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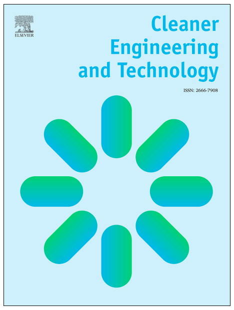

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# Pilot scale production of *Hermetia illucens* (L.) larvae and frass using former foodstuffs

Anton Gligorescu[a], Laura Ioana Macavei[b], Bjarne Foged Larsen[c], Rikke Markfoged[a], Christian Holst Fischer[a], Jakob Dig Koch[a], Kim Jensen[d], Lars-Henrik Lau Heckmann[e], Jan Værum Nørgaard[d], Lara Maistrello[b,f,]*

[a] Division of Environmental Technology, Danish Technological Institute, Kongsvang Alle 29, DK-8000 Aarhus, Denmark.

[b] Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy

[c] Daka Denmark A/S, Lundagervej 21, 8722 Hedensted, Denmark

[d] Department of Animal Science, Aarhus University, Blichers Allé 20, DK-8830 Tjele, Denmark

[e] Skov A/S, Hedelund 4, Glyngoere, DK-7870 Roslev, Denmark

[f] Centre BIOGEST-SITEIA, University of Modena and Reggio Emilia, Piazzale Europa 1, 42124 Reggio Emilia, Italy

**corresponding author*: Lara Maistrello, Department of Life Sciences, Centre BIOGEST-SITEIA, University of Modena and Reggio Emilia, Via G. Amendola 2, 42122 Reggio Emilia, Italy;

*e-mail address*: lara.maistrello@unimore.it

**7647 words**

## Abstract

The food and feed sector requires new sustainable sources of protein and innovative solutions for upcycling of food waste (former foodstuffs), which today is downcycled into energy or even wasted. This study aimed at evaluating the use of former foodstuff waste streams as feed substrate for *Hermetia illucens* (L.) larvae (black soldier fly larvae, BSFL) under long-term and semi-industrial conditions. Different foodstuff-based mixtures and different stocking BSFL densities were used during 20 batches, and quality and safety assessments were performed on the main outputs, namely BSFL production performance, frass impurities, larval and frass nutrient profiles and heavy metal content. About 1400 kg of former foodstuffs (fresh weight) were used to produce 239 kg BSFL and 230 kg frass. The production of BSFL reared on former foodstuffs was highly efficient, with feed conversion rates (FCR) ranging between 2.3 and 5.5 (dry matter basis). The optimization experiment revealed that former foodstuffs-based mixture and high larval density (10 larvae/cm$^2$) lead to highly efficient (FCR: 2.6) and heavy metal-free production of BSFL and frass. The quality of the derived BSFL meal was high in terms of protein and amino acids. Furthermore, the quality of the technical frass was high in terms of N, P, and K levels and minimal packaging material residuals (<2.65%). This investigation suggests that nutrients in former foodstuffs can be successfully and safely recycled in production of BSFL.

## Keywords

Black soldier fly larvae; insect-based bioconversion; soil improver; food waste, biowaste valorization; former foodstuffs

## 1. Introduction

A third of the total food produced is known to end up as waste (FAO, 2019). Moreover, it is estimated that by 2050 the global population will increase to 9.7 billion people (FAO, 2019), while the amount of waste generated will reach 3400 billion kg (Silpa et al., 2018), making food waste an unstructured, cross-cutting and persistent problem (Närvänen et al., 2020). The food sector requires innovative and sustainable production systems that can reduce waste and increase nutrient recovery by nutrient cycling. Such a solution can be found in the form of insect bioconversion systems, which can upcycle a high amount of heterogeneous substrates from organic waste streams into high value protein and lipids in form of insect larval biomass, suitable as food and feed. The obtained insect frass (residual substrate from the production) could be successfully used as fertilizer (Bortolini et al., 2020).

A promising insect candidate are the larvae of *Hermetia illucens* – commonly known as the black soldier fly (BSF) - which during the last years have gained high popularity due to i) their ability to be reared on a large variety of organic waste streams, such as municipal waste, livestock manure (Surendra et al., 2020), brewery waste or by-products from the food industry (Barbi et al., 2020), and ii) due to their vast applicability in food, feed and non-food sectors.

