

## Research Paper

# Effect of fecal matter co-digestion with kitchen waste on *Hermetia illucens*' larval weight and protein content

Rosemary M. Matheka<sup>a,b,\*</sup>, James M. Raude<sup>a</sup>, Sylvia I. Murunga<sup>c</sup>, Joy N. Riungu<sup>b</sup> and Simon M. Wandera<sup>d</sup>

<sup>a</sup> Soil, Water and Environmental Engineering Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

<sup>b</sup> Civil and Environmental Engineering Department, Meru University of Science and Technology, P.O. Box 972-60200, Meru, Kenya

<sup>c</sup> Agricultural and Biosystems Engineering Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

<sup>d</sup> Civil Engineering Department, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

\*Corresponding author. E-mail: rmwende@must.ac.ke

### ABSTRACT

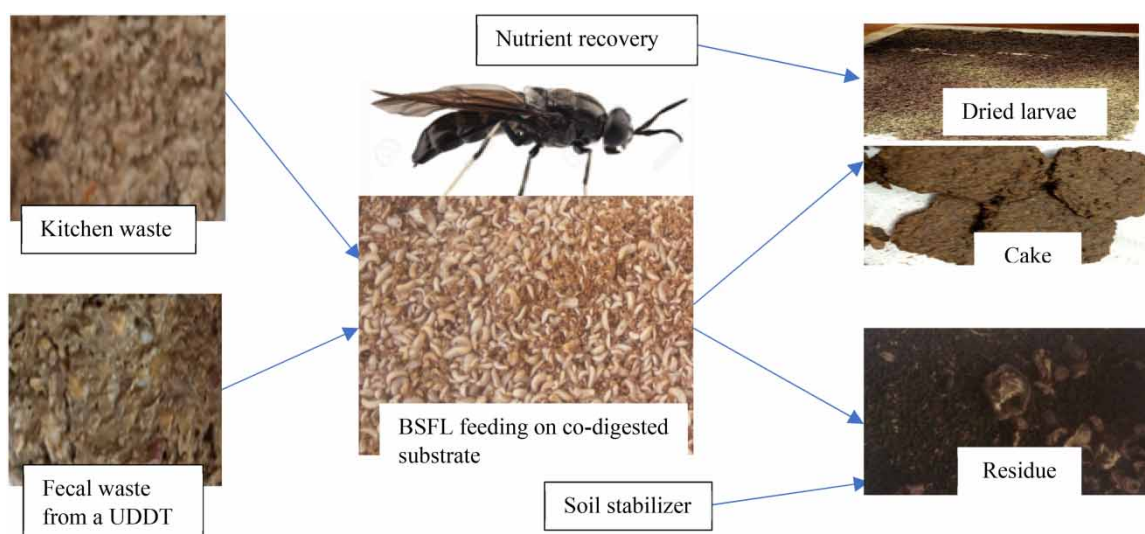
Sustainable treatment of fecal matter is a long-standing challenge in sanitation, particularly in developing countries. Although recent developments have seen the evolution of innovative onsite sanitation technologies, the treatment of fecal waste collected still remains a daunting task. This study evaluated the effect of formulated feedstock: fecal matter from urine-diverting dry toilets and kitchen waste (1:0, 1:1, 2:1, 4:1, and 0:1) on waste weight reduction, *Hermetia illucens*' larval weight gain, and crude protein content during co-digestion. Samples were collected after every 2 days for larval weight determination and protein content analysis using the Kjeldahl method of nitrogen determination. The waste reduction index (WRI) was determined after 50% pupation. The larvae grew on all substrates yielding 32.97–38.6% dry matter (DM) protein content and larval weight ranging from 1.12 to 1.70 g per five larvae. Results indicate that a 1:1 co-digestion ratio resulted in a high WRI (3.61), DM crude protein content (38.6%), and larval weight (1.70 g per five larvae). This study used the circular economy-based approach which provides a win-win situation to sanitation provision and environmental management while realizing products with potential for livelihood improvement.

**Key words:** circular economy, co-digestion, kitchen waste, sanitation

### HIGHLIGHTS

- A system for simultaneous co-digestion of the urine-diverting dry toilet fecal matter and kitchen waste for animal feed protein ingredients was investigated.
- *Hermetia illucens*' larvae developed in all rearing substrates provided despite the substrates' elemental composition.
- Co-digestion of fecal matter increased process performance of *H. illucens*' larvae.
- Co-digestion significantly increased larval protein content and weight.

## GRAPHICAL ABSTRACT



## INTRODUCTION

Universal access to safe and equitable sanitation is fundamental for promoting good hygiene behavior, health, and well-being of communities. However, ensuring the provision of effective, safe, equitable, and sustainable sanitation, particularly in developing countries has remained a global challenge (United Nations 2018). Worldwide, 4.5 billion people lack safely managed sanitation facilities and 892 million are still practicing open defecation (United Nations 2018). Regionally, more than 60% of the African population have no access to improved sanitation, and 40% of the rural population practice open defecation (Lalander *et al.* 2013). Locally, only 12% of the Kenyan population have access to sewerage services (Mansour *et al.* 2017). Previous efforts only focused on toilet provision neglecting the management of the entire sanitation service chain: that is user interface, containment and emptying, transport, treatment, and safe reuse/disposal (Gensch *et al.* 2018). However, for well-planned central areas, sewer systems are appropriate. Besides, sewer systems are not practical in fast-growing low-income areas due to their dependence on large water quantities, long planning horizons, expensive sewer networks (capital and maintenance cost), and stable institutions (Strande 2014; Larsen *et al.* 2016). Therefore, there is a need for holistic research on multi-technology and multidisciplinary approaches for sustainable stabilization of fecal sludge from onsite sanitation systems (OSS).

According to Strande (2014), 2.7 billion people globally depend on onsite sanitation technologies for their sanitation needs producing fecal sludge which requires safe disposal *in situ*, or transport and treatment offsite. Onsite sanitation facilities include pit latrines with slabs, composting toilets, ventilated improved pit latrines (VIP), pour-flush pit latrines, and urine-diverting dry toilets (UDDTs) (Strande 2014; Gensch *et al.* 2018). Onsite sanitation technologies are potentially sustainable and resilient due to the fact that they are more adaptable to demographic and environmental changes and allow for recovery of energy, nutrients, and water resources (Larsen *et al.* 2016; Gensch *et al.* 2018). They also expand opportunities for the involvement of the private sector and humanitarian organizations in the collection and safe reuse of resources in order to complement lacking public services (Diener *et al.* 2014; Strande 2014; Gensch *et al.* 2018). However, only 18% of the products from domestic OSS facilities are treated worldwide (United Nations 2018). Therefore, emphasis should be given to improve OSS which encourage safe fecal sludge treatment and disposal so as to reduce the use of pit latrines that use the 'fill and abandon strategy'.

