conversely, the KW treatment showed the highest total feed intake and feeding rate per larvae. Overall, the FR treatment provided the most favourable growth conditions among these feeds. It resulted in the best optimal growth rates, highest percentages of weight gain and length gain, best growth per day, high specific growth rate (SGR), and the most efficient feed conversion to biomass, with a low FCR. The CF treatment had the lowest %WG and %LG and the lowest grams of food consumed per larvae.

Figure 1, which gives the growth as average weight of the BSFL growing on the different feeds until the prepupal stage was reached, the different curve gradients clearly show differences in growth rates between treatments. Figure 1 also

Table 2 Performance parameters of the different batches of BSFL according to feed provided

BSFL batch	FR1	FR2	FROT	OT	CF1	CF2	KW1	KW2	SE
Days	31	11	12	19	19	21	42	24	19
No of larvae	496	816	3500	9879	3500	1972	2189	5570	2172
Larval density[1]	0.23	0.39	1.69	4.76	1.69	0.95	1.05	2.68	1.05
Init. BSFL Wt. (g)	0.06	0.06	0.07	0.06	0.07	0.06	0.05	0.06	0.05
Final biomass (g)	144	109	350	1927	428	272	427	558	242
End BSFL Wt. (g)	0.29	0.13	0.10	0.20	0.12	0.14	0.20	0.10	0.12
%WG/day	12.36	11.55	4.29	11.56	3.97	5.89	6.72	3.41	6.55
SGR (%/day)	5.10	7.50	3.50	6.10	3.00	3.80	3.20	2.50	4.30
Initial length (cm)	1.20	1.10	1.30	1.30	1.30	1.20	0.80	1.00	1.10
End length (cm)	2.20	1.60	1.60	1.90	1.50	1.60	1.70	1.40	1.50
%LG/day	2.57	4.06	1.75	2.52	1.18	1.95	2.86	1.99	1.80
Total feed (g)	472	175	573	2290	1400	950	8098	6062	414
Feed rate (g/day)	15.20	15.90	47.80	120.50	73.70	45.20	192.80	252.60	21.80
Feed/larva (g/day)	0.03	0.02	0.01	0.01	0.02	0.02	0.09	0.05	0.01
Wet FCR	4.10	2.90	4.80	1.70	7.50	6.40	25.70	24.10	3.30

FR frass only, *FROT* frass and oats, *OT* oats, *CF* chicken feed, *KW* kitchen waste, *SE* seeds

[1]Expressed as number of larvae occupying 1 cm^2 of the culture container

Table 3 Average values of different performance parameters of the replicated batches of BSFL according to feed provided

	FR	CF	KW	p-value
Days	21.0 ± 14.10	20.0 ± 1.40	33.0 ± 12.70	0.506
No of larvae	656.00 ± 226.30	2736.00 ± 1080.50	3879.50 ± 2390.70	0.247
Final biomass (g)	126.50^{a} ± 24.80	350.00^{ab} ± 110.30	492.50^{b} ± 92.60	0.050
Initial BSFL weight (g)	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.354
End BSFL weight (g)	0.21 ± 0.11	0.13 ± 0.01	0.15 ± 0.07	0.613
%WG/day	11.96^{b} ± 0.57	4.93^{a} ± 1.36	5.07^{a} ± 2.34	0.034
Wet SGR (%/day)	6.30 ± 1.70	3.40 ± 0.57	2.85 ± 0.49	0.090
Initial length (cm)	1.15 ± 0.07	1.25 ± 0.07	0.90 ± 0.14	0.081
End length (cm)	1.90 ± 0.42	1.55 ± 0.07	1.55 ± 0.21	0.447
% LG/day	3.32 ± 1.05	1.57 ± 0.54	2.43 ± 0.62	0.223
Total feed (g)	323.50^{a} ± 210.00	1175.00^{a} ± 318.20	7080.00^{b} ± 1439.70	0.008
Feed rate (g/day)	15.55^{a} ± 0.49	59.45^{a} ± 20.15	222.70^{b} ± 42.28	0.009
Feed/larva (g/day)	0.03 ± 0.08	0.02 ± 0.001	0.07 ± 0.03	0.154
Wet FCR	3.50 ± 0.85	6.95 ± 0.78	24.90 ± 1.13	0.102[1]