Effective treatment and conversion of municipal solid organic waste and industrial organic waste has been achieved by BSF larvae (BSFL) Surendra et al., 2020). The production of BSFL in industrial setups is already in place across the globe and is growing at rapid rates (Ojha et al., 2020). According to the European interest organization for insect producers (IPIFF), the annual production of insect protein in Europe is expected to be 3,000-5000 million kg/year in 2030, of which BSFL will make up about 80% (IPIFF vision paper, 2018). The global BSFL market is expected to reach 2.1 billion euro by 2030 (Meticulous Market Research Pvt. Ltd., 2020).

The performances of BSFL depend on the quality of the feed and on the production conditions (pH, density, temperature, humidity, etc.) (Singh and Kumari, 2019). As other holometabolous insects, the BSFL are adjusting their growth rate and nutrient accretion with the main goal of accumulating enough reserves for the adult stage and ensuring reproduction (Gold et al., 2018). According to Gold et al. (2018), the rearing of BSFL on a more balanced waste-based feed with ratio of 1:1 of protein (14-19%) to non-fiber carbohydrate (13-15%), showed high improvement in the performances of BSFL compared to the individual waste streams (mill by-products, canteen waste, human feces, slaughterhouse waste, cow manure, vegetable canteen waste). Differences in BSFL performance are also associated to different waste streams, while the protein content (dry matter, DM) of BSFL was found to be similar, ranging between 39% to 44% when reared on food waste, human feces, slaughterhouse waste, fruit and vegetable waste (Lalander et al., 2019). On the contrary, the BSFL lipid content seems to be strongly affected by the feed quality and by the larval density (Barragan- Fonseca et al., 2018). Pre-treated feeding substrates with high amounts of tannins and total phenolic compounds resulted in low-weight larvae, and consequently in a low biomass conversion ratio (Isibika et al., 2019). The utilization of balanced feeds developed on different waste streams could stabilize the BSFL rearing and assure sustainable production of both insect protein and insect oil.

The frass from BSF has potential as a sustainable soil amendment (Schmitt and de Vries, 2020), as shown by Setti et. (2019). When compared with composting of untreated food waste, bioconversion by BSFL is more environmentally and economically efficient (Ites et al., 2020). However, when reared on waste streams such as former foodstuffs, the insect frass might contain packaging materials or potential hazardous compounds (e.g. heavy metals) (Lievens et al., 2021). In case of high moist content of such waste stream, the separation of BSFL from the frass might be challenging and time consuming (Lalander et al., 2020). Moreover, one quarter of the biomass production can be lost during this step (Guo et al., 2021). In this study, former foodstuffs are distinguished from general food waste and household waste by being defined as pre-consumer food products of plant or animal origins, which were intended to be used as food. This may be an important feature for feed substrates in the legislative regulation of future BSFL production.

The objectives of this study were to document the use of former foodstuff waste streams as feed substrate for BSFL under long-term and semi-industrial conditions and to assess the production of BSFL feed on former foodstuff enhanced with different waste streams and reared at different stocking densities. These objectives were addressed in experiments evaluating production performances (feed conversion rate, larval biomass production, percent reduction of initial substrate, BSFL meal and BSFL oil production, frass production), larval quality (protein content and amino acid profile of BSFL meal), and product safety on BSFL and frass (heavy metal presence and impurity content).

## 2. Materials and methods

### *2.1 BSF colony*

*Hermetia illucens* larvae were obtained from a colony established in 2017 at the Danish Technological Institute, Aarhus, Denmark. The colony was maintained under constant conditions in a climate-controlled room at 28°C, 60% relative humidity., a photoperiod of 14:10 Light:Dark cycles and provisioned commercial chicken feed (PacoStar19, DLG, Denmark) as substrate.

### *2.2 Long-term BSFL production*

Twenty consecutive batches (B1-B20) of BSFL and frass were produced on former foodstuffs over a period of 1.5 years at the Danish Technological Institute, Aarhus. The production was performed at pilot scale, using trays of 60 x 40 x 20 cm, under controlled condition at 27±1°C and a relative humidity 45±9%. Fresh former foodstuff was multiple collected from Daka ReFood (Hedensted, Denmark). The biomasses originate typically from food production industry, central warehouse, supermarkets, and dairy production. Currently the former foodstuff is processed into a pulp to be used for biogas production. During the fractionation processing step, the former foodstuffs, including packaging materials, are loaded into a pulper and mixed with water. Consequently, the biomass originate typically from food production industry, central warehouse, supermarkets, and dairy production are squeezed through perforated separation plate (2 mm sieves), and thus divided into 2 fractions: large fragments (>2mm), consisting mainly of fibers and packaging residues, and small fractions (<2mm) consisting mainly of organic materials called bio-pulp.