The use of a circular economy approach through resource-oriented sanitation can address multiple challenges related to fecal sludge accumulation (Lohri *et al.* 2017) and depleting resources. Recent developments have seen the emergence of cost-effective bio-resource-based technologies such as UDDT. UDDTs take advantage of the human body anatomy which excretes urine and feces separately. Urine and feces are separately collected and cover materials such as ash, lime, and sawdust are added to the toilet after every use to keep the fecal matter dry, eliminate odor, and flies. Moreover, resource recovery

from UDDT is a potential alternative to increase the value of OSS beyond mitigating both human and environmental health risks (Rieck *et al.* 2012). It also assures nutrient recovery from fecal waste reflecting a shift to rating human excreta as a resource, not a waste. The resources recovered from human feces can be re-integrated into the ecosystems thereby offsetting global pressure on nutrient, energy, and water systems. However, a post-treatment step is required for pathogen inactivation since the addition of ash/lime alone is inadequate (Rieck *et al.* 2012). Several treatment technologies have been developed for OSS-generated waste: vermicomposting, ammonia treatment, black soldier fly (BSF) composting, solar drying and thermal drying, and pelletizing. However, their potential for application remains limited (Strande 2014). Although BSF has been used for organic waste treatment, their potential for fecal waste treatment is limited (Strande 2014). This study sought to explore UDDT-generated fecal matter (FM) treatment via BSF technology.

The BSF larvae (BSFL) are saprophagous insects found in tropical, subtropical, and warm temperate zones (Dortmans *et al.* 2017). Bioconversion of organic waste using the BSF technology has been noted to reduce the microbial load. A study by Lalander *et al.* (2013) reported a 6 log<sub>10</sub> reduction in *Salmonella* spp. in human feces in 8 days after using BSFL for the treatment of fecal matter from OSS. However, BSFL have minimal effects on *Ascaris* ova (Lalander *et al.* 2013). In BSF processing facilities, the larvae feed on decomposing organic material, growing from a few millimeters to approximately 2.5 cm while achieving up to 80% waste reduction (WR) (Dortmans *et al.* 2017). The larvae are harvested using a mechanical agitator to separate them from the residue.

BSF technology has the potential to outperform composting and vermicomposting of organic waste in tropical regions (Lohri *et al.* 2017). In addition, BSF offers a cost-effective potential alternative for recycling human feces, fruit and vegetable, pig manure, municipal solid waste, kitchen waste (KW), and other biodegradable waste into valuable products (Diener *et al.* 2011; Nguyen *et al.* 2015; Lalander *et al.* 2016). A study by Chen *et al.* (2019) reported that BSFL treatment is an alternative for decreasing CH<sub>4</sub>, N<sub>2</sub>O, and NH<sub>3</sub> emissions (by 72.63–99.99, 99.68–99.91, and 82.30–89.92%, respectively) and reducing the global warming potential. Besides, the digestate could be utilized in the farms as zoo compost (Lalander *et al.* 2016) which increases carbon sequestration. Therefore, this fly's ferocious feeding on biowaste presents an opportunity for the achievement of multiple sustainable development goals (SDGs) through the development of a circular food economy.

Literature has reported that the larvae consist of ±35% protein and ±30% crude fat (Dortmans *et al.* 2017). Gold *et al.* (2020) reported a larval protein content ranging from 26.7 to 38.7% with human feces and different waste formulations as the BSF larval feed. However, the impacts of the nutritive composition of the substrate on the BSF larval weight and protein content are unknown. For the purpose of stabilizing the BSF conversion process and increasing the larval yield, co-digestion has been considered as an efficient method (Rehman & Razzaq 2017). Thus, this research aimed to add knowledge on the effect of the nutritive composition of UDDT fecal matter and its co-digestion mixtures on WR, increase in larval weight during BSFL treatment, and the harvested biomass protein content.

## MATERIALS AND METHODS

### Materials

#### BSF larvae

The experiments were carried out at the Meru University of Science and Technology Sanitation Research Institute (MUST SRI). The BSFL used in the study were supplied from the MUST SRI breeding area at 5 days of age.

#### UDDT fecal waste sample collection

UDDT fecal waste (UDDT-FW) samples used for this study were obtained from UDDT within Kunene Primary school, a rural public school in Meru, Kenya, with rural setting food-based diets. Within each toilet facility, a 20 L container was used for the collection of feces, with approximately 10 g sawdust added after every toilet use. UDDT-FW collection is on a daily basis, where used containers are collected and replaced with clean ones. From a batch consisting of eight containers, four containers were randomly selected, the contents were thoroughly mixed so as to have a homogeneous mix. Fifteen kilograms of UDDT-FW was then drawn and further mixing was carried out in order to obtain a homogenous sample.

#### KW sample collection

Mixed KW was obtained from the MUST cafeteria. Approximately 15 kg of the KW was collected which contained vegetable, fruit, and food waste in about equal proportions.

## Experimental method

Five treatments (three replicates per treatment) were prepared with different mixing ratios of fecal to KW as presented in Table 1. The mixtures were homogenized to mimic the pretreatments used in BSFL treatment facilities (Dortmans *et al.* 2017). Plastic containers (260 mm\*130 mm\*110 mm) were used as treatment units. To save on space, individual containers were stacked upon each with ventilation frames between them to allow free air circulation.

