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Biowaste treatment with black soldier fly larvae: Increasing performance through the formulation of biowastes based on protein and carbohydrates

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ABSTRACT

A key challenge for black soldier fly larvae (BSFL) treatment is its variable reliability and efficiency when applied to different biowastes. Similar to other biowaste treatment technologies, co-conversion could compensate for variability in the composition of biowastes. Using detailed nutrient analyses, this study assessed whether mixing biowastes to similar protein and non-fibre carbohydrate (NFC) contents increased the performance and reduced the variability of BSFL treatment in comparison to the treatment of individual wastes. The biowastes examined were mill by-products, human faeces, poultry slaughterhouse waste, cow manure, and canteen waste. Biowaste formulations had a protein-to-NFC ratio of 1:1, a protein content of 14–19%, and a NFC content of 13–15% (dry mass). Performance parameters that were assessed included survival and bioconversion rate, waste reduction, and waste conversion and protein conversion efficiency. In comparison to poultry feed (benchmark), vegetable canteen waste showed the best performance and cow manure performed worst. Formulations showed significantly improved performance and lower variability in comparison to the individual wastes. However, variability in performance was higher than expected for the formulations. One reason for this variability could be different fibre and lipid contents, which correlated with the performance results of the formulations. Overall, this research provides baseline knowledge and guidance on how BSFL treatment facilities may systematically operate using biowastes of varying types and compositions.

1. Introduction

The treatment of biowaste by black soldier fly larvae (BSFL) is an emerging waste management technology (Čičková et al., 2015; Gold et al. 2018b; Zurbrügg et al., 2018). This process converts waste into larval biomass, reduces waste dry mass and generates the raw materials for the production of soil conditioner and fertilizer (Setti et al., 2019), lubricants and biodiesel (Leong et al., 2016; Li et al., 2011), pharmaceuticals (Vilcinskas, 2013) and animal feeds (Barragán-Fonseca et al., 2017; Makkar et al., 2014; Sánchez-Muros et al., 2014; Wang and Shelomi, 2017).

A key challenge for BSFL biowaste treatment is its variable reliability and efficiency. Currently, performance—as measured by bioconversion rate, larval weight, and larval biomass composition (e.g. protein and lipid content)—varies both when using the same type of biowaste (e.g. different vegetable wastes) and when treating different types (e.g. vegetable waste compared to mill by-products) (as summarised by Gold et al., 2018a). The sustainable operation of BSFL biowaste treatment facilities likely depends on the use of different waste types of varying quantity and composition. Homogenous or highly nutritious biowastes such as food industry

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by-products (e.g. bread and mill by-products) or canteen and restaurant wastes are often already used elsewhere (e.g. as animal feeds and for energy recovery). In addition, poor waste management practices, such as a lack of organic waste segregation, incentives for landfill disposal, and complex collection and transport logistics, often hinder access to high-quality wastes. Importantly, the use of different wastes from different sources will adversely affect the day-to-day operation (e.g. running over or under capacity) of BSFL treatment facilities with concurrent impacts on BSFL growth and waste treatment performance. This affects the sustainability (Mertenat et al., 2019; Smetana et al., 2019, 2016) and scalability of this technology and the down-stream application of products (e.g. live-feed for aquaculture) (Gold et al., 2018a).

Similar to other animal species used for production, the nutrient content of biowaste is hypothesised to have the largest influence on performance under similar operating conditions (e.g. feeding rate, larval density and temperature) (Nguyen et al., 2013; Oonincx et al., 2015a; Tinder et al., 2017). Factors determining the nutritional quality of biowaste include the density, ratio and type of nutrients it contains. Nutrients considered to be decisive include the sum of all macronutrients, organic matter, protein, non-fibre carbohydrates (NFC), fibre and lipids (Barragán-Fonseca et al., 2018a, 2018b; Gold et al., 2018a; Lalander et al., 2018). For example, manures are typically low in organic matter and fibre, restaurant and canteen wastes are rich in NFC and lipids, and fruit and vegetable wastes are low in proteins (Gold et al., 2018a). In response to these different nutritional conditions, fly larvae adjust their growth rate and nutrient accretion, with the main goal of accumulating enough reserves to complete the non-feeding life-stages of metamorphosis and adulthood (Danielsen et al., 2013; Gold et al., 2018a). Similar to other animals, an insufficient amount or an unfavourable ratio of nutrients prolongs development, reduces growth and related biomass production, and limits the efficiency of waste reduction (Danielsen et al., 2013).

BSFL feeding experiments and assessments of the midgut (the main organ involved in digestion) suggest that protein, NFC and lipids are highly digestible by BSFL and, therefore, their supply enhances performance (Barragán-Fonseca et al. 2018a; Beniers and Graham, 2019; Bonelli et al., 2019; Lalander et al., 2018). In contrast, fibre including cellulose and lignin are less digestible and tend to decrease larval growth rates (Liu et al., 2018). Among these nutrients, several studies have concluded that the protein (and amino acid) content of biowastes is most important. For example, Lalander et al. (2018) concluded that protein has the greatest impact on the development time to prepupa. Beniers and Graham (2019) also observed that protein has greater importance for larval weight than NFC. As amino acids allow larvae to proceed to the next instar (Gold et al., 2018b) and BSFL accumulate lipids during later instars (as energy reserves for later life-stages) (Liu et al., 2017), waste with a greater protein content can also increase larval lipid content. Research on the common fruit fly larvae (*Drosophila melanogaster*) indicates that fly larvae control their feeding with respect to protein and may overfeed on other nutrients such as NFC (Almeida de Carvalho and Mirth, 2017). This further emphasises that protein is so essential for development. For BSFL, protein supply may influence larval weight and lipid content when receiving low-protein and high-carbohydrate feeds as carbohydrates may be converted into body lipids (Pimentel et al., 2017; Spranghers et al. 2017). By contrast, lipids in biowaste can impede or promote larval development. Nguyen et al. (2013) suspected that excess lipids in fish offal may decrease larval development; however, results from Oonincx et al. (2015a) for food industry by-products and from Nguyen et al. (2013) for liver and kitchen waste suggest that lipids can also increase performance as the energy density of the feed is increased (Brouwer, 1965). Oonincx et al. (2015a) also observed high feed-conversion efficiencies for feeds with a high lipid and protein content. The ash content of biowaste positively correlates with larval ash content (Spranghers et al. 2017) and negatively with bioconversion rates (Lalander et al., 2018).

A reliable high-performance BSFL treatment for biowaste requires strategies that build on existing knowledge of the influence of variable waste nutrient compositions on larval performance. Similar to other biowaste treatment options such as anaerobic digestion or composting, co-conversion, i.e. the treatment of a mixture of several biowastes, could increase performance and reduce variability (Li et al., 2009). Specifically, mixing multiple biowastes can provide a more nutritious and balanced feed for larval growth. Rehman et al. (2017) and Nyakeri et al. (2019) observed that mixing cow and human manure with food wastes and food production by-products (e.g. soybean curd residue and banana peels) increased larval weight compared to these individual wastes. Similar to composting (i.e. carbon-to-nitrogen ratio) and anaerobic digestion (e.g. methane potential), a systematic approach to co-conversion based on biowaste nutrients could compensate for the variability in biowaste composition.