The bio-pulp was stored at -18°C in a freezer before being used in the production and samples from each bio-pulp batch were collected and analyzed to determine DM, ash, crude protein (N x 6.25), lipid and fiber, as shown in Table 1.

**Table 1.**

Characteristics of bio-pulp used in the production of 20 batches of BSFL and frass.

| Batches | DM (% as-is) | Ash (DM basis) | Protein (DM basis) | Lipid (DM basis) | Fiber (DM basis) |
|---|---|---|---|---|---|
| B1 | 16 | 7 | 24 | 23 | 7 |
| B2 | 12 | 19 | 29 | 27 | 9 |
| B3 | 16 | 19 | NA | NA | NA |
| B4 | 20 | 8 | 20 | 24 | 7 |
| B5 | 17 | 11 | 23 | 24 | 7 |
| B6 | 15 | 12 | 26 | 30 | 6 |
| B7 | 17 | 7 | 27 | 32 | 6 |
| B8 | 23 | 5 | 20 | 25 | 5 |
| B9 | 18 | 5 | 25 | 29 | 6 |
| B10 | 13 | 8 | 19 | 37 | 6 |
| B11 | 13 | 8 | 19 | 37 | 6 |
| B12 | 19 | 7 | 21 | 44 | 6 |
| B13 | 21 | 8 | 22 | 39 | 7 |
| B14 | 20 | 7 | 20 | 42 | 6 |
| B15 | 21 | 8 | 27 | 34 | 11 |
| B16 | 20 | 12 | 28 | 22 | 12 |
| B17* | 23 | 7 | 13 | 19 | 3 |
| B18 | 19 | 8 | 16 | 23 | 4 |
| B19 | 28 | 6 | 24 | 18 | 11 |
| B20 | 26 | 9 | 12 | 21 | 2 |

*The macronutrient and ash content are average values from six different treatments evaluating different feed mixtures and BSFL densities. NA: not available.

During the production period, different parameters (e.g., feeding strategies and availability, feed quality, larval density, time at harvest) were optimized during multiple batches. The overall utilized bio-pulp, and the produced BSFL biomass and frass were assessed for individual batches, on a fresh weight basis. while the feed conversion rate (FCR) on a DM basis was considered in accordance with (Oonincx et al., 2015). A series of optimization experiments were considered in order to identify the best zootechnic conditions (i.e., larval densities, feeding strategies, feed enhancement with other waste streams, substrate porosity and viscosity, separation procedures etc.), and to optimize the production.

### *2.3 Feed optimization and BSFL density experiment*

#### *2.3.1 Experimental design*

This sub-experiment was conducted during batch 17 and considered two factors: i) three different waste mixtures developed from former foodstuffs, named mixture A, B, C (Table 2), and ii) two larval densities of 7 or 10 larvae/cm². The densities were chosen based on preliminary results of different density experiments conducted during the production. The feed mixtures were obtained by mixing two types of bio-pulp with husk and coffee grounds considering the products DM. The bio-pulps were the biomass collected at Daka ReFood (Bio-pulp 1) and a high fiber and packaging residue bio-pulp from pulping process (Bio-pulp 2). The mixtures were conducted to reduce the high viscosity seen in the bio-pulp during the Covid-19 spring outbreak, and to assess the possibility of using other waste streams to enhance the physical properties of the bio-pulp (lowering the viscosity and enhancing porosity), while securing a high nutrient content. Each of the six treatments consisted of five replicates.

**Table 2.**
Composition of feed mixtures and their content of dry matter (DM), ash, crude protein, lipid, and carbohydrate, C/N ratio and essential amino acids.

| | Mixture A | Mixture B | Mixture C |
|---|---|---|---|
| Bio-pulp 1 (%) | 80 | 92 | 80 |
| Bio-pulp 2 (%) | 20 | - | - |
| Husk (%) | - | 8 | - |
| Coffee grounds (%) | - | - | 20 |
| | | | |
| DM (% as-is) | 19.06 | 27.30 | 22.04 |
| Ash content (% of DM) | 8.16 | 6.34 | 6.47 |
| Crude protein (% of DM) | 22.70 | 21.10 | 19.78 |
| Crude lipids (% of DM) | 30.54 | 20.74 | 26.94 |
| Carbohydrates* (% of DM) | 38.60 | 51.83 | 46.80 |
| CHO/Crude protein (ratio) | 1.70 | 2.46 | 2.37 |
| Arginine (% of DM) | 0.72 | 0.38 | 0.41 |
| Histidine (% of DM) | 0.44 | 0.25 | 0.34 |
| Isoleucine (% of DM) | 0.86 | 0.51 | 0.70 |
| Leucine (% of DM) | 1.47 | 0.86 | 1.32 |
| Lysine (% of DM) | 0.96 | 0.58 | 0.66 |
| Methionine (% of DM) | 0.38 | 0.22 | 0.28 |
| Phenylalanine (% of DM) | 0.81 | 0.47 | 0.74 |
| Threonine (% of DM) | 0.77 | 0.46 | 0.57 |
| Tryptophan (% of DM) | 0.22 | 0.12 | 0.21 |