Substrates' calcium, iron, copper, potassium, magnesium, sodium, phosphorus, and zinc contents were estimated according to the Poitevin (2012) procedures. The carbohydrate content was determined by the method of difference following the FAO (2003) procedures. Protein based on total nitrogen was analyzed using the Kjeldahl method of nitrogen determination and a conversion factor of 6.25 was used (AOAC 1990). Fat extraction was done using the Soxhlet extraction method (Gopalasatheeskumar 2018). The substrates were characterized for different elements as reported in Table 2. To analyze the pH and electrical conductivity (EC), fresh samples mixed with distilled water at a ratio of 1:10 (weight of wet sample/volume of distilled water) were used and the pH and EC of the sample were measured using a Multiparameter Water Quality Meter (pH Meter) (MK900-CN, China) calibrated in the range of 4.01–10.01. The moisture content was determined by fresh sample weight reduction after drying at 105 °C in an oven (Memmert UN 30-240 V Universal Oven) for 24 h. Ash content was determined gravimetrically after sample incineration by a muffle furnace (Model: JK-SX2-5-12N). Five grams of 9-day old larvae were added into each 1 kg feed substrate at approximately 70–85% moisture content which was determined using the gravimetric method as reported by Shukla *et al.* (2014).

**Table 1** | Percentage of fecal and KW in different feeding substrates

Substrate	Fecal waste (%)	KW (%)
a	100	0
b	50	50
c	67	33
d	80	20
e	0	100

Each feeding substrate had three replicates weighing 1,000 g each.

**Table 2** | Characteristics of feed substrates in this study (mean ± standard deviation)

Parameter	Substrate a	Substrate b	Substrate c	Substrate d	Substrate e	p-value
Crude protein (%)	41.50 ± 0.04	35.31 ± 0.06	40.51 ± 0.02	40.26 ± 0.03	25.94 ± 0.19	6.94E-19
Crude fat (%)	10.77 ± 0.02	9.62 ± 0.19	10.41 ± 0.001	11.09 ± 0.002	7.80 ± 0.19	3.14E-12
Calcium	23.78 ± 0.07	22.81 ± 0.15	22.54 ± 0.02	22.9 ± 0.51	22.4 ± 0.08	0.000316
Phosphorus	1.69 ± 0.01	8.36 ± 0.07	8.39 ± 0.009	6.7 ± 0.004	0.62 ± 0.02	1.09E-20
Copper	3.38 ± 0.06	2.49 ± 0.03	2.22 ± 0.1	1.42 ± 0.16	1.83 ± 0.11	4.28E-09
Zinc	0.48 ± 0.01	0.12 ± 0.01	0.12 ± 0.003	0.16 ± 0.007	0.15 ± 0.02	1.6E-12
Carbohydrates	36.51 ± 0.60	41.34 ± 0.406	36.93 ± 0.525	35.74 ± 0.86	49.05 ± 1.15	4.76E-09
pH	8.74 ± 0.004	7.21 ± 0.002	7.71 ± 0.004	7.93 ± 0.008	4.18 ± 0.004	3.66E-26
Moisture content (%)	83.37 ± 0.15	82.15 ± 0.285	81.52 ± 0.708	82.45 ± 0.036	80.37 ± 0.52	7.03E-05
Total solids	16.6 ± 0.152	17.85 ± 0.28	18.48 ± 0.71	17.55 ± 0.04	19.63 ± 0.52	6.94E-05
TVS	57.87 ± 1.2	78.66 ± 2.16	81.3 ± 4.93	87.83 ± 2.2	89.1 ± 1.55	4.75*10 <sup>-7</sup>
EC (µS)	471.67 ± 2.08	672.33 ± 1.15	695.67 ± 1.53	657 ± 2	805.33 ± 2.08	1.39E-18
Sodium	31.38 ± 0.42	44.51 ± 0.58	34.51 ± 0.23	32.29 ± 0.13	35.77 ± 0.21	1.05*10 <sup>-15</sup>
Potassium	20.21 ± 0.17	18.12 ± 0.21	19.29 ± 0.14	16.64 ± 0.31	14.57 ± 0.19	1.35*10 <sup>-10</sup>
Magnesium	15.21 ± 0.15	17.92 ± 0.21	17.23 ± 0.25	17.62 ± 0.25	10.10 ± 0.24	4.75*10 <sup>-12</sup>
Iron	6.55 ± 0.35	5.01 ± 0.09	4.26 ± 0.35	3.1 ± 0.17	1.44 ± 0.05	1.87*10 <sup>-9</sup>

The treatment performance of BSFL in converting waste to larval biomass was estimated by measuring the larval development time, the WR based on substrate weight, waste reduction index (WRI), bioconversion and feed conversion rate (FCR) on a wet mass basis. WR was calculated to determine the overall material reduction as described by Meneguz *et al.* (2018) in the following equation:

$$WR = \left( \frac{FC}{W} \right) * 100\% \quad (1)$$

where WR is the waste reduction (as a percentage), FC is the feed consumed (in grams), and  $W$  is the initial feed weight (in grams).

The residual substrates and the total final larval biomass were weighed so as to evaluate the larval efficiency in consuming and metabolizing the growing substrates. WRI in Equation (3) (Meneguz *et al.* 2018) was calculated using the overall degradation  $D$  in Equation (3) (Meneguz *et al.* 2018) divided by the number of days the larvae used to reduce the given amount of waste

$$D = \frac{(W - R)}{W} \quad (2)$$

$$WRI = \left( \frac{D}{t} \right) * 100\% \quad (3)$$

where  $D$  is the overall degradation,  $W$  is the total feed applied (in grams),  $R$  is the residue left after bioconversion (non-digested substrate + excretion products) (in grams), WRI is the waste reduction index (as a percentage), and  $t$  is bioconversion time (in days).

The larval yield was determined from the difference between final and initial larval yields, and the bioconversion rate (BR) was calculated according to Diener *et al.* (2009) and Gold *et al.* (2020) in the following equation:

$$BR = \left( \frac{LY}{W} \right) * 100\% \quad (4)$$

where BR is the bioconversion rate (as a percentage), LY is the larvae yield (in grams), and  $W$  is the total feed applied (in grams).

### Statistical analysis

Statistical analysis was done using SPSS software (SPSS Inc., Chicago, IL, USA). Data obtained from the three replicates per treatment were combined by computing their mean values, standard deviations, and standard errors. The variables comprised total feed added, total residue collected, WR, prepupal mean weight, and prepupal yield. The bioconversion, FCR, and WRI were calculated for actual yield, and the mean values and standard deviation were then presented in the form of either a table or a figure. One-way analysis of variance (ANOVA) with a 95% confidence interval was used to establish whether a statistically significant difference occurred between feed substrates followed by Tukey's honest significant difference test as a *post hoc* analysis (Lalander *et al.* 2019). The significant difference between the compared values was indicated by a  $p$ -value of <0.05.

