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Bioconversion of faecal and kitchen waste using black soldier fly larvae (*Hermetia illucens*): Mass Balance process

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ABSTRACT

Novel technologies to convert faecal waste into valuable nutrients provide a win-win situation in enhancing the closed sanitation loop, and providing safe sanitation. In this study, laboratory-scale experiments were set to examine the applicability of the Black Soldier Fly in bioconversion of organic matter (faecal matter (FM) and kitchen waste (KW) while producing larvae biomass rich in protein and fat compounds. To determine the mass balance process, each of the feed substrates (500g) in triplicate and 3 grams of 6-day-old larvae (844 larvae) were introduced. The larval developmental time to 50% pupation, survival rate (SR), waste reduction rates (WR), prepupal yield, bioconversion rate (BCR), feed conversion rate (FCR), and efficiency of digested feed (ECD) were monitored in triplicate at the end of the experiments for mass balance process. Mass balance determination (triplicate) yielded average prepupal yield of 70.43±0.02g and 56.77±0.01g, with protein content per unit ranging from 32.23% to 41.26% and 20.06% to 37.13% on faecal and kitchen waste respectively. The ECD of 17.63±0.01% and 12.05±0.00%, waste reduction of 79.91% and 92.24% from faecal waste and kitchen waste respectively were obtained. From the findings, both substrates were palatable as BSFL feeds. The study findings show the potential of using BSF larvae technology to valorise faecal and kitchen waste and produce larval biomass rich in proteins and fats.

Introduction

The amount of organic waste is exponentially increasing with regard to the improved standards of living and population growth putting immense pressure on the existing sanitation infrastructures and the environment making faecal sludge management a challenge (Capone *et al.*, 2020). Engineers has focused on conventional sewer systems, which due to their high capital, maintenance and operational cost and their reliance on vast amounts of water have proved to be less practical in developing countries (Matheka *et al.*, 2021). Onsite sanitation has thus been the predominant form of excreta management serving about 2.7 million people globally (Harada *et al.*, 2016). However, the high dependence on onsite technologies along with minimal technological development for faecal waste

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management has caused a sanitation crisis (Riungu, 2021). As a result, 50% of the faecal generated in developing countries is disposed haphazardly into the environment causing contamination, eutrophication, global warming and surcharge drainage systems (Seleman *et al*., 2019). In addressing the faecal waste menace in developing countries, greater attention should be placed on innovating sustainable technological options that couple the safe management of waste and promotes a closed-loop economy.

Resource-oriented approaches have been embraced including black soldier fly, co-composting and vermicomposting. BSF treatment has been used in the conversion of several types of organic waste such as faecal waste (Nyakeri *et al*., 2019), restaurant waste (Pérez-Pacheco *et al*., 2022) and fruits and vegetables (Hopkins *et al*., 2021). The larvae convert organic waste into high-value products which can serve as an excellent source of protein in animal feed and their saprophagous feeding activity reduces the waste dry mass (residue) significantly (Lalender *et al*., 2013). The BSF can develop into larvae or prepupae containing up to 33% fat and 36% to 48% protein (St-Hilaire *et al*., 2007). Furthermore, literature has reported waste reduction rates ranging from 43%-73% on human faeces (Siddiqui *et al*.,2022) and bioconversion rates ranging from 11%-31.8% making BSF a promising alternative in organic waste management (Gold *et al*., 2020; Lalander *et al*., 2013).

The mass balance process converts waste into larval biomass and leaves behind a residue that can be used as a soil conditioner. The approach divides the total feed consumed by larvae into the undigested material mass (residue), the mass of the diet feed used for homeostasis and the larval biomass harvested (Manurung *et al*., 2018). For instance, feeding of 200mg/larva/day using 1110.4mg DM resulted in 30.8 mg (2.77%) larval biomass increase with protein content of 25.7%, leaving 1002.08mg (90.24%) of the residue and 77.52mg (6.98%) of metabolism biomass in 20 days (Supriyatna *et al*., 2018). Yang and Liu (2014) study recorded 9.58-9.64% larval biomass and 74.45-83.71% residue using 3250 eggs of *Chrysomya megacephala* in the treatment of 1 kilogram of pig manure. The mass balance process designs the system for biomass production and helps predict diet digestibility (Kinasih *et al*., 2018). However, the fate of nutritional spectra is poorly understood. There is also a maximum amount of feed that larvae digest within a certain developmental time before they pupate (Manurung et al., 2016). It is therefore important to understand the flow of energy during the treatment process to determine how suitable is the BSF treatment technology.