The formulation of appropriate biowaste mixtures based on nutrients requires the reliable determination of composition using parameters that are relevant for BSFL growth. Such an approach—and also incorporating cost considerations—is widely applied for feed formulation in commercial livestock production (McDonald et al., 2011). Barragán-Fonseca et al. (2018b) were the first to formulate feeds for BSFL with similar protein and NFC contents using combinations of food industry by-products; however, larvae still performed significantly different between these waste mixtures. These authors concluded that nutrient quality (e.g. amino acid content, type of NFC and fibre) must be considered to reduce this variability. Previous studies have not considered this sufficiently when determining biowaste composition. For example, biowaste has been characterised based on its carbon, nitrogen and protein content (using generic nitrogen-to-protein conversion factors) or the sum of other nutrients to estimate its NFC content (Barragán-Fonseca et al. 2018b; Lalander et al., 2018). Nitrogen may not, however, be an accurate measure as it may also include non-protein nitrogenous compounds of low nutritional value such as urea, ammonia, nitrate and nitrite (Chen et al., 2017). Similarly, carbon includes digestible fibre such as cellulose and lignin. The importance of these compounds is difficult to assess as the extent to which non-protein nitrogen and fibre are used by the gut microbes of BSFL is not yet known. Thus, generic nitrogen-to-protein conversion factors may overestimate protein content. In addition, carbon may greatly overestimate NFC when the ash, lipid, protein and fibre content is subtracted from 100% rather only than the sum of digestible carbohydrates such as glucose and starch.

This study aimed to assess the performance of BSFL treatment as applied to different waste formulations prepared from six types of biowaste following the determination of their respective nutritional composition. It was hypothesised that biowaste formulations with a similar protein and NFC content would increase performance and reduce variability in comparison to the individual wastes. Thereby, this research sought to generate knowledge and advice on how BSFL treatment facilities may best operate with biowaste of varying type and composition.

2. Materials and methods

2.1. Biowastes used in the feeding experiments

Six different types of biowaste were used in feeding experiments, namely mill by-products, human faeces, poultry slaughterhouse waste, cow manure, and canteen and vegetable canteen

waste. Two different batches of human faeces were used as BSFL treatment performance was unexpectedly high with the first batch. Mill by-products were obtained from a Swiss wheat-milling company. The human faeces were obtained from dry toilets separating urine and faeces at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) in Dübendorf, Switzerland. The poultry slaughterhouse waste consisted of discarded body parts (feet, head, liver, stomach, and intestine) from a poultry slaughterhouse of Micarna, a leading meat processing company in Switzerland. The cow manure was obtained from a farm near Zurich, Switzerland. The vegetable waste was obtained from the Eawag canteen and consisted of a mixture of vegetables with and without salad dressing. The difference between the vegetable canteen waste and the canteen waste was that the latter had the addition of sausage and other meat offal.

Following their collection, the wastes were homogenised with a kitchen blender to mimic the pre-treatments used in BSFL treatment facilities (Dortmans et al., 2017), and moisture content was determined in duplicate with a halogen moisture analyser (BM-65, Phoenix instrument, Garbsen, Germany) The wastes were then portioned into plastic bags, frozen and stored at −20 °C until the start of the feeding experiments (Diener et al., 2009; Lalander et al., 2018; Myers et al., 2008; Nguyen et al., 2015). The wastes were thawed at 4 °C for 24 h and brought to the experimental temperature of 28 °C prior to each feeding experiment.

2.2. Composition of the experimental biowastes

Oven-dried (105 °C) wastes and poultry feed (used as a high-performance benchmark) were analysed for gross nutrient composition, moisture content and pH using standard procedures (AOAC 1997; Van Soest et al., 1991). The second batch of human faeces was only analysed for protein, lipid and organic matter content. Moisture and organic matter were determined in quintuplicate with an automatic thermogravimetric determinator (TGA-701, Leco, St. Joseph, MI, USA). Nitrogen content was determined in triplicate using a C/N analyser (Type TruMac CN, Leco Cooperation, St. Joseph, MI, USA). Fibre fractions including neutral (NDF) and acid detergent fibre (ADF) were assessed in duplicate using a fibrebag system (Fibretherm, Gerhardt Analytical Systems, Germany) according to methods 6.5.1 and 6.5.2 of the Association of German Agricultural Analytic and Research Institutes (Naumann et al., 2012). Lipids were analysed from ether extracts from freeze-dried samples by Eurofins Scientific, Schönenwerd, Switzerland, according to Regulation (EC) No 152/2009 (European Commission (EC) 2009). The extraction solvent used was petroleum ether at 40–60 °C following hydrolysis with 3 M hydrochloric acid. pH was analysed with a portable meter and pH probe (HQ40d, Hach Lange GmbH, Switzerland).

Amino acids were analysed in triplicate in freeze-dried samples (Çevikkalp et al., 2016; Kwanyuen and Burton, 2010; White et al., 1986; Zhang et al., 2009). The samples were hydrolysed at 110 °C for 16–24 h with 5 M sodium hydroxide (tryptophan) or 6 M hydrochloric acid containing 0.1% phenol (for all other amino acids). For tryptophan, the hydrolysed samples were subsequently neutralised, diluted and analysed by RP-HPLC-FLD using an Agilent 1200 series LC-system including a fluorescent detector (FLD) (Agilent Technologies, Santa Clara, USA) and a C18 analytical Pico Tag amino acid analysis column (3.9 × 150 mm) in combination with a Nova-Pak C18 guard column (3.9 × 20 mm) (Waters AG, Baden, Switzerland). The fluorescence detector was operated at an excitation wavelength of 280 nm and an emission wavelength of 340 nm. For all other amino acids, the hydrolysed amino acids were transformed into their phenylthiocarbamyl derivatives with phenyl isocyanate and analysed by RP-HPLC using an Agilent 1100 series LC-system including a diode array detector (DAD) operated at 254 nm (Agilent Technologies, Santa Clara, USA) and the same column as above. α-methyl-DL-tryptophan and L-norleucine was used as an internal standard. The HPLC results were corrected with the respective recovery rates of the internal standards. Only results with an internal standard recovery >70% were considered further. More details on amino acid analyses are included in the Supplementary Material.

Glucose and starch were determined in triplicate using freeze-dried samples with a commercial enzyme assay (Megazyme, 2019). In brief, glucose was removed from each sample with ethanol. Then, following centrifugation, the glucose concentration was determined in the supernatant and the pellet was used for starch analysis. Resistant starch was converted into maltodextrins in potassium hydroxide. Amylase and amyloglucosidase were used to hydrolyse the remaining starch into glucose. Glucose was then quantified with a spectrometer (Genesys 10S, Thermo Fisher Scientific, USA) in comparison to a glucose standard.