### Prediction model

Batch experiments were done to describe the treatment of BSF larvae in substrates made of FW:KW at different ratios: 1:0, 1:1, 2:1, 4:1, and 0:1. Since the maximum weight gain and protein content that could be obtained from the different substrates were unknown, the modified Gompertz model (Algapani *et al.* 2016) represented by Equation (5) was used to simulate the experimental data:

$$P = P_0 \cdot \exp \left\{ - \exp \left[ \frac{r_{\max} \cdot e}{P_0} \cdot (t_0 - t) + 1 \right] \right\} \quad (5)$$

where  $P$  is the cumulative product volume at treatment time  $t$ ;  $P_0$  denotes the product potential of the substrate;  $r_{\max}$  indicates the maximum conversion rate (tangent to the curve) at time  $t_0$ ; and  $t_0$  is the lag time in days which falls where  $P = P_0 \cdot \exp(-e)$  as reported by Tjerve & Tjerve (2017).  $e$  indicates the natural logarithm. The constants  $P_0$ ,  $r_{\max}$ , and  $t_0$  were fixed by the MATLAB nonlinear fitting program. The constants  $P_0$ ,  $r_{\max}$ , and  $t_0$  were fixed by the MATLAB nonlinear fitting program. BSF undergoes five life stages: egg, larvae, prepupa, pupa, and adult (Dortmans *et al.* 2017); thus, the data collected were not continuous data since growth ends. The experiments were done in triplicate. Therefore, the first dataset was used to determine the parameters, the second dataset was used for simulation of the parameters, and the third dataset validated the model.

## RESULTS AND DISCUSSION

Since this study aims to investigate the effect of the nutritive composition of fecal matter and its co-conversion mixtures on BSFL performance efficiency, the nutrient composition of the feed substrates was preliminarily determined. The nutritive characteristics varied significantly among the feed substrates (Table 2).

All rearing substrates were suitable for larval growth due to the fact that the crude protein content of the substrates varied between 25.94% for pure food waste and 41.5% for fecal waste (Table 2) on a dry matter (DM) basis. The findings of 41.5% DM crude protein for fecal matter in this study differ with 35.5 and 38.8% DM crude protein reported by Lalander *et al.* (2019) and Rose *et al.* (2015), respectively. The high crude protein content was likely protein from the microbial biomass in the gut. In addition, the dietary feed intake would influence the fecal crude protein content. KW had the highest total volatile solids (TVS) content (89.1% on DM) and FW the lowest (57.85% on DM). Unlike undigested KW, low TVS in FW was due to biodegradation in the human digestive system for growth and development.

For FW and the co-digested substrates, the initial pH ranged between 7.21 and 8.74. These findings are within the pH range of 6.0–10.0 reported by Ma *et al.* (2017) for BSFL bioconversion of organic waste. Formulating different substrates improved pH, nutritive, and organic composition of the co-conversion substrates compared with the individual substrates. The high dietary moisture content of the rearing substrates makes it easy for the larvae to feed since the kind of the macerating mouthparts allow the larvae to scrape off the feed.

### WR, prepupal yield, bioconversion rates, and WRI

Formulating different types of biowaste has the potential to increase the process performance of BSFL (Gold *et al.* 2018) since the formulations are more nutritively balanced. All the feed substrates provided were consumed by the BSFL for their growth and development despite the different nutritive content. However, the WR, prepupal yield, bioconversion, FCR, and WRI were strongly affected by the co-conversion mixtures used in the feed substrate (Table 3).

There was a statistically significant difference between WR in the different substrates ( $P = 0.001$ ). WR was significantly higher in the substrate e ( $91.5 \pm 1.3$ ) than in substrates a ( $83.3 \pm 1.8$ ), b ( $86.6 \pm 1.5$ ), c ( $85.3 \pm 1.5$ ), and d ( $84.3 \pm 0.4$ ) on a wet basis. In a study by Diener *et al.* (2011), growing substrates such as chicken feed, market waste, and municipal organic waste resulted in 66.4–78.9% WR. For fecal sludge, the WR on a dry basis was 73% (Lalander *et al.* 2013). Nguyen *et al.* (2015) highlighted that WR in KW, fish rendering, and a mixture of fruits and vegetables were 67.9, 74.2, and 98.9%, respectively. This shows that WR is affected by the substrate type. In addition, WR obtained in this study is similar to previously reported results. A co-conversion mix-ratio of 1:1 resulted in a good combination for BSF larval biomass production and efficient WR, which is consistent with the results reported by Diener *et al.* (2011). This was caused by the more balanced

**Table 3** | Effect of different substrates on *H. illucens*'s WR, prepupal yield, bioconversion, FCR, and WRI

Sample	Residue (g)	WR (%)	Prepupal yield (g)	Bio-conversion (%)	FCR	WRI
a	167.0	83.30 (1.81)	127 (12.29) <sup>a</sup>	12.70 <sup>a</sup>	6.61 <sup>a</sup>	3.47 <sup>a</sup>
b	133.7	86.60 (1.45)	226 (13.15) <sup>b</sup>	<b>22.60<sup>b</sup></b>	<b>3.87<sup>b</sup></b>	<b>3.61<sup>b</sup></b>
c	164.0	85.30 (1.55)	220 (15.87) <sup>b</sup>	22.00 <sup>b</sup>	3.89 <sup>b</sup>	3.56 <sup>ab</sup>
d	153.0	84.70 (0.44)	157.3 (8.51) <sup>a</sup>	15.70 <sup>a</sup>	5.39 <sup>a</sup>	3.53 <sup>ab</sup>
e	85.0	91.50 (1.30)	146 (7.80) <sup>a</sup>	14.60 <sup>a</sup>	6.28 <sup>a</sup>	2.86 <sup>c</sup>

Values are in mean (SD);  $n = 3$ , values bearing different superscripted alphabets differ from each other at  $p < 0.05$ .

The bold values indicate the substrate that resulted in the best performance efficiency. That is, good reduction efficiency is indicated by high WRI values, good feed conversion rate is indicated by the lowest FCR, and good bio-conversion is indicated by the highest bioconversion efficiency.

nutrients in the formulation. FM was low in organic matter and carbohydrates and high in protein content, while KW was rich in organic matter and carbohydrates and low in protein content. In this study, the WRI was high in the formulated substrates than in the individual substrates which indicates that the formulation increased larval performance. The findings are similar to the results reported by Gold *et al.* (2020).