Materials and Methods

The study was carried out at Meru University of Science and Technology Sanitation Research Institute.

Black Soldier Fly

The black soldier fly larvae were obtained from the Meru University of Science and Technology Sanitation Research Institute (MUST-SRI) breeding unit. The study utilized 6-day-old larvae to reduce mortality and ease handling during the early stages of the experiment.

Faecal Waste Substrate Collection

The fresh human faecal was obtained from a container-based sanitation facility installed in MUST-SRI. A 20-litre container was used in the facility for faecal collection with about 10g wheat bran added after every toilet use to reduce odour and keep it appealing for the next user. The faecal matter was collected 2 days interval where used containers were swapped with clean ones. The collected faecal matter was thoroughly mixed using a wooden rod for homogeneity then weighed and transferred into the experimental feeding troughs for the treatment process.

Kitchen Waste Substrate Collection

Kitchen waste comprising of equal proportion (0.3kg each) of food remains (rice, ugali, meat,

sweet potatoes, Irish potatoes, beans, green grams and chapatti), fruits (bananas and oranges), and vegetable waste (cabbage, kales and tomatoes) were obtained from MUST student cafeteria. The fruits and vegetables were chopped into small sizes (1cm) to reduce the surface area to volume ratio to allow BSF larvae to work on them well. After this, the kitchen waste constituents were thoroughly mixed to achieve a unified substrate composition.

Experimental Set-Up

A quantitative (laboratory) experimental study was applied the to determine mass balance process across the treatment process. Two treatments (3 replicates each) were set up for both kitchen and faecal waste in plastic troughs measuring (18 x 9 x 6 cm). Moisture content was analysed prior to the treatment to ensure that the substrates were within the optimal BSF working range (60-85%) using Eq.1 according to Meneguz *et al.* (2018). The fresh samples of (kitchen waste and faecal waste) were analysed for pH using a PHSJ-3F pH meter (MK900-CN, China). A 500g of each unified substrate (kitchen and faecal waste) was supplied in the feeding troughs in batches and treated using 3g of six-day -old larvae. The duration of the treatment experiment was ascertained by the time 50% of the larvae reach maturity. The substrate was characterized for protein, fat, carbohydrates and ash at the start and end of the experiment. The larvae were manually separated from the frass (residue) after maturity (turned into prepupa). The harvested eggs were collected after 2 days and the shells that were left behind after adult emergence were also collected, oven dried for protein analysis as by formulae shown in figure 1.

$$Moisture\ Content\ (\%) = \left(1 - \frac{Dry\ weight - Crucible\ weight}{Sample\ weight}\right) \times 100 \quad \text{.....} \quad \text{Eq.1}$$

Larval survival rate (SR), larval development time, prepupal yield, waste reduction rates (WR), bioconversion rate (BR), and efficiency of digested feed (ECD) were used to determine the mass balance of the BSF treatment process.

$$Survival\ rate\ \% = \frac{\text{Larvae.end}}{\text{larvae.beg}} \times 100\% \quad \text{.....} \quad \text{Eq.2}$$

Where; Larvae. end number of larvae at the end, Larvae. beg-number of larvae at the beginning (Van Der Fels-Klerx *et al.*, 2016).