Protein was calculated by multiplication of the nitrogen results with specific conversion factors, namely 5.6 for poultry feed (based on results for maize and soybean meal) (Sriperm et al., 2011), 4.3 for cow manure (Chen et al., 2017), 5.4 for mill by-products (based on results for cereals) (Mariotti et al., 2008), 5.4 for canteen waste, 5.0 for vegetable canteen waste, and 5.0 for poultry slaughterhouse waste (based on results for meat, fish, cereals and vegetables) (Mariotti et al., 2008). No conversion factors were available for human faeces and so this was estimated as the ratio of the sum of all amino acids divided by the nitrogen content. Samples of human faeces (mixed with sawdust) (Nyakeri et al., 2019) and pit latrine sludge provided by Sanergy, Nairobi, Kenya, were also included in the analysis to cover the typical variability of human faeces and faecal sludge (Gold et al., 2017b, 2017a). Caloric content was estimated by multiplying the mean results for lipids, NFC and protein with their gross caloric content of 9.4, 5.4, and 4.1 kcal/g, respectively (Merrill, 1973; Wu, 2016). Hemicelluloses were determined as the difference between NDF and ADF. ADF was assumed to be a reliable estimate of cellulose and lignin content. The sum of glucose and starch was assumed to reflect the total NFC.

2.3. Formulation of the biowaste mixtures

For the feeding experiments, either the six individual wastes or six mixtures of the wastes (Table 1) were used. The mixtures were based on the composition of the biowastes and aimed to achieve a protein-to-NFC ratio of approximately 1:1 (DM) considering the low content of NFC (Barragán-Fonseca et al. 2018b; Cammack and Tomberlin, 2017). In contrast to Barragán-Fonseca et al. (2018b), no high-value ingredients such as sunflower oil or cellulose were added to balance the unavoidable variability in fibre and lipid content as this is not typically practicable for cost reasons.

The formulations were generated using Visual Basic for Applications in Microsoft Excel and were always based on mill by-products complemented with two to three other wastes. The formulations were prepared from thawed wastes on the day of feeding and were mixed thoroughly. Formulation 3 was prepared with two different batches of human faeces based on the nutrient composition of the first batch. These batches appeared to have a similar composition based on their lipid (20.9 and 19.3% DM), crude protein (20.5 and 21.8% DM) and ash (13.7 and 15.8% DM) content. In the following discussions, the two human faeces formulations are referred to as formulation F3 (1) and formulation F3 (2), respectively.

Table 2 shows the realised nutrient composition of the six formulations, calculated based on the proportions shown in Table 1 and the results of the analyses of the individual biowastes. The

Table 1
Dry mass proportion of individual wastes in the biowaste formulations (F1-F6).

Formulation	F1	F2	F3	F4	F5	F6
Mill by-products	23	37	51	60	33	65
Canteen waste	–	7	–	20	33	–
Human faeces	16	–	14	20	–	–
Poultry slaughterhouse waste	–	–	–	–	–	22
Cow manure	11	35	34	–	–	12
Vegetable canteen waste	50	21	–	–	33	–

Table 2
Mean dry mass nutrient contents of the different biowaste formulations (F1-F6) based on the percent dry mass proportion of individual wastes in the biowaste formulations (Table 1) and the composition of their constituent wastes (Table 3).

	Proteins	Non-fibre carbohydrates	Fibres	Lipids	Organic matter	Moisture content
Formulation 1 (F1)	13.8	13.6	38.5	19.0	90.5	80.8
Formulation 2 (F2)	14.0	13.0	48.7	11.2	88.9	81.5
Formulation 3 (F3)*	14.0	12.7	50.1	5.9	88.4	79.9
Formulation 4 (F4)	19.1	15.8	43.8	13.0	92.1	72.5
Formulation 5 (F5)	19.6	15.4	39.8	22.3	93.1	76.9
Formulation 6 (F6)	19.0	15.4	45.8	12.0	92.1	73.9
Mean	**16.6 (2.9)**	**14.3 (1.4)**	**44.5 (4.7)**	**13.9 (5.9)**	**90.7 (2.2)**	**77.6 (3.7)**

In parentheses: standard deviation.
* Formulation (3) 1.

formulations contained between 14 and 19% DM of protein and between 13 and 15% DM of NFC.

2.4. Feeding experiments

Feeding experiments were designed as outlined by Lalander et al. (2018) and Liu et al. (2018). Three individual sets of experiments with different batches of larvae were carried out. First with the individual wastes (experiment 1) and then with formulations 1 to 3 (experiment 2) and finally with formulations 4 to 6 (experiment 3). Larvae were obtained from the BSFL research colony at Eawag maintained according to Dortmans et al. (2017). The BSFL hatched within 24 h and were first fed *ad libitum* with poultry feed (UFA 625, UFA AG, Switzerland) for 12–14 d until they reached a mean individual weight of 3.8 ± 0.5 mg DM. The larvae had a similar content of carbon (55–56% DM), protein (36–38% DM) and ash (13–14% DM) across the experiments (Section 2.5). From these populations, 4 to 5 × 80 randomly selected larvae per treatment were manually counted and placed in plastic containers (7.5 cm diameter, 11 cm height) with individual wastes or waste formulations, giving a larval density of approximately 2 larvae/cm^2. Larvae were also freeze-dried for the analysis of larval composition. The experimental containers were covered with paper towels or mosquito nets and randomly arranged in a climate chamber (HPP 260, Memmert GmbH, Germany) providing a steady microclimate of 28 °C and 70% relative humidity. Feed was provided every 3 d. Considering the increase in the nutrient requirements of BSFL with growth (Nyakeri et al., 2019), the feeding rate was increased over the 9-day experiment from 15 to 25 and 40 mg DM/larva per day on days 0, 3 and 6, respectively. Due to the expected improved nutritional quality of the formulations, the feeding rate was lowered by 25% for each feeding in experiment two and three.

In contrast to previous studies, which have typically terminated experiments after the first appearance of prepupae (Bosch et al., 2019; Lalander et al., 2018), all experiments were terminated after 9 d, before the appearance of prepupae. Prepupae are richer in chitin and lipids and, therefore, not optimal for animal feed applications (Nyakeri et al., 2019). Larvae were manually separated from the residue, cleaned with tap water, and dried with paper towels. Subsequently, larvae were manually counted, weighed and freeze-dried. Residues were dried in a laboratory oven at 80 °C. Both the dried larvae and the residues were then weighed and stored at 4 °C.

2.5. Analysis of larval composition

The dried larvae were milled and treatment replicates were combined equally by mass. Samples were then analysed in triplicate for DM, carbon and nitrogen content using the same analysers as for the wastes. Larval protein content was calculated as the nitrogen content × 4.67 following Janssen et al. (2017). Carbon content was divided by the total amount of organic matter; as lipids typically contain more carbon than proteins and carbohydrates (Brouwer, 1965), the ratio of carbon-to-organic matter was used as an indicator of larval lipid content.