There was a statistically significant difference between the larval yield in the feed substrates ( $P < 0.05$ ). The larval yield was the highest in substrate b and the lowest in a. All the co-conversion mixtures showed a significant increase in larval production from the fecal matter (Table 3) due to improved buffer capacity and nutrient balance. This resulted in the establishment of a positive synergism that impacts larval biological growth. The FCR was positively affected by the substrate formulation compared with the individual feed substrates due to the fact that co-digestion balances the macro- and micronutrients and increases the organic loading of the substrates. In this study, the bioconversion rate was 14.6% DM for KW compared with 15–23% DM for canteen waste as reported by Gold *et al.* (2020). For the formulations, the bioconversion rate was 15–17% DM as reported by Gold *et al.* (2020) compared with 15.7–22.6% DM in this study.

Apart from the nutrient concentration among the formulations, microbial communities and numbers could have contributed to the variations in BSFL treatment performance. This was expected since microbes differ between biowastes and are influential in biowaste decomposition and BSF larval development (Gold *et al.* 2018). However, this was not part of this research.

### Larval weight

The larval growth and development time are influenced by factors such as feed availability (Diener *et al.* 2009), nutrient availability, and feed characteristics (Rose *et al.* 2015). The  $p = 1.23 \times 10^{-24} < p = 0.05$  indicates that different substrates affected the BSF larval weight. Table 4 shows that the  $P_0$  of mixed co-conversion substrates was higher compared with the individual FM and KW substrates since the kinematics factors are dependent on the substrate used.

The  $P_0$  of substrate c was close to that of substrate d and significantly higher than that of substrates a and e. Substrate b resulted in the highest weight gain ( $P_0$ ) and a significantly higher prepupal yield. The steep slope for substrate b (Figure 1) indicates that less time was utilized by the larvae to attain its maximum larval weight compared with the other substrates.

In addition, the larvae easily converted the feed to its own body mass thus increasing the larval weight. Mixed co-conversion substrates showed a significantly improved larval weight and performance in comparison to the individual substrates. This is attributed to the sufficiently high TVS and protein content in the co-conversion mixtures which support both larval growth and development. It was noted that larval weights were significantly higher where the initial pH ranged between 7.21 and 8.74, and the lowest when the initial pH was 4.81. This indicates that the initial pH significantly affects larval weight gain and biological growth rate. Thus, co-digestion of FW increased both the alkalinity capacity and production of larvae, while reducing the fat concentration. Inhibition of the fats was beneficial for gut microbiota development which plays a vital role in nutrient biodegradation for larval development. For the KW which had low pH, the larvae adjusted through alkalization of the substrate caused by the release of ammonium ions and ammonia (Alidadi *et al.* 2016)

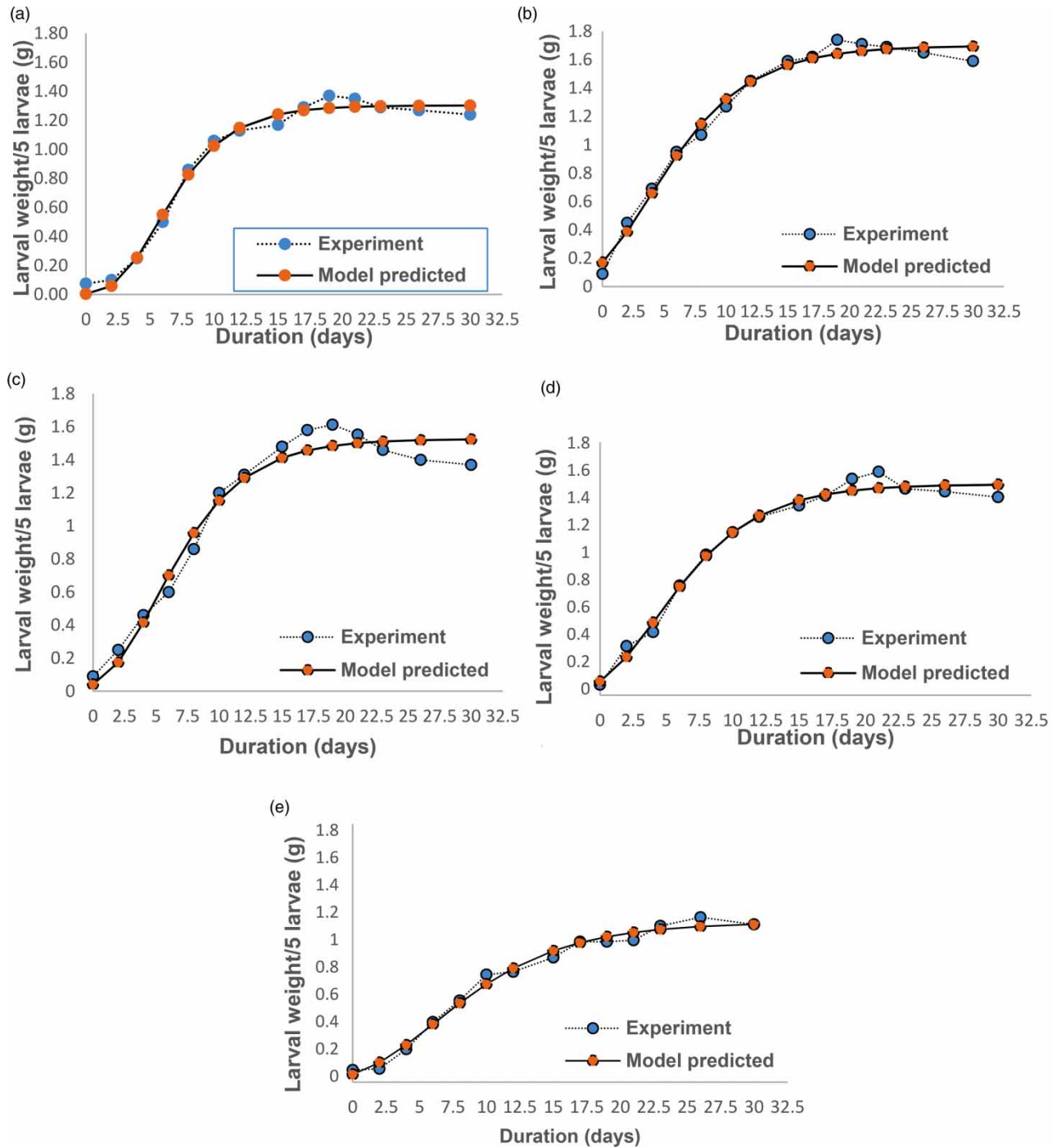
However, comparing the larval weight (Table 4) and WR results of the substrates (Table 3) demonstrates that higher WR did not necessarily result in higher larval weight. Nevertheless, the results reveal the ability of BSFL to manage fecal waste-producing larval biomass which is beneficial in closing the loop between the waste and lost nutrients. Adopting BSFL technology for fecal waste management is capable of eliminating the end-of-pipe technology in wastewater treatment. The BSFL technology results in a sustainable reduction of pollution and production of animal feed protein sources.