Waste reduction rate was calculated using Eq. 3.4 (Mertenat *et al.*, 2019).

$$Waste\ Reduction\ \% = \left(\frac{Feed\ Concumed\ (g)}{Initial\ feed\ weight\ (g)}\right) \times 100 \quad \text{.....} \quad \text{Eq.3}$$

The Bioconversion rate was calculated using Eq. 3.4 (Dortmans *et al.*, 2015).

$$Bioconversion\ Rate\ \% = \left(\frac{Larval\ Yield\ (g)}{Total\ feed\ applied\ (g)}\right) \times 100 \quad \text{.....} \quad \text{Eq.4}$$

The ability of BSF larvae to digest faecal matter and kitchen waste will be determined through the Efficiency of digested feed (ECD) based on Diener *et al.* (2009)

$$B = (I - F) - M$$

$$ECD = B/(I - F) \quad \text{.....} \quad \text{Eq.5}$$

Where; B-total amount of feed used for growth, I-total amount of food offered during the experiment, F-total amount of residue (undigested food and excretory food), M-amount of food metabolized by larvae (Calculated by mass balance)

Figure 1: *Formulae for protein analysis*

	Age	pH	MC (%)	Protein (%)	Fat (%)	Carbs (µg/ml)	Ash (%)
FM	0	6.27±0.00	73.83±3.4	29.21±0.06	12.5±0.00	0.91±0.16	20.00±0.00
	22	8.81±0.00	53.28±2.64	9.61±0.10	2.97±0.21	0.15±0.02	8.48±0.09
KW	0	3.09±0.00	77.14±0.16	17.42±0.09	20.01±0.00	1.22± 2.82	9.67±0.00
	22	6.24±0.16	54.34±0.70	8.72±0.00	4.42±0.33	0.66±0.06	1.44±0.00

Table 1: *Chemical composition of feed substrates*

pH, moisture content (MC), crude protein, crude fats, ash and carbohydrates in kitchen waste substrate were measured on dry weight. fm-faecal matter, kw-kitchen waste, day 0-initial day, day 22-final day

Chemical analysis

The feed substrate was analyzed for protein, fat, carbohydrates and ash content where protein content was determined by a micro Kjeldahl method using a standard conversion factor of 6.25 (Barragan *et al.* 2017) and ether content was determined using the Soxhlet method (Lalander *et al.* 2013). Ash content was gravimetrically determined after sample incineration by a muffle furnace (Model: JK-SX2-5-12N).

Results and discussion

Both the substrates (faecal and kitchen waste) displayed proper growth conditions for the BSF larvae with the initial crude proteins of 29.21% and 17.43% which reduced to 9.61 and 8.72% on DM basis for faecal waste and kitchen waste respectively. Crude protein is an essential macronutrient in BSF larval growth and development and it is necessary for supporting the accumulation of protein in BSF body cells. The initial fat content was 12.50% and 20.01% which reduced to 2.97 and 4.42% on a DM basis respectively. The substrates' ash content is an important parameter for measuring nutrient content reduction (Matheka *et al.*, 2021). See Table 1.

The ash is the inorganic and indigestible component of a substrate, and in the faecal matter, it arises from non-digestible nutrients like vitamins (Rose *et al.*, 2015). The ash recorded for this study was 20.00 and 9.67% which reduced to 8.48 and 1.44% on DM for faecal waste and kitchen waste respectively.