Means in a row followed by superscript a or b are significantly different from each other ($p < 0.05$), whereas superscript ab is not significantly different to either a or b. Means followed by the same superscript are not significantly different. Results are presented as means with ± standard deviation

FR frass only, *CF* chicken feed, *KW* kitchen waste

[1]Kruskal-Wallis

[2]t-test (Frass, Chicken Feed)

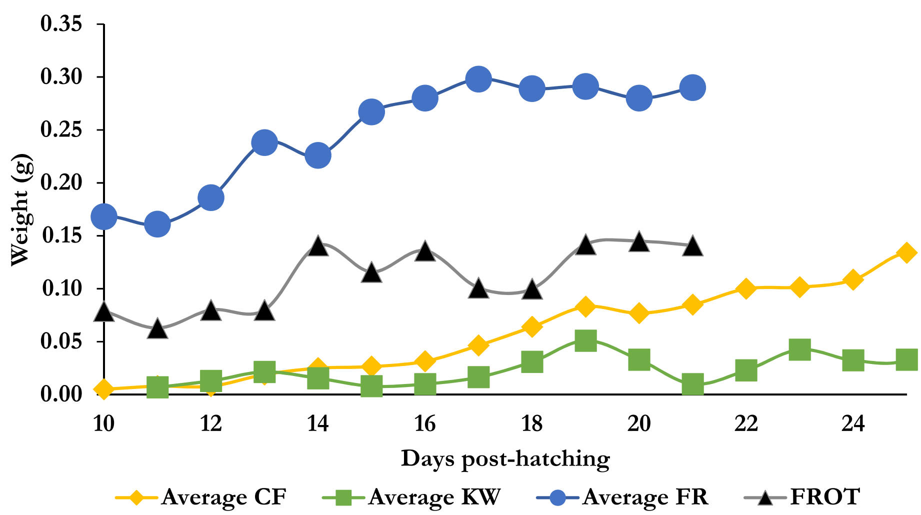

Fig. 1 Growth performance up to prepupal stages of BSFL on different feeds; *FR* frass only, *FROT* frass and oats, *CF* chicken feed, *KW* kitchen waste. Curves for oats and seed were comparable to those for chicken feed and kitchen waste. Data not shown. The * sign indicates when pupation occurred. Days-post hatching is shown until the 25th day, as growth progress for the remainder of the days was very similar to the first 25 days

shows that the number of days required to reach prepupal phase varied a lot between the different treatments being approximately 12 days shorter for BSFL feeding on frass (FR), frass and oats (FROT) as well as chicken feed (CF) compared to BSFL reared on kitchen waste (KW). The final weight and length of the larvae when this stage was reached, was also greater for these three previous feeds. There were also clear differences in the length and diameter of the prepupae obtained from each feed (Table 4); for the larvae reared on chicken feed (CF) an average length of 2.08 and a diameter of 0.70 cm were registered. This is compared to 1.74 cm length and 0.56 cm diameter for the BSFL reared on kitchen waste (KW).

FCRs were also very different between treatments, varying from 24.90 for the BSFL reared on KW to 3.50 for the BSFL reared on the frass. Statistical analysis of the BSFL batches fed FR, CF and KW determined that there was a significant difference in the %WG (Table 3), with BSFL fed on FR showing the biggest growth weight gain.

Table 5 gives the protein and lipid contents measured on a dry matter basis for all BSFL trials. Table 6 gives the average percentage values for protein and lipid content and standard deviation for the different feeds, tested in duplicates.