### Crude protein content

Concerning nutrient recovery with BSFL, harvested larval biomass could be used either as an animal feed ingredient (Wang & Shelomi 2017) or for biodiesel production (Wong *et al.* 2019). From the study, it could be seen that different substrates have

**Table 4** | Kinetic parameters of larval weight per five larvae of the experiment by modified Gompertz model

Parameter	a (1:0)	b (1:1)	c (2:1)	d (4:1)	e (0:1)
$P_0$	1.303	1.698	1.526	1.502	1.115
$r_{\max}$	0.153	0.138	0.144	0.130	0.075
$t_0$	2.408	0.000	1.117	0.026	0.585
$R^2$	0.989	0.987	0.969	0.986	0.989



**Figure 1** | Model simulation of the larval weight.

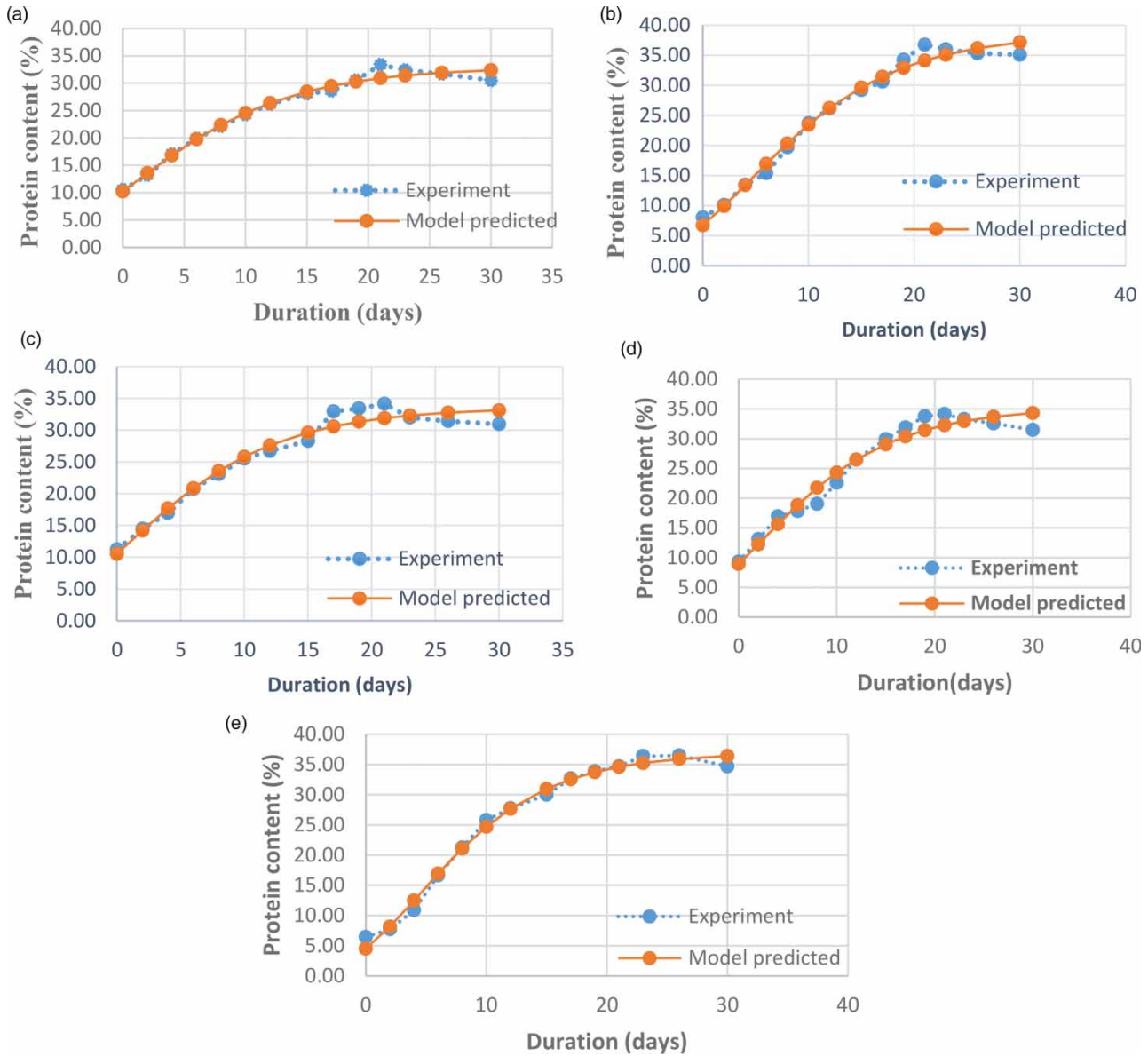
different protein content potentials ( $P_0$ ) as shown in Table 5. Therefore, the model was used to predict the maximum crude protein potential and the time required to attain the maximum conversion rate of the different substrates, as simulated in Figure 2. From Table 5,  $t_0 = 0$  indicates that the larvae had accumulated some protein before the start of the experiment and  $r_{\max}$  shows the ease of conversion of the feed by the BSFL.