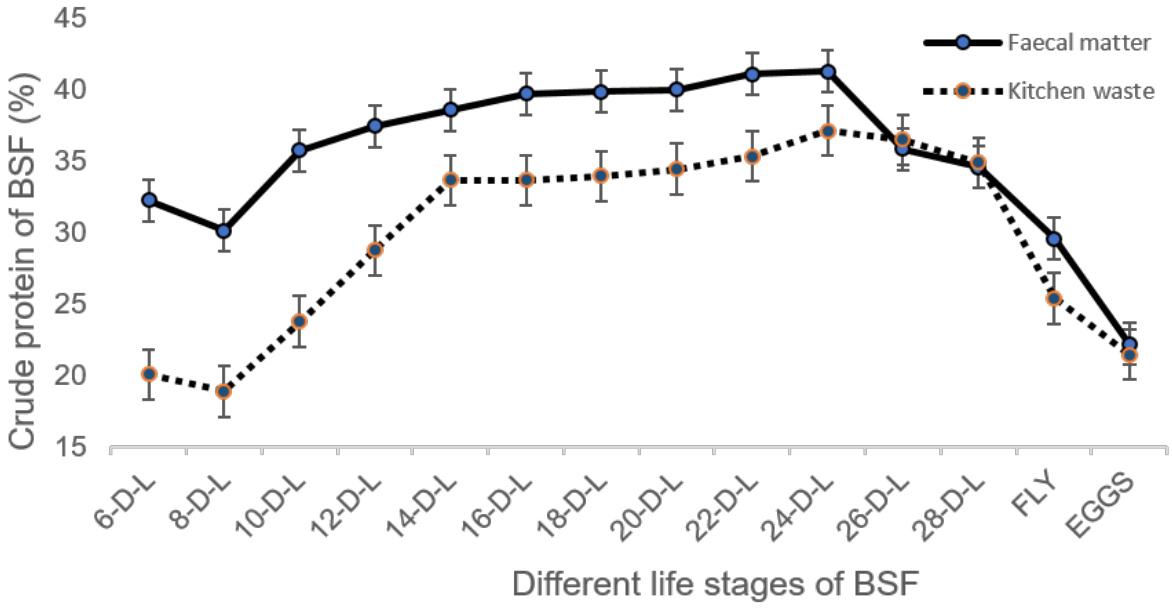

Figure 2: *Changes in BSF protein content during the life cycle*

Changes in BSF nutritional content during the life cycle. The nutrient was calculated in dry weight, D-L = Day-old- Larvae

The Dynamic fluctuations of the protein content of the BSF life cycle

The nutrient variation of the entire black soldier fly life cycle (egg-adult) reared on faecal and kitchen waste was recorded in Table 2. The crude protein content of BSF reared on faecal waste fluctuated non-uniformly from day 0 to day 22, with day 0 recording 32.23% and reducing to 30.11% on day 2. However, the protein increased from day 4 (35.73%) steadily to day 18 (41.26%) reaching its peak and then reducing to 34.55% in day 22. The same trend was exhibited from BSF reared on kitchen waste with an initial CP of 20.06% on day 0 which increased from day 2 (18.88%) steadily to day 18 (37.13%) reaching its peak and then reduced to 34.90% on day 22 as illustrated in figure 2.

BSF performed well on both feed substrates with the larval and prepupal protein content ranging from 32.23% to 41.26% and 20.06% to 37.13% on faecal and kitchen waste respectively.

Mass Balance model of the treatment process

Mass balance indicates the flow of energy from the feeding substrates into larval biomass, the residue and metabolic processes (Supriyatna *et al*., 2018). This helps the researcher to know the mass flow (what enters and leaves the treatment system). The experiment used 3.0 grams of larvae (844 larvae) on 500 grams of feed substrates (faecal and kitchen waste).

Faecal waste recorded a mass balance of 70.43g (14.09%) of larval biomass increase leaving behind 100.43g (20.09%) of residue. During the treatment process, the larvae converted 329.14g (65.83%) of the initial mass into metabolic processes whereas kitchen waste recorded a mass balance of 56.77g (11.35%) of larval biomass increase leaving behind 28.79g (5.75%) of residue and larvae converted 414.44g (82.9%) of the initial mass into metabolic processes as shown in Figure 3. The present study findings from faecal matter varied with Bank *et al*. (2014) values of 108g (wet weight) of prepupal yield, 260g of residue after treating 480kg (ww) of fresh human faeces. Findings on kitchen waste differed with Diener *et al*. (2011) results of 17.8kg (DW) of prepupal yield, 48kg (DW) of residue from the treatment of 151kg (DW) of municipal organic waste, and Supriyatna *et al*. (2018) values of 200mg/larva/day feeding rate using 1110.4mg DM of BSFL on cassava peels resulted in 30.8 mg (2.77%) larval biomass increase, leaving 1002.08mg (90.24%) of residue and larvae converted 77.52mg (6.98%) to metabolism in 20 days. The lower larval biomass reported by Supriyatna *et al*. (2018) could be ascribed to casava peels used which contained higher amount of lignin-cellulose causing poor substrate digestibility which could have affected the feeding of larvae leading to lower total larval biomass and a lot of residue.