2.6. Determination of the performance of BSFL treatment

Larval counts, and residue and larvae dry weights, were used to calculate five BSFL performance parameters. First, larval survival rates were calculated using Eq. (1) as the ratio of larvae at the end ($larvae_{end}$) and the beginning ($larvae_{beg}$) of the experiments (Van Der Fels-Klerx et al., 2016).

$$\text{Survival rate } (\%) = \frac{larvae_{end}}{larvae_{beg}} \times 100 \quad (1)$$

Waste reduction was calculated using Eq. (2) as the ratio of residue dry mass ($residue_{mass}$) to the dry mass of total feed ($feed_{mass}$) provided (Diener et al., 2009):

$$\text{Waste reduction } (\%\ \text{DM}) = \left(1 - \frac{residue_{mass}(g)}{feed_{mass}(g)}\right) \times 100 \quad (2)$$

The bioconversion rate was calculated using Eq. (3), for which the larval dry weight gain ($larval_{gain}$) was calculated as the difference between the final larval dry weight and the initial larval dry weight multiplied by the number of larvae at the end of the experiment:

$$\text{Bioconversion rate } (\%\ \text{DM}) = \frac{larvae_{gain}(g)}{feed_{mass}(g)} \times 100 \quad (3)$$

Waste conversion efficiency (Liu et al., 2018), also called efficiency of conversion of ingested/digested food (Diener et al., 2009; Oonincx et al., 2015b), was calculated using Eq. (4):

$$\text{Waste conversion efficiency (\% DM)} = \frac{larvae_{gain}(g)}{feed_{mass}(g) - residue_{mass}(g)} \times 100 \quad (4)$$

Finally, the protein conversion efficiency was calculated using Eq. (5) as the ratio of the amount of larval protein accumulated ($protein_{gain}$) to feed provided ($feed_{mass}$). Larval protein accumulated was calculated as the difference between the amount of final larval protein and the initial larval protein multiplied by $larvae_{end}$. The amount of larval protein was calculated by multiplying the larval protein content with the larval weight:

$$\text{Protein conversion efficiency (\% DM)} = \frac{protein_{gain}(g)}{feed_{mass}(g)} \quad (5)$$

2.7. Performance benchmark

As in previous research, poultry feed (60% moisture content) was fed to larvae in parallel to the individual biowastes and biowaste formulations as a high-performance benchmark (Lalander et al., 2018). As shown in Fig. 1, the results for poultry feed varied between experiments but no single experiment stood out as being different across all of the performance parameters. Even though larvae had a similar weight and composition at the start of the experiment, variability between experiments could be due to differences in age, feeding rates or other confounding factors (e.g. differences in airflow in the climate chamber due to varying numbers of containers).

To ensure a consistent basis for comparison between the three experiments, and between the individual wastes and waste formulations, performance parameters were also expressed as percentage differences (Fig. 1) in comparison to the results for the poultry feed using Eq. (6). For this, the results for each performance parameter ($Performance_{treatment}$) were subtracted from the median result obtained using the poultry feed ($performance_{benchmarkl}$) over all three experiments:

$$\text{Performance in \% to benchmark} = \frac{performance_{treatment} - median\ performance_{benchmark}}{median\ performance_{benchmark}} \times 100 \quad (6)$$

Even though all of the parameters were corrected by the mass of total feed provided, variations in feeding rate, which differed between experiments, could influence performance results. Therefore, poultry feed and mill by-products were also fed to larvae at two different feeding rates: 27 mg DM/larva per day as used in experiment 1 and 20 mg DM/larva per day as used in experiments 2 and 3. This comparison, included in Supplementary Fig. S1, did not reveal an influence of feeding rate on performance, thus justifying the comparisons across the experiments.

2.8. Data analyses and statistics

Data were analysed using R software and RStudio version 1.1.463 (RStudio Inc., Boston, MA, USA). The mean, median, standard deviation, and range (difference of maximum and minimum) of the biowaste composition and performance parameters were calculated. Significance of differences in mean nutrient composition and mean performance parameters between the individual wastes and the waste formulations were tested using analysis of variance (ANOVA) followed by pairwise Tukey post-hoc comparisons. Due to the small size sample size per group (n = 3–5), normality and homogeneity of variance were assessed visually by residuals versus fits and Q-Q plots (Ricci et al., 2019). These graphs are shown in Supplementary Figs. S2–S4. A conservative p-value of <0.01 was chosen to declare significance due to the small sample size per group, which could lead to misinterpretation of model assumptions. The Mann–Whitney *U* test ($p < 0.05$) used to identify significant differences between the distributions of performance parameters of all the individual wastes (n = 29) in comparison to all the waste formulations (n = 29). The Levene and Shapiro-Wilk test ($p < 0.05$) identified that the data in those two groups violated the assumptions for parametric tests. Following visual assessment of normality (Supplementary Fig. S5), Pearson correlation coefficients ($p < 0.01$) were also calculated to identify linear dependencies between biowaste composition and feeding experiment results.

3. Results and discussion

3.1. Biowaste composition

Nutrient composition varied significantly between the biowastes (Table 3). The results for amino acids are included in Supplementary Table S1. The biowastes showed large variability

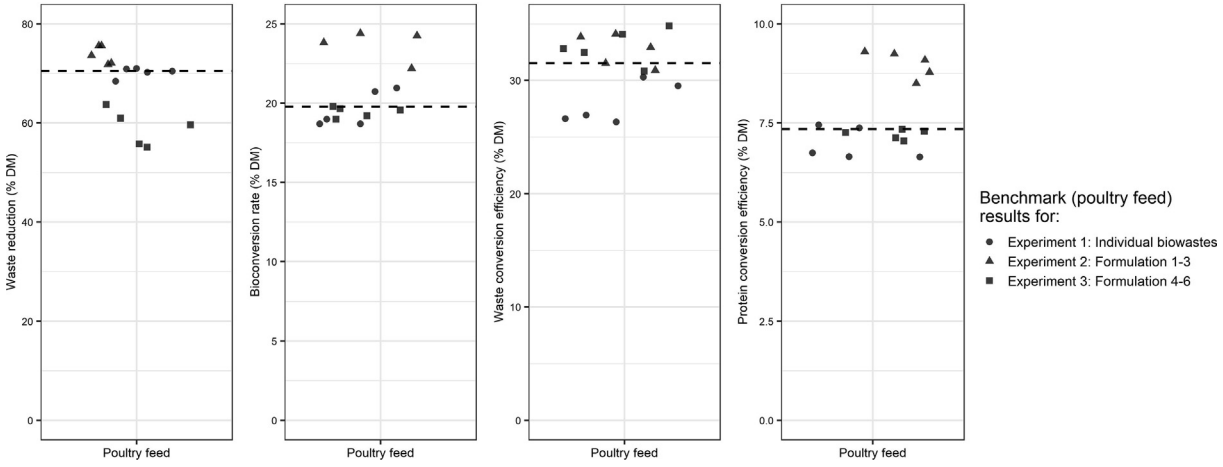

Fig. 1. Performance of BSFL fed on poultry feed used as a high-performance benchmark in the three experiments. Medians are shown as dashed lines. Performance results of the biowastes and formulations were expressed relative to these medians using Eq. 6.

Table 3
Mean nutrient composition of individual biowastes as percent of dry mass, moisture content in percent, pH, and caloric content as kcal per 100 g dry biowaste.