Table 4 The average lengths (L) and diameters (D) ± standard deviation of the BSFL at their prepupal stage were precisely determined using ImageJ 1.53e photographic image interpretation of the larvae against a mm scale

Feed type	Image	L (cm)	D (cm)
Frass (FR)		2.06 ± 0.07	0.66 ± 0.04
Frass + oats (FROT)		1.89 ± 0.11	0.62 ± 0.07
Oats (OT)		2.05 ± 0.12	0.63 ± 0.06
Chicken feed (CF)		2.08 ± 0.08	0.70 ± 0.04
Kitchen waste (KW)		1.74 ± 0.05	0.56 ± 0.02
Seed (SE)		1.77 ± 0.15	0.60 ± 0.05
	2 cm		

A scale bar of 2 cm is additionally shown. The largest length (L) and diameter (D) were obtained by the CF feed and the lowest dimensions were obtained by the KW feed

Table 5 Nutritional profile of all the BSFL fed on frass (FR), frass and oats (FROT); oats (OT); chicken feed (CF); and kitchen waste (KW) and bird seeds (SE)

BSFL batch	FR1	FR2	FROT	OT	CF1	CF2	KW1	KW2	SE
% Protein	38.40	49.7	41.25	39.95	40.30	39.35	39.95	38.40	45.20
% Protein (lipid-free)	55.25	59.24	60.39	54.10	56.28	53.39	50.06	54.78	62.20
% Lipid	30.50	16.10	28.40	26.30	20.20	29.90	26.20	27.30	31.70
% Ash	10.00	11.50	9.70	10.90	10.60	9.60	n.d	6.70	5.80

All measurements are on a dry weight basis. n.d, no data

Table 6 Nutritional profile of the BSFL fed on frass (FR), chicken feed (CF) and kitchen waste (KW)

BSFL batch	FR	CF	KW	p-value[2]
% Protein	44.05 ± 7.99	39.83 ± 0.67	39.18 ± 1.10	0.586
% Protein (lipid-free)	57.25 ± 2.82	54.84 ± 2.04	52.42 ± 3.34	0.353
% Lipid	23.30 ± 1.18	25.05 ± 6.86	26.75 ± 0.78	0.893
% Ash	10.75 ± 1.06	10.10 ± 0.71	6.70 ± 0.71	0.546

Results are presented as means with standard deviation in brackets. The highest % protein (including lipids) and % protein (lipid-free) content was obtained by the FR treatment. The other two treatments had lower values. Conversely, the % lipid content was the highest for the KW treatment and the lowest for the FR treatment. Percentage ash content was the highest for the FR and CF treatments, and lowest for KW

[2]t-test (Frass, Chicken Feed)

Standard t-test gave no significant difference in protein and lipid content for BSFL fed on FR, CF and KW. The highest % protein was obtained by FR2, whilst the highest % protein (lipid free) by SE. The lowest % protein content was obtained by FR1 and KW2, but FR1 had slightly higher % protein (lipid free). The highest % lipid was obtained by SE and the lowest by FR2; FR2 had the highest ash content and SE had the lowest ash content. The above results are further summarised in Fig. 2 which compares the nutritional profile of harvested BSFL prepupae on a dry matter basis to highlight that the FR treatment had the highest protein content (including lipid), as well as having the lowest lipid content. The KW had the lowest amount of protein content and the lowest amount of lipid content.

4 Discussion

The MWL achieved an average mass of 0.12 g which is comparable to the available literature; 0.13 g [19] and 0. 19 g [20]. The results obtained for the feed conversion ratio (FCR) in this study, which ranged from 5.8 to 11.6, were also similar to the values seen in other studies [11, 21, 22]. Different MWL batches generated varying quantities of frass,

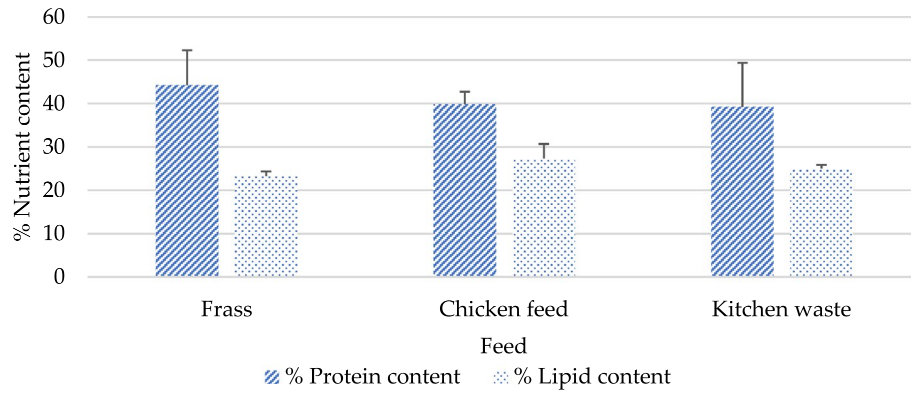

Fig. 2 Nutritional profile of harvested BSFL prepupae on a dry matter basis with the error bars showing the standard deviations