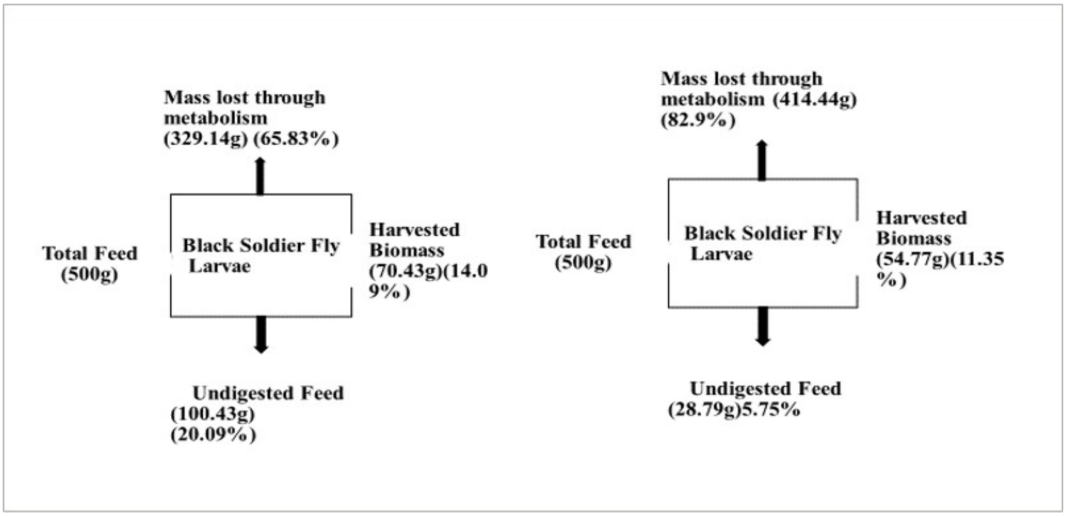

Figure 3: *Mass balance across the treatment process,= Mean ± standard deviation (n = 3)*

Parameters	Faecal waste	Kitchen waste
SR	99.53±0.002	98.50±1.74E-14
Larval biomass	70.43±0.02	56.77±0.01
Waste reduction	79.91±0.01	94.24±0.03
Bioconversion rate	14.09±0.00	11.35±0.00
Residue	100.43±0.04	28.79±0.17
FCR	4.67±0.00	7.30±0.00
Metabolism	329.14±0.06	414.44±0.16
Digestibility (ECD)	17.63±0.01	12.05±0.00
Time	22±0.00	22±0.00

Table 2: *Effects of Rearing substrates on BSF larvae survival rate, Waste Reduction, Bioconversion rate, feed conversion rate (FCR), digestibility (ECD), metabolic process and pre-pupal yield, Mean ± standard deviation (n = 3)*
SR- survival rate, WR-waste reduction, BCR-bioconversion rate, FCR-feed conversion rate
*All parameters were measured on wet weight

Parameters on Mass Balance Process

The survival rate, larval weight gain, waste reduction, bioconversion rates, feed conversion rates, digestibility, prepupal yield, developmental time and residue were observed as shown in Table 2

Waste Reduction Rates

The waste reduction rate is an important parameter in the recycling of waste as it indicates the ability of larvae to upcycle organic waste. Waste reduction was higher on kitchen waste (94.24%) on faecal matter and (79.91%) on kitchen waste on wet basis. The present findings on faecal waste reduction rates differed with 83.3% from faecal (Matheka *et al*., 2021), 73% from faecal sludge (Lalander *et al*., 2013). The variability in feeding regimes either batch (Matheka *et al*., 2021) or continuous (Gold *et al*., 2020) and source of faeces could be the source of variations. Kitchen waste reduction rate varied with waste reduction rates of 65% for kitchen waste (Liu *et al*., 2018), 85% for food waste (Chirere *et al*., 2021), 53.7% for kitchen waste (Nana *et al*., 2019).The influence of the number and amount of feed could be a source of variations