Wastes	pH	Moisture content	Protein	Non-fibre carbohydrates			Fibre			Lipids	Organic matter	P:NFC* ratio	Caloric** content
				Total	Glucose	Starch	Total	Cellulose & lignin	Hemicellulose				
Mill by-products	6.2 (0.1)	70.0	14.5^{d} (0.3)	23.2^{b} (0.2)	1.7^{b} (0.0)	21.2^{b} (0.6)	51.7 (0.9)	22.1 (1.0)	29.6 (1.9)	3.0	93.8^{c} (1.3)	1:2	211
Canteen waste	4.3 (0.0)	74.0 (1.2)	32.2^{b} (0.8)	7.5^{d} (0.7)	3.5^{a} (0.4)	4.0^{d} (0.4)	36.2 (1.4)	22.8 (0.6)	13.4 (0.9)	34.9	93.0^{c} (0.7)	4:1	501
Human faeces (1)	6.0 (0.0)	76.7 (0.9)	20.1^{c} (0.9)	1.7^{e} (0.1)	1.0^{bc} (0.0)	0.7^{e} (0.1)	27.9 (0.6)	19.5 (0.3)	8.4 (0.6)	20.9	86.4^{b} (0.3)	12:1	288
Poultry slaughterhouse waste	5.7 (0.1)	66.7 (1.2)	37.3^{a} (0.5)	0.3^{e} (0.1)	0.2^{d} (0.1)	0.1^{e} (0.0)	20.8 (1.9)	9.3 (0.9)	11.5 (2.7)	42.9	94.0^{c} (1.3)	152:1	557
Cow manure	7.2 (0.1)	87.0 (0.2)	11.1^{e} (0.4)	1.8^{e} (0.6)	0.7^{cd} (0.3)	1.0^{e} (0.4)	58.4 (0.4)	40.9 (1.7)	17.4 (1.2)	4.4	80.7^{a} (0.5)	7:1	96
Vegetable canteen waste	3.8 (0.0)	82.7 (0.1)	12.1^{e} (0.1)	15.5^{c} (0.9)	3.7^{a} (0.3)	11.6^{c} (0.6)	31.5 (1.8)	24.0 (1.5)	7.5 (0.3)	28.9	92.4^{c} (0.5)	1:1	404
Mean	**5.5 (1.3)**	**74.5 (10.0)**	**21.2 (10.2)**	**8.3 (9.2)**	**1.8 (1.5)**	**6.4 (8.4)**	**37.7 (14.5)**	**23.1 (10.2)**	**14.6 (8.2)**	**22.5 (16.3)**	**90.1 (5.4)**	**29:1**	**343(177)**
Poultry feed (benchmark)	5.7 (0.0)	60.0	19.1^{c} (0.7)	28.5^{a} (0.8)	0.5^{cd} (0.2)	27.5^{a} (1.4)	22.0 (1.0)	8.6 (0.0)	13.5 (1.1)	4.8	98.2^{ab}(4.0)	1:2	274

In parenthesis: standard deviation for samples where n ≥ 3 and differences between analyses where n = 2.
results with no shared letter are significantly different from each other.
* P:NFC = ratio of protein to non-fibre carbohydrates (NFC).
** gross caloric content of protein, NFC, and lipids.

with respect to protein content, which was highest in poultry slaughterhouse waste, canteen waste and human faeces, and lowest in cow manure, vegetable canteen waste and mill by-products. Protein quality may also differ. In contrast to the other wastes, the protein in human faeces and cow manure was likely protein from gut microbial biomass (Rose et al., 2015).

This was the first study in which nitrogen-to-protein conversion factors were determined for human faeces and faecal sludge. Human faeces collected in Zurich and pit latrine sludge collected in Nairobi had conversion factors of 3.9 and 3.8, respectively. These conversion factors are comparable to those for animal manures in the range of 2.8–4.3 (Chen et al., 2017). In comparison, human faeces collected in Nairobi had less non-protein nitrogen, with a conversion factor of 5.2. Potential reasons for this difference could be the differing diets between the residents of Zurich and Nairobi (Rose et al., 2015) or storage conditions (e.g. temperatures) leading to the volatilisation of nitrogen. Overall, these results confirm that multiplying nitrogen results with the generic factor of 6.25 (i.e. the inverse of the mean nitrogen content of protein) can greatly overestimate true protein (i.e. amino acid) content (Mariotti et al., 2008). This is well established but has not been implemented even in recent BSFL research (Lalander et al., 2018; Liu et al., 2018).

Wastes were low in glucose, starch and total NFC. NFC was highest in the mill by-products and vegetable canteen waste. The addition of meat to the vegetable canteen waste increased protein content from below 15 to over 30% DM but concurrently decreased NFC content by half. Cow manure, human faeces, and slaughterhouse waste had almost no NFC. This was expected as animal tissue contains only very small amounts of glycogen and most NFC is digested or fermented in the gut of humans and animals (Riesenfeld et al., 1980). The sum of glucose and starch was much lower than when NFC was calculated as the difference between DM and ash, protein, fibre, and lipids. For example, the calculated value for human faeces was 17% DM compared to 1.7% DM for glucose plus starch (Rose et al., 2015; Spranghers et al., 2017). This indicates that there are either large amounts of non-sugar-non-starch-non-fibre organic matter or that there is an accumulation of analytical error in the gross nutrient measurements, or both. Overall, in the present study, low NFC was the reason why the protein-to-carbohydrate ratios in the waste formulations did not exceed 1:1 and that the mean NFC contents did not exceed 14% DM. The corresponding values described by Barragán-Fonseca et al. (2018b) and Cammack and Tomberlin (2017) were 1:1 to 1:2 and 21–30% DM, respectively.

The content of lipids, fibre and ash also varied among the wastes. Poultry slaughterhouse waste had a high lipid content and low ash and fibre content; the opposite was true for cow manure and mill by-products. The addition of meat to the canteen waste markedly increased the lipid content, while both of the canteen wastes were low in ash. Also, both batches of human faeces were rich in lipids, with values exceeding 20% DM. These results are high considering values ranging from 2 to 21% DM have been reported in the literature. The lipid content of the human faeces and pit latrine sludge samples collected in Nairobi were 9.4 and 16.6% DM, respectively. This suggests that the amount of lipids in faecal sludge can vary depending on management practices (e.g. residence time in the containment and the addition of sawdust) (Gold et al., 2017b), the presence of unabsorbed lipids, endogenous lipid losses (e.g. bile) and microbial processes (Aylward and Wood, 1962; Rose et al., 2015). Overall, the data show that the cow manure, human faeces, and poultry slaughterhouse wastes had low amounts of digestible nutrients and high protein-to-NFC ratios, whereas both the canteen wastes were rich in digestible nutrients and had a high caloric content However, it is unknown how much of this energy can be harnessed by the fly larvae. Similar to mill by-products, the vegetable canteen waste was balanced or slightly

NFC biased with proteins and NFC ratios of 1:1 and 1:2, respectively. Thus, these wastes were expected to perform best in feeding experiments when offered alone (Barragán-Fonseca et al. 2018b; Cammack and Tomberlin, 2017). For the other biowastes, mixing those with complementary nutrient compositions was expected to be advantageous.