From the results, co-digestion improved the reliability of the substrates by balancing both macro- and micronutrients in the rearing substrates since the nutritional composition of BSFL is highly influenced by the rearing substrate. Unlike KW, fecal



**Table 5** | Kinetic parameters of protein content of the experiment by modified Gompertz model

Parameter	a (1:0)	b (1:1)	c (2:1)	d (4:1)	e (0:1)
$P_0$	32.97	38.71	33.57	35.27	36.95
$r_{max}$	1.674	1.784	1.833	1.693	2.243
$t_0$	0	0	0	0	0
$R^2$	0.9828	0.9853	0.9652	0.9826	0.9914



**Figure 2** | Model simulation of crude protein content.

matter is low in the TVS content. Therefore, co-digestion increased the TVS in the formulated substrates resulting in improved feed conversion for larval growth. From our study, FW contained the highest crude protein content and the lowest TVS and yet resulted in the lowest larval crude protein. Possibly, the larvae accumulated substantial protein quantity for growth while

consuming minimal energy which resulted in low biomass protein content. Our findings show that the BSFL protein content ranged between 32.97 and 38.71% on a DM basis which is within the range of 31.9–46.3% reported by Diener *et al.* (2009) and Spranghers *et al.* (2017). FM co-digestion helps to optimize the nutrient balance of the rearing substrates, enhance WR, and larvae growth.

From the study, the results suggest that a co-conversion ratio of 1:1 was more appropriate for both larval biomass production and waste treatment. This reveals that BSFL can be reliable for fecal waste management and the treatment can be improved through formulating different organic wastes depending on their initial nutritive characteristics. Bioconversion of fecal waste is beneficial due to its availability, affordability, zero competitiveness for food/feed, and the need for sustainable waste management procedures. However, the characteristics of fecal matter are dependent on the source (Rose *et al.* 2015), which can affect an industrial-scale recycling facility.

## CONCLUSION

This study shows that the performance and protein content of BSF larvae are highly affected by the characteristics of the growing substrate provided. The larvae served effectively in the dual roles of high-protein biomass production and waste minimization. Thus, the study shows that BSFL co-digestion can be used for fecal waste recycling and management for nutrient recovery and re-integration into the food chain and bio-fertilizer production. Overall, using the circular economy approach for fecal waste management would result in reduced environmental pollution, improved sanitation, and sustainable economic growth. Future research should assess if BSFL prefer feed substrates with numerous bacterial species or higher bacterial loads and their effects on bioconversion efficiency. Moreover, the effect of the nutritive composition of the inoculum on BSF bioconversion should be investigated.

## CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

## FUNDING

The authors thank the Meru University of Science and Technology (MUST) Sanitation Research Institute (SRI) for the continuous supply of BSF larvae used in this work.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## REFERENCES

- Algapani, D. E., Qiao, W., Su, M., di Pumpo, F., Wandera, S. M., Adani, F. & Dong, R. 2016 [Bio-hydrolysis and bio-hydrogen production from food waste by thermophilic and hyperthermophilic anaerobic process](https://doi.org/10.1016/j.biortech.2016.06.016). *Bioresource Technology* **216**, 768–777. <https://doi.org/10.1016/j.biortech.2016.06.016>.
- Alidadi, H., Hosseinzadeh, A., Najafpoor, A. A., Esmaili, H., Zanganeh, J., Takabi, D. M. & Piranloo, F. G. 2016 [Waste recycling by vermicomposting: maturity and quality assessment via dehydrogenase enzyme activity, lignin, water soluble carbon, nitrogen, phosphorous and other indicators](https://doi.org/10.1016/j.jenvman.2016.07.025). *Journal of Environmental Management* **182**, 134–140. <https://doi.org/10.1016/j.jenvman.2016.07.025>.
- AOAC 1990 *Official Methods of Analysis: Animal Feed*, Vol. 1, 15th edn. K. Helrich, ed. Association of Official Analytical Chemistry, Virginia.
- Chen, J., Hou, D., Pang, W., Nowar, E. E., Tomberlin, J. K., Hu, R., Chen, H., Xie, J., Zhang, J., Yu, Z. & Li, Q. 2019 [Effect of moisture content on greenhouse gas and NH<sub>3</sub> emissions from pig manure converted by black soldier fly](https://doi.org/10.1016/j.scitotenv.2019.133840). *Science of the Total Environment* **697**, 133840. <https://doi.org/10.1016/j.scitotenv.2019.133840>.
- Diener, S., Zurbrugg, C. & Tockner, K. 2009 [Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates](https://doi.org/10.1177/0734242X09103838). *Waste Management and Research* **27** (6), 603–610. <https://doi.org/10.1177/0734242X09103838>.
- Diener, S., Zurbrugg, C., Roa Gutiérrez, F., Nguyen, D. H., Morel, A., Koottatep, T. & Tockner, K. 2011 Black soldier fly larvae for organic waste treatment – prospects and constraints. In: *Proceedings of the WasteSafe 2011 – 2nd International Conference on Solid Waste Management in the Developing Countries*, Vol. 52 (2), pp. 978–984.
- Diener, S., Semiyaga, S., Niwagaba, C. B., Muspratt, A. M., Gning, J. B., Mbéguéré, M., Ennin, J. E., Zurbrugg, C. & Strande, L. 2014 [A value proposition: resource recovery from faecal sludge – can it be the driver for improved sanitation?](https://doi.org/10.1016/j.resconrec.2014.04.005) *Resources, Conservation and Recycling* **88**, 32–38. <https://doi.org/10.1016/j.resconrec.2014.04.005>.