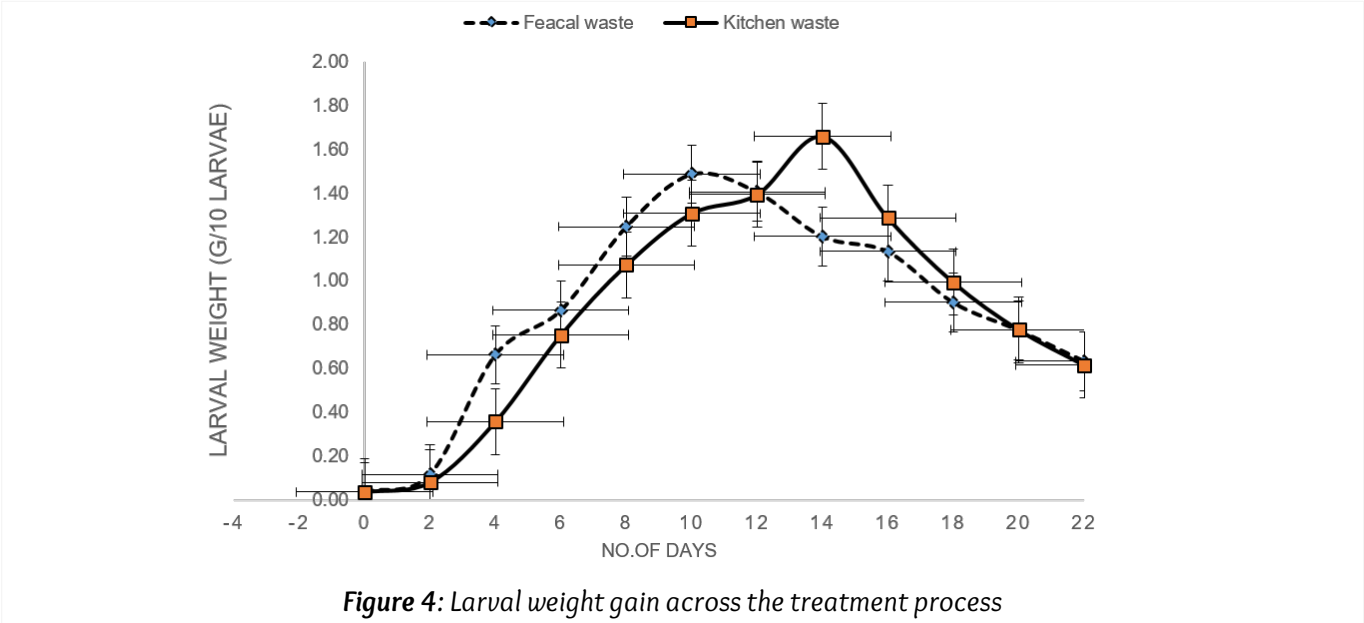

***Figure 4**: Larval weight gain across the treatment process*

Bio-conversion Rates (BCR) and Feed Conversion Rates (FCR)

The larvae performance efficiency on substrate consumption is indicated by the bioconversion rates. The BCR values of 14.09% reported on faecal matter in the present study differed with 11% from human faeces, 2.3% of BCR on primary sludge, 0.2% on digested sludge recorded by (Lalander *et al., 2019). Bioconversion rate of 7.7-27*.9% from food waste (Salomone et al., 2017; Ermolaev et al., 2019), 14.6% from kitchen waste by Matheka et al. (2021) varied with to the BCR of the present study findings of 11.35% on kitchen waste on a wet basis. The effect of moisture content on substrates, feeding rates, nutrient quality and larval density could have caused differences in reported studies.