3.2. Treatment performance of individual biowastes

All individual biowastes supported the development of BSFL (Table 4). The mean survival rates were 90–99% and were not significantly different between the biowaste types. They were also comparable to those found in previous research, where survival rates were shown to be above 80% (summarised by Rehman et al., 2017). Lalander et al. (2018) reported survival rates for different biowastes in the range of 81 to 100%, except for wastewater sludge which supported survival rates of only 39 to 81%. These results suggest that the experimental conditions applied in the present study were suitable and confirms that BSFL can develop on a wide variety of biowastes. That said, BSFL treatment performance varied widely between the different biowastes (Fig. 2). Mean performances values were significantly different among most of the biowastes and those that performed best were not always the same for each performance parameter. Waste reduction and protein conversion efficiency were lower for all of the wastes in comparison to the poultry feed, and cow manure had the poorest performance in all of the performance parameters.

Using vegetable canteen waste and mill by-products resulted in the highest waste reduction even though values were still 17 to 20% lower than for the poultry feed. This could be due to the high NFC content of these two wastes, which are easily digested and absorbed into the haemolymph of fly larvae (Bonelli et al., 2019; Pimentel et al., 2018). However, comparison of the waste reduction and larval weight results of mill by-products and human faeces demonstrate that this higher waste reduction did not necessarily result in higher larval weight. The level of waste reduction in the mill by-products exceeded the sum of easily digestible nutrients which are assumed to be reflected by the sum or protein, NFC and lipids (Table 3) based on the morphofunctional features of the BSFL midgut reported by Bonelli et al. (2019). This suggests that some fibre, likely hemicelluloses, were decomposed during BSFL treatment. Gold et al., 2018b also observed some decomposition of hemicelluloses in BSFL treatment with artificial diets, but this happened to a much smaller extend than that reported by Rehman et al. (2017) with cow manure. Such differences in digestibility have not yet been considered in biowaste formulation and could lead to unexpected performance results when designing formulations based on the glucose and starch content of NFC alone.

Human faeces supported a bioconversion rate that was comparable to the poultry feed (and this was higher for human faeces (1) and lower for human faeces (2)), despite having a much lower waste reduction. This was due to an 85% higher waste conversion efficiency than with the poultry feed. BSFL showed a significantly lower performance using poultry slaughterhouse waste than human faeces. The lowest performance was found for cow manure, which was low in protein, NFC, and lipids. In contrast, the human faeces and poultry slaughterhouse wastes were high in protein and lipids. These results thus suggest that NFC is less important for larval development than high overall nutrient content.

The protein conversion efficiencies were less variable and trends were different in comparison to the other performance parameters. Human faeces (1) and vegetable canteen waste had the highest bioconversion rate but not the highest protein conversion efficiency. This was due to varying larval composition arising from the different wastes and their associated larval weights. Larval protein content was notably higher when fed on mill by-products and canteen waste than on human faeces and vegetable canteen waste (Table 4). Larvae fed with human faeces and vegetable canteen waste likely incorporated more lipids, as indicated by a higher proportion of carbon in organic matter in comparison to the mill by-products and the canteen waste. Larva growing on human faeces contained the most ash.

Considering these findings, the most promising biowaste thus depends on the objective of the BSFL treatment. Mill by-products and vegetable canteen waste performed best with respect to waste treatment whereas human faeces and vegetable canteen waste were more favourable with respect to larval biomass production efficiency. The most protein per unit of biowaste was produced using the mill by-products and the canteen waste. Thus, for facilities targeting insect protein meal production, these wastes would be favourable. Poultry slaughterhouse waste and cow manure resulted in generally poor performance. However, not all wastes

Table 4
Mean performance of BSFL treatment on the different biowastes and formulations.

					Larval biomass composition		
	Survival rate %	Larval weight mg DM	Waste reduction % DM	Bioconversion rate % DM	Protein % DM	Ash % DM	Carbon % OM*
Individual wastes							
Mill by-products	96.2 (1.5)	41.7 (0.9)	56.4 (1.2)	14.9 (0.3)	42.1 (0.4)	7.3	58.0 (0.7)
Canteen waste	92.3 (3.1)	44.2 (5.9)	37.9 (3.8)	15.3 (2.1)	36.1 (0.3)	5.2	62.8 (0.3)
Human faeces (1)	99.1 (0.6)	58.8 (1.7)	39.1 (1.5)	22.7 (0.6)	26.7 (0.4)	13.6	65.8 (0.1)
Human faeces (2)	96.2 (2.5)	50.2 (1.2)	48.6 (0.3)	18.8 (0.8)	27.1 (0.1)	13.1	65.1 (0.6)
Poultry slaughterhouse waste	90.7 (2.9)	39.4 (0.7)	30.7 (4.7)	13.4 (0.5)	31.5 (0.7)	4.1	64.6 (0.4)
Cow manure	89.8 (7.5)	14.3 (0.4)	12.7 (0.9)	3.8 (0.2)	36.2 (0.2)	23.1	56.1 (0.3)
Vegetable canteen waste	97.5 (2.7)	59.1 (2.6)	58.4 (1.4)	22.7 (1.1)	24.5 (0.2)	5.1	65.4 (0.1)
Poultry feed (benchmark)	97.9 (2.1)	55.6 (5.1)	67.7 (6.9)	21.0 (2.4)	36.3 (0.8)	12.2	60.4 (0.7)
Waste formulations							
F 1	99.8 (0.6)	64.2 (1.1)	64.1 (0.6)	31.8 (0.6)	25.2 (0.3)	8.0	65.8 (0.2)
F 2	97.8 (3.7)	39.1 (0.3)	51.1 (0.7)	20.9 (0.9)	33.9 (0.5)	11.4	60.9 (0.4)
F 3 (1)	100.0 (0.0)	29.7 (1.2)	45.3 (1.1)	16.4 (0.7)	38.7 (0.3)	16.1	56.9 (0.3)
F 3 (2)	99.7 (0.6)	29.2 (2.0)	49.2 (1.6)	14.5 (1.1)	39.0 (0.6)	15.9	56.9 (0.6)
F 4	98.0 (1.4)	48.9 (2.4)	58.3 (1.1)	22.9 (1.1)	36.9 (0.3)	8.3	61.7 (0.5)
F 5	97.0 (3.4)	62.8 (1.6)	65.2 (2.0)	30.9 (1.6)	28.6 (0.3)	4.9	65.3 (0.7)
F 6	99.0 (1.0)	39.8 (2.1)	56.6 (0.7)	19.8 (1.1)	38.1 (0.3)	8.1	61.0 (0.2)

In parenthesis: standard deviation for samples where $n \geq 3$.
* OM = organic matter.