probably due to the varying quantities and types of wet feed. The waste reduction index (WRI) of the MWL was also calculated, as this describes the ability of the larvae to reduce feeding substrates; higher values show a greater ability to reduce the organic matter, with B1 being most efficient to break down the organic matter. The efficiency of conversion of digested food (ECD) refers to the efficiency of conversion of digested food. A large value indicates high food conversion efficiency, and once again B1 had the best efficiency. No literature data is available about frass production and use by mealworms. The frass generated by the MWL clearly contained significant nutritional value which enabled its subsequent usage as feed for the BSFL. The nutrient composition, as well as the bacterial and fungal content of the frass is determined by the original feed provided to the MWL [23]. As frass is commonly used as a fertiliser for plants, the main components of frass nutrient analysis are the nitrogen, phosphorus and potassium contents. As reported by [24] frass from MWL contains levels of nitrogen, phosphorus and potassium as high as those found in poultry manure. It has been shown that BSFL are capable of surviving and growing effectively on poultry manure [25], thus it is not surprising that MWL frass also has the capacity to sustain BSFL. Despite the numerical differences, there was no statistical difference in the specific growth rate (SGR) performances of BSFL grown on the different growth substrates (but there were significant differences in the %WG/day). SGR was the highest for FR2, at 7.5%/day. FR1 was the third highest at 5.1, only bettered by OT with 6.1. Chicken feed gave SGRs of 3.0%/day (CF1) and 3.8%/day (CF2). Generally, larvae with high SGR (more than 4.3%/day) had lower FCRs. In contrast, larvae that had low SGR (less than 4.3%/day) had higher FCRs. Therefore, those larvae capable of converting the most feed into biomass were able to increase their weights. This indicated that the frass and oats had a good nutritional profile. There were no significant differences between the different FCR values of the larvae fed the different feeds, but again there were noticeable variations. The BSFL batch with the lowest FCR was batch OT (1.7) fed on oats, and the highest was KW1 (25.7) fed on kitchen waste. Frass-reared larvae had FCR values of 2.9 (FR2) and 4.1 (FR1). Their FCR was one of the lowest amongst all the batches, signifying the efficiency of converting this frass into biomass. This result indicates the efficiency of converting this frass into biomass. One hypothesis for the favourable FCRs obtained with frass is that the frass was already broken down into fine particles aiding the digestion process of the BSFL. Because the MWL were provided with a variety of organic kitchen waste, the frass produced may have contained enough nutrients to sustain the BSFL. Whilst the best FCR result was obtained with oats, an excellent FCR of 1.7, possibly due to the very efficient way that BSFL use high protein content feed [11], the frass-fed larvae showed a much better FCR compared to the BSFL fed on organic kitchen waste in this study, and compared well overall with FCRs achieved in various publications of BSFL reared on organic kitchen waste which was found to be between 2.0 and 7.4 [11, 26, 27]. The high FCR obtained with the kitchen waste could be explained by insufficient nutrient content in the waste provided in this experiment or a consequence of the method of preparation involving blending which resulted in a high moisture feed. This resulted in a diminished accessibility of the complete nutrient profile in the waste due to its dilution with the water which in turn increased feed consumption by the larvae to compensate for the deficiency. Frass and oats demonstrated an ideal combination of low FCR and high SGR values, indicating their high nutrient content. This suggests that these feed sources efficiently support biomass production in larvae. A feed with such nutritional richness requires less consumption to fulfil larval nutrient requirements, as it facilitates efficient conversion into biomass without the need for excessive intake to compensate for nutrient deficiencies. Interestingly, the BSFL batch with the heaviest and longest prepupae obtained in the study was batch FR1, which was reared on frass, with an average larval length comparatively longer than what many studies have found with BSFL larvae reared on different feeds (not frass) [28–31]. In terms of production duration, the number of days taken by BSFL fed oats and average number of days taken by BSFL fed frass or chicken feed (21 and 20 days respectively) to reach prepupae were slightly longer than the time achieved by Liu et al. [10], but on par with other studies [13, 14]. In contrast, KW2 took 42 days to reach prepupae phase, although they were considerably heavier and longer than those of [13] and [32]. The statistical tests did not show up any significant difference between the nutrient contents of the various batches of BSFL. Frass reared larvae, on average, had the highest protein profile and the lowest fat content, with FR2 containing the highest protein value at 50.2% and the lowest fat content at 16.1%. These results suggest that frass-fed larvae, are on par with the commercial nutrient criteria of BSFL, which vary from 36 to 63% protein and 7–39% fat [33, 34].