FCR shows the proportion of substrate consumed, assimilated and turned into biomass. A lower FCR value indicates a higher efficiency of conversion of substrate to biomass. The FCR was 4.67% on faecal matter which varied from 2-16% on human faeces recorded by Banks *et al. (2014).* The FCR in the present study indicated that the faecal substrate was effectively degraded and highly turned into larval biomass. Kitchen waste recorded an FCR of 7.30% which was in close range with 6.3% from kitchen waste (Matheka et al., 2021).

Efficiency of Digested Feed (ECD)

The ability of larvae to digest the feeding substrates is termed digestibility. A higher digestibility indicates that the substrate is easier to digest and be converted into energy (Kinasih *et al*., 2018). The present study (table 2) recorded a digestibility of 17.62% on faecal waste and kitchen waste reported 12.05% of ECD. These findings varied with 12-21% reported on cassava peels (Supriyatna *et al*., 2018) and 5.69-10.85% on rice straw (Manurung *et al*., 2016). From the findings, faecal waste was easier to digest because its nutrient had been broken down into smaller units by the human digestive tract enzymes. However, the lower digestibility obtained on kitchen waste could be attributed to the mass of undigested food that made it hard for larvae to break down the food particles thus most food was lost through transpiration.

Survival Rate and Developmental Time

The survival rate was (99.53%) on faecal waste and 98.50% on kitchen waste which varied with 47% from kitchen waste (Nguyen *et al*., 2015), 82.9%-95% recorded by Bohm *et al*. (2022). The variation in the survival rate could be due to different food sources which induce adaptive chemical, physical and biological changes in the digestive tracts of BSFL (Sun *et al*., 2022). The development time on faecal and kitchen waste was 22 days which closely ranged with 19-24 days on restaurant and kitchen waste (Nguyen *et al*., 2015; Sprangers *et al*., 2017), 22 days on kitchen waste (Klammsteiner *et al*., 2021), 8-12 days from faecal waste reported by Banks *et al*. (2014). The variations among studies could be attributed to difference in the pupation rate for the comparable studies either 40% pupation rate or 50% pupation rate (Lalender *et al*.,2019).

Larval Growth Rate and Prepupal Yield

The larval weight gain increased steadily on all feed substrates and reduced gradually after reaching optimum weight. The larval weight increased gradually from day 0 to day 4, then rapidly achieving the peak larval weight gain of 1.49g on the 10^{th} day of the larval development on faecal waste. However, growth on kitchen waste was slower from day 0 to day 10 but it picked up achieving peak larval weight gain of 1.66g on the 14^{th} day of the larval development. The larval weight began to reduce after reaching their optimum weights as shown in Figure 4.

A slow growth observed on kitchen waste from day 0 to day 8 could be attributed to the pH of 3.09 to 3.94 which was lower compared to optimal pH values (6-10) and hence could not support the enzymes necessary for the breakdown of food for larval development (Salam *et al*. 2022). Thus, larvae were less active and

sluggish and had to adjust the pH through alkalization. The polymeric structure made kitchen waste adhesive in the first 4 days that BSF larvae could not move freely. The prepupal yield was faecal waste (70.43g) and kitchen waste (56.77g) which differ from 127g and 146g obtained by Matheka *et al*. (2021) for faecal and kitchen waste respectively. The average larval weights from faecal waste were heavier due to the nutrients contained in faecal substrates such as proteins and carbohydrates.

Conclusion

The flow of energy when treating faecal and kitchen waste indicated the suitability of the BSF technology in the treatment of organic waste since the larval biomass generated could be used as animal feed and the residue could be used as fertilizer. Faecal waste resulted in larval with the highest biomass with less mass being converted into metabolic processes. In contrast, larvae on kitchen waste converted a high amount of waste into metabolic processes but the prepupal yield was lower. Faecal waste performed best in relation to bioconversion rate, feed conversion rate, larval biomass production and digestibility. The knowledge acquired from this research will be important for developing rearing protocols to optimize the bioconversion of BSF insects in various applications.

Conflict of Interest

The authors have declared that no competing interests exist.

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