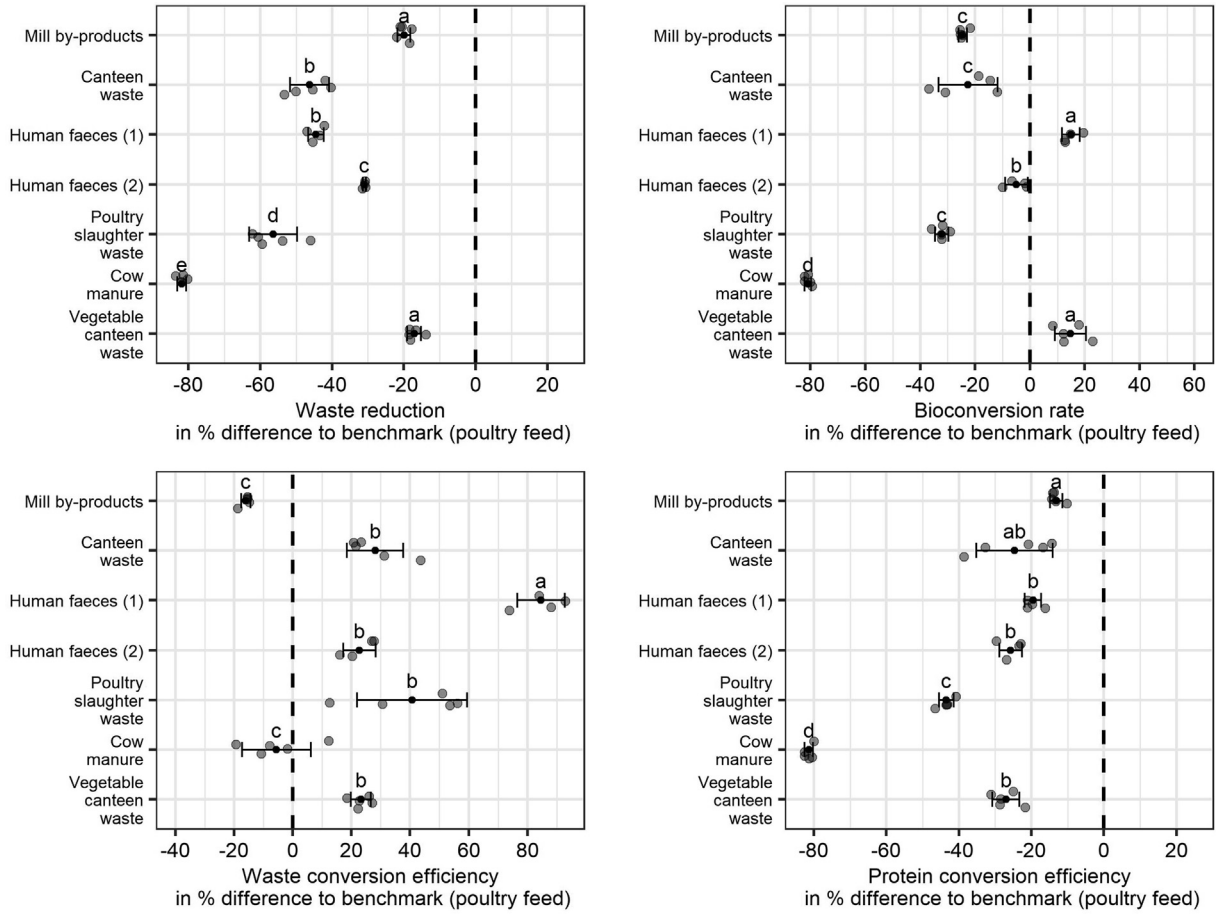

Fig. 2. Effects of the different individual wastes on waste reduction (top, left), bioconversion rate (top, right), waste conversion efficiency (bottom, left) and protein conversion efficiency (bottom, right) in comparison to the benchmark poultry feed (dashed vertical line). Means, standard deviations and results per replicate are displayed. Performance results with no shared letter are significantly different from each other. All results are given in dry mass.

can be employed in BSFL treatment facilities for animal feed production given legal resolutions (Lähteenmäki-Uutela et al., 2017). For example, in the European Union, only mill by-products and pre-consumer wastes (in nutrient composition similar to canteen wastes) can currently be used (European Commission (EC), 2017).

Food, restaurant, and canteen wastes also resulted in the highest—and animal manures the lowest—BSFL treatment performance in previous studies (Lalander et al., 2018; Nyakeri et al., 2019; Oonincx et al., 2015b). Lalander et al. (2018) reported a bioconversion rate of 14% DM for food waste in comparison to 15–23% DM for canteen wastes in this study. The corresponding values for waste reduction were 55% DM in comparison to 38–58% DM. For human faeces, the bioconversion rate was 11% DM as reported by Lalander et al. (2018) compared with 19–23% DM in the present study, and waste reduction data were 48% DM in comparison to 39–49% DM, respectively. In contrast to the present study, Lalander et al. (2018) observed a higher BSFL performance using slaughterhouse waste compared to food waste and human faeces. Values for waste reduction in the literature for cow manure range from 29 to 58% DM (Miranda et al., 2019; Myers et al., 2008; Rehman et al., 2017) and bioconversion rates range from 2 to 6% DM (Miranda et al., 2019; Rehman et al., 2017). This compares with a 13% DM waste reduction and a 4% DM bioconversion rate observed in the present study. These differences confirm that predicting larval performance exclusively based on the type of biowaste is not reliable and can lead to greatly over- or underestimated performance. Such variation is likely to result not only from variable biowaste composition (i.e. nutrient and microbial numbers and communities) but also differences in experimental setups. To help address this, international standards for BSFL feeding experiments could allow for better comparisons across studies.

3.3. Treatment performance of biowaste formulations

The performance of the BSFL grown on the different waste formulations was significantly different despite targeting a similar protein and NFC content and ratio (Table 4; Fig. 3). Overall, using a formulation significantly increased performance compared to individual wastes. Distributions were different between the waste formulations and individual wastes for survival rate, waste reduction, bioconversion rate and protein conversion efficiency but not for waste conversion efficiency.

Feeding BSFL with the waste formulations resulted in higher survival rates in comparison to the individual wastes, and ranged from 97 to 100%. Despite a 25% lower feeding rate, the median larval weight was 43.5 mg DM for the formulations and 40.1 mg DM for the individual wastes. The median of the survival rate was 99% for the formulations and 95% for the individual biowastes. Individual wastes resulted in the median waste reduction and bioconversion rate being lower, by 45.4 and 25.0%, respectively, compared to poultry feed. In comparison, the median waste reduction was only 18.5% lower—and the bioconversion rate even 8.6% higher—for the formulations compared to the poultry feed. The median protein conversion efficiency was 28.5% lower for the individual biowastes and 8.4% higher for the formulations comparison to the poultry