For a multitrophic insect production system to be financially feasible, it has to generate a product from a species capable of sustaining itself with the waste products produced by another component of the integrated system [35, 36]. In any multitrophic system, it has to be ensured that the lower trophic level systems can sustain the higher trophic levels (Hughes and Black, [37]). Thus, for the upscaling multitrophic system investigated in this study, the MWL must produce enough frass to sustain a population of BSFL. In turn, the BSFL must have the nutritional profile to be used for its ultimate purpose, such as animal feed. This production goal has to be achieved in the shortest possible time with the maximum efficiency (maximum biomass with minimum FCR) and with minimum energy and space utilisation as possible.

Such a multitrophic system using frass is potentially a zero-waste system where all the nutrients are reutilised. The initial input is partly a waste product generated by humans and is converted into a commercially viable and valuable product. This form of multitrophic system diminishes the ecological impacts, serving as an essential solution to the global organic waste conundrum. Furthermore, any by-products, such as the frass produced by the BSFL, could themselves be used, for example, as a fertiliser [38]. The results obtained from this research can be used to calculate potential data pertaining to large-scale productions, keeping in mind that despite having a promising outcome, small-scale results might not necessarily be translatable to a large scale. Considering an annual BSFL production of 25,000 tonnes, and using the FCR obtained by the FR batches, about 87,500 tonnes of frass would be needed to grow 25,000 tonnes of BSFL. The average mealworm mass obtained here was 0.13 g, producing about 0.14 g frass. To produce 87,500 tonnes of frass about 81,250 tonnes of MWL would be required. The question arising is whether it is more advantageous to use the frass instead of raw organic kitchen waste directly to achieve the BSFL production goals. Using the frass on its own or as a supplement makes use of the waste product of MWL production and may stabilise BSFL production, in terms of nutritional profile and supply, once the system is fully characterised and established. Globally it is estimated that nearly half of the fruits and vegetables produced are wasted, amounting to 1.3 billion tonnes annually [39] so there is clearly no lack of raw materials whichever route is taken.

A better characterisation of the nutritional composition of the feeds provided to the MWL and BSFL would have enabled a better understanding of the growth performance and nutrient content of the larvae produced in this study. This would have provided in-formation on how the different feeds given to the MWL affected the nutritional quality of the frass produced, and subsequently the performance of the BSFL. Ultimately, this study has only touched upon the topic and other parameters, including daily feeding rate and larval density for example, need to be studied to understand and optimise the production parameters of such a multitrophic system.

5 Conclusions

The study demonstrated the viability and performance advantages of a multitrophic system utilizing MWL frass (FR) as feed for BSFL production, with the potential to create a zero-waste culture system. However, scaling up to an industrial level may pose challenges due to the large quantity of frass required, and further research is necessary to ensure the feasibility of implementing such a multitrophic system on a commercial scale. A mixed feed system incorporating FR and other organic waste feed as in FROT, may lend itself to a more feasible multitrophic system on an industrial scale.

Author contributions A.D. carried out the main experiments under supervision of J.B;A.D. and J.B contributed equally to the written manuscript; S.D. checked the advanced draft of the manuscript and made suggestions for improvement;. J.B., A.D. and S.D. reviewed the manuscript several times, Final corrections were made by J.B. and A.D.

Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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