- Dortmans, B., Diener, S., Verstappen, B. & Zurbrügg, C. 2017 *Black Soldier Fly Biowaste Processing: A Step-by-Step Guide*. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf. <https://doi.org/10.1117/12.464354>.
- FAO 2003 *Food and Agriculture Organization of the United Nations. Economic Record*. <https://doi.org/10.1111/j.1475-4932.1946.tb01187.x>.
- Gensch, R., Jennings, A., Renggli, S. & Reymond, P. 2018 *Compendium of Sanitation Technologies in Emergencies*, 1st edn. German WASH Network (GWN), Swiss Federal Institute of Aquatic Science and Technology (Eawag), Global WASH Cluster (GWC) and Sustainable Sanitation Alliance (SuSanA). Available from: [http://www.bmz.de/de/zentrales\\_downloadarchiv/Presse/emergency-sanitation-compendium.pdf](http://www.bmz.de/de/zentrales_downloadarchiv/Presse/emergency-sanitation-compendium.pdf).
- Gold, M., Tomberlin, J. K., Diener, S., Zurbrügg, C. & Mathys, A. 2018 *Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review*. *Waste Management* **82**, 302–318. <https://doi.org/10.1016/j.wasman.2018.10.022>.
- Gold, M., Marie, C., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S. & Mathys, A. 2020 *Biowaste treatment with black soldier fly larvae: increasing performance through the formulation of biowastes based on protein and carbohydrates*. *Waste Management* **102**, 319–329. <https://doi.org/10.1016/j.wasman.2019.10.036>.
- Gopalasatheeskumar, K. 2018 Significant role of soxhlet extraction process in phytochemical research. *Mintage Journal of Pharmaceutical & Medical Sciences* **7**, 43–47.
- Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A. & Vinnerås, B. 2013 *Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) – from a hygiene aspect*. *Science of the Total Environment* **458–460**, 312–318. <https://doi.org/10.1016/j.scitotenv.2013.04.033>.
- Lalander, C. H., Fidjeland, J., Diener, S., Eriksson, S., Lalander, C. H., Fidjeland, J., Diener, S. & Eriksson, S. 2016 *High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling*. <https://doi.org/10.1007/s13593-014-0235-4>.
- Lalander, C., Diener, S., Zurbrügg, C. & Vinnerås, B. 2019 *Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*)*. *Journal of Cleaner Production* **208**, 211–219. <https://doi.org/10.1016/j.jclepro.2018.10.017>.
- Larsen, T. A., Hoffmann, S., Lüthi, C., Truffer, B. & Maurer, M. 2016 *Emerging solutions to the water challenges of an urbanizing world*. *Science* **352** (6288), 928–933. <https://doi.org/10.1126/science.aad8641>.
- Lohri, C. R., Diener, S., Zabaleta, I., Mertenat, A. & Zurbrügg, C. 2017 *Treatment technologies for urban solid biowaste to create value products: a review with focus on low- and middle- income settings*. *Reviews in Environmental Science and Bio/Technology* **16**, 81–130. <https://doi.org/10.1007/s11157-017-9422-5>.
- Ma, J., Lei, Y., Rehman, K. U., Yu, Z., Zhang, J., Li, W., Li, Q., Tomberlin, J. K. & Zheng, L. 2017 *Dynamic effects of initial PH of substrate on biological growth and metamorphosis of black soldier fly (Diptera: Stratiomyidae)*. *Environmental Entomology* **XX** (X), 1–7. <https://doi.org/10.1093/ee/nvx186>.
- Mansour, G., Oyaya, C. & Owor, M. 2017 *Situation Analysis of the Urban Sanitation Sector in Kenya*.
- Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. & Gasco, L. 2018 *Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae*. *Journal of the Science of Food and Agriculture* **98**, 5776–5784. <https://doi.org/10.1002/jsfa.9127>.
- Nguyen, T. T. X., Tomberlin, J. K. & Vanlaerhoven, S. 2015 *Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste*. *Environmental Entomology* **44** (2), 406–410. <https://doi.org/10.1093/ee/nvv002>.
- Poitevin, E. 2012 *Determination of calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc in fortified food products by microwave digestion and inductively coupled plasma-optical emission spectrometry: single-laboratory validation and ring tri*. *Journal of AOAC International* **92** (5). [https://doi.org/10.5740/jaoacint.CS2011\\_14](https://doi.org/10.5740/jaoacint.CS2011_14).
- Rehman, H. A. & Razaq, R. 2017 *Benefits of biochar on the agriculture and environment – a review*. *Journal of Environmental Analytical Chemistry* **04** (03), 3–5. <https://doi.org/10.4172/2380-2391.1000207>.
- Rieck, C., von Müenich, E. & Hoffman, H. 2012 *Technology review of urine-diverting dry toilets (UDDTs)*. *Deutsche Gesellschaft Für Internationale Zusammenarbeit (GIZ) GmbH* 1–60. Available from: [papers2://publication/uuid/A119E662-AA96-4E2A-A116-7577686E2B61](https://publication/uuid/A119E662-AA96-4E2A-A116-7577686E2B61).
- Rose, C., Parker, A., Jefferson, B. & Cartmell, E. 2015 *The characterization of feces and urine: a review of the literature to inform advanced treatment technology*. *Critical Reviews in Environmental Science and Technology* **45** (17), 1827–1879. <https://doi.org/10.1080/10643389.2014.1000761>.
- Shukla, A., Panchal, H., Mishra, M., Patel, P. R., Srivastava, H. S., Patel, P. & Shukla, A. K. 2014 *Soil moisture estimation using gravimetric technique and FDR probe technique: a comparative analysis*. *AJRFANS* **8** (1), 89–92.
- Sprangers, T., Ottoboni, M., Klootwijk, C., Ovyin, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S. 2017 *Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates*. *Journal of the Science of Food and Agriculture* **97**, 2594–2600. <https://doi.org/10.1002/jsfa.8081>.
- Strande, L. 2014 *The global situation*. In: *Faecal Sludge Management* (Strande, L., Ronteltap, M. & Brdjanovic, D., eds). IWA Publishing, pp. 1–14. Available from: [http://www.sswm.info/sites/default/files/reference\\_attachments/EAWAG\\_SANDEC\\_2008\\_Module\\_5\\_FSM\\_Lecture.pdf](http://www.sswm.info/sites/default/files/reference_attachments/EAWAG_SANDEC_2008_Module_5_FSM_Lecture.pdf).
- Tjerve, K. M. C. & Tjerve, E. 2017 *The use of gompertz models in growth analyses, and new Gompertz-model approach: an addition to the unified-Richards family*. *PLoS One* **12** (6), 1–17. <https://doi.org/10.1371/journal.pone.0178691>.
- United Nations 2018 *Sustainable Development Goal 6 Synthesis Report on Water and Sanitation 2018*. United Nations, New York.

- Wang, Y.-S. & Shelomi, M. 2017 Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods* **6** (10), 91. <https://doi.org/10.3390/foods6100091>.
- Wong, C.-y., Rosli, S.-s., Uemura, Y. & Ho, Y. C. 2019 Potential protein and biodiesel sources from black soldier fly larvae: insights of larval harvesting instar and fermented feeding medium. *Energies* **12** (1570), 1–15. <https://doi.org/10.3390/en12081570>.

First received 5 May 2021; accepted in revised form 14 July 2021. Available online 28 July 2021