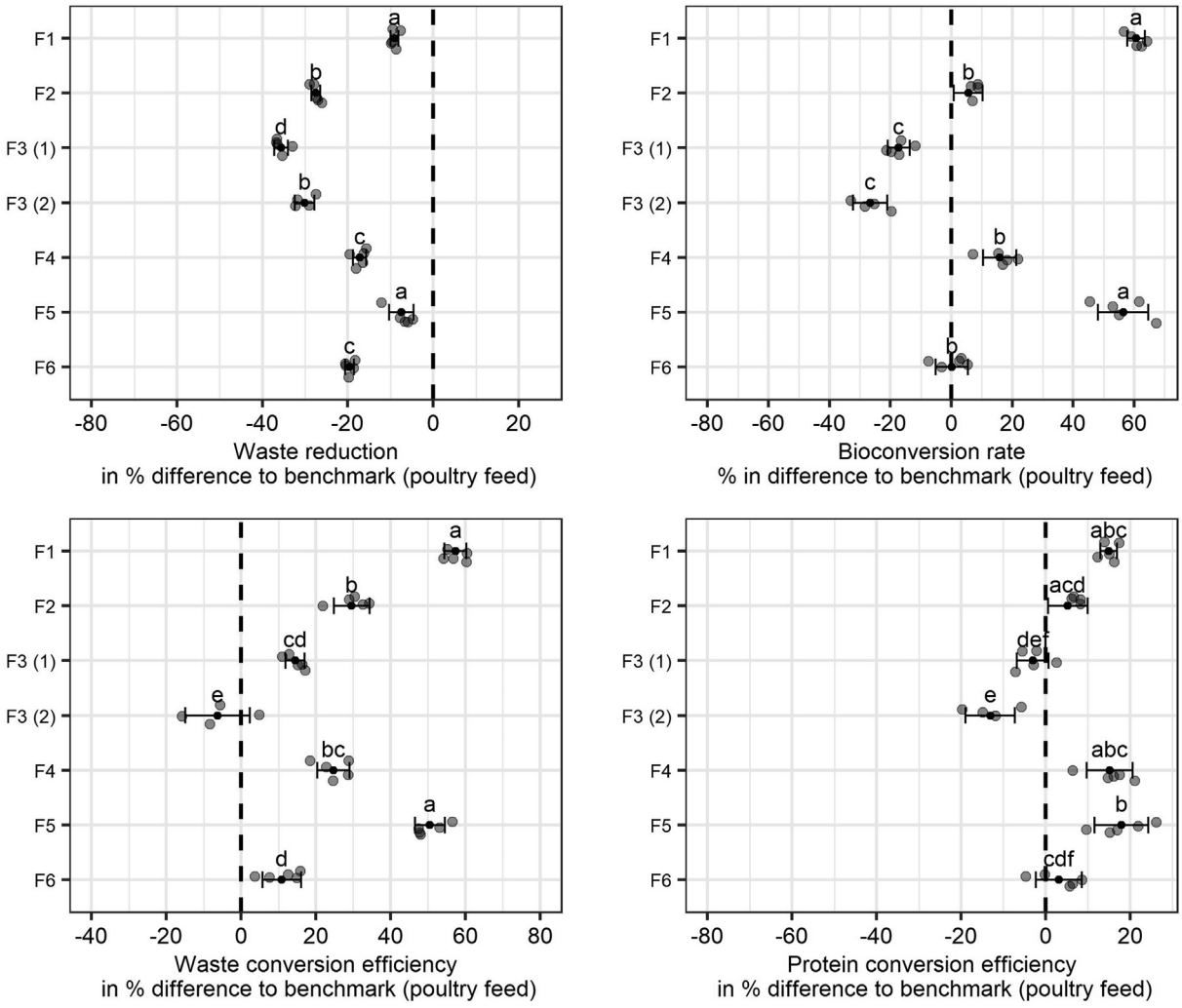

Fig. 3. Effects of the different waste formulations on the waste reduction (top, left), bioconversion rate (top, right), waste conversion efficiency (bottom, left) and protein conversion efficiency (bottom, right) of BSFL in comparison to poultry feed (dashed vertical line). Means, standard deviations and results per replicate are displayed. Performance results with no shared letter are significantly different from each other. All results are given in dry mass.

feed. These results suggest that the performance of BSFL treatment facilities can be increased by designing biowaste mixtures based on similar protein and NFC contents.

By comparing Figs. 2 and 3 it also becomes apparent that the use of formulations decreased the variability in performance. In comparison to the poultry feed, the results for the formulations had a range of 28% for waste reduction, 87% for bioconversion rate, 64% for waste conversion efficiency and 31% for protein conversion efficiency. In comparison, individual biowaste produced a range of 65% for waste reduction, 96% for bioconversion rate, 101% for waste conversion efficiency and 68% for protein conversion efficiency. This suggests that formulating different biowastes based on their initial nutrient composition can improve the reliability of BSFL treatment facilities. Although formulations, on average, contained less NFC, protein and lipids and more fibre than the individual wastes (see Tables 2 and 3), they were more balanced in nutrients without the absence or excess of NFC (as was the case for human faeces, cow manure and poultry slaughterhouse waste), protein (poultry slaughterhouse waste) and fibre (cow manure and mill by-products). It should be stated that all of the formulations included at least 50% mill by-products or canteen waste or both, and these were the wastes that supported high BSFL performance when used individually.

Variability in the performance parameters was, nevertheless, higher than expected in the formulations; bioconversion rates were expected to be similar between the formulations as protein and NFC appear to have the greatest influence on larval development (Barragán-Fonseca et al. 2018a, Barragán-Fonseca et al. 2018b; Cammack and Tomberlin, 2017). However, variable bioconversion rates (with a range of 87%) are not practical for BSFL treatment facilities. Such variability between formulations could be due to variable fibre and lipid contents. In the formulation feeding experiments, for example, lipid correlated positively and fibre negatively with waste reduction ($R^2 = 0.96$, $p < 0.01$ for lipids, $R^2 = -0.97$ and $p = <0.01$ for fibre) and the bioconversion rate ($R^2 = 0.96$, $p = <0.01$ for lipids, $R^2 = -0.95$, $p < 0.01$ for fibre). Formulations 1 and 5 resulted in the greatest waste reduction and bioconversion rate. These formulations were highest in lipids and lowest in fibre (see Tables 2 and 4) due to the high proportion of canteen wastes (see Table 1). In contrast, formulations 2 and 3 had the lowest lipid and highest fibre content due to a high proportion of human faeces and cow manure. This suggests that the variability in performance could be further reduced by keeping content of lipids and fibre within narrower limits. However, maintaining all macronutrients within fixed limits is difficult in practice considering that wastes typically have variable amounts of each macronutrient.

In addition to different lipid and fibre contents between the formulations, biowaste microbial numbers and communities could have been contributing to the variable BSFL treatment performance despite a similar protein and NFC content. This was not part of this study but can be expected considering that microbes can influence biowaste decomposition (De Smet et al., 2018; Gold et al., 2018a) and larval growth and typically differ between biowastes (Bruno et al., 2018; Ryckeboer et al., 2003; Wynants et al., 2019).

Similar to the larvae grown on the individual wastes, larval protein content was variable between the formulations (Table 4). Larvae fed on the formulations with a lower bioconversion rate tended to have a higher protein content. Protein efficiency was not significantly different between formulations 1, 4, and 5, and between formulations 2, 3, and 6, with the latter having a lower protein conversion efficiency, overall.

4. Conclusions

Given reliable biowaste compositional data, the formulation of mixed biowaste offers a promising systematic approach for the more efficient and predictable operation of black soldier fly larvae (BSFL) treatment facilities using a range of biowastes. Formulating biowaste mixtures in such a way that similar protein and non-fibre carbohydrate (NFC) contents are achieved can be expected to increase BFSL treatment performance and to reduce performance variability. Performance variability could be further reduced by keeping lipids and fibre within narrower limits. Future research should investigate whether these bench-scale results are transferrable to industry-scale BSFL treatment plants with higher larval densities and feed temperatures. Benefits of biowaste formulations need to be balanced with the additional resources required for biowaste analysis and the needed technologies to produce formulations as part of biowaste pre-treatment (e.g. scales, shredder, dewatering, mixer and tank).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2019.10.036.

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