| | | | |
|---|---|---|---|
| Valine (% of DM) | 1.10 | 0.65 | 0.94 |

*The carbohydrate was estimated by subtracting the other macronutrients from 100%, using the Weende method.

About 14,000 and 20,000 6 days old BSFL belonging to the two larval densities (7 and 10 larvae/cm²) were placed in plastic trays (40 x 60 x 20 cm, N=30) and fed with a total of 0.4 g fresh substrate per larva for 10 days until the first prepupae were observed. The mixtures were administrated in two tranches: 75% at the beginning of the experiment and the remaining 25% during day 8 of the experiment. The trays were maintained under controlled laboratory conditions (26.88 ± 0.84°C and 60 % relative humidity) and rotated every second day until harvest, to ensure similar conditions in all trays.

At harvest, the content of individual trays (BSFL and frass) was separated using two steps: i) firstly, separating the fine particles of the BSFL frass (technical frass) from the larvae and the remaining residues using a manual sieve with a 2 mm mesh; ii) secondly, the BSFL and the remaining residues were placed on a sieve of 4 mm until the larvae migrated into a collecting tray leaving the remaining residues (discharged frass). The fresh weight of the larval biomass, technical frass and discharged frass as well the total frass were determined for each individual tray. To determine the DM content, samples of larvae (20 individuals) and technical frass (10 g) belonging to individual trays were collected and placed in an oven at 105°C for 24 hours. Subsequently, for the BSFL biomass and for the technical frass, the trays/replicates belonging to the same treatment were mixed and a pooled sample of either BSFL or technical frass (approx. 500 g) were taken for each treatment and further stored in a freezer at -20°C for chemical analysis. Chemical analyses were performed on: i) BSFL quality (lipid, crude protein, and essential amino acid content; amino acids only on mixture A at high density); ii) technical frass quality (N, P and K content), and iii) product safety (Pb, Cd, Hg and As content). All analyses were conducted at Eurofins Steins Laboratorium A/S (Ladelundvej 85, DK-6600 Vejen, Denmark).

### *2.3.2 Production performances*

The BSFL biomass for each treatment was assessed by evaluating the fresh weight and larval DM content of individual trays. The BSFL lipid and crude protein content was determined for each treatment (pooled sample) and used together with the larval biomass (DM) to calculate potential BSFL oil and BSFL meal production for each tray. The total frass and technical frass production for each tray was determined based on the weight of the technical frass post separation, while the total frass was obtained by summing the two types of obtained frass (technical and discharged frass).

The number of juveniles and BSFL at harvest were determined using a weight approach. Consequently, the number of juveniles and BSFL were used to determine the survival rate for individual trays. The FCR was calculated on a DM basis for each treatment, whereas the substrate reduction was calculated following eq. 1.

$$Substrate\ reduction\ (\%) = 100 - \left(\frac{Total\ frass\ (g)}{Total\ feed\ (g)} * 100\right) \qquad eq.\ 1$$

*2.3.3 Product quality*

The BSFL crude protein content was used together with BSFL biomass (DM) and calculated BSFL meal production for individual treatments in order to estimate the BSFL meal protein content following eq. 2.

$$Protein\ content\ of\ BSFL\ meal\ (\%) = \frac{BSFL\ protein\ content\ (\%) * BSFL\ biomass\ (g\ DM)}{BSFL\ meal\ (g\ DM)} \qquad eq.\ 2$$

The essential amino acid (AA) profile of BSFL reared on mixture A at high density was analyzed on a DM basis and used to estimate the AA of BSFL meal. The N, P, and K content (%) of technical frass was estimated for individual treatments based on the pooled sample.

*2.3.4 Product safety*

The heavy metals (Pb, Cd, Hg, and As) contained in both BSFL and technical frass were analyzed at treatment levels based on the pooled samples. The impurity content in technical frass was estimated for each tray using a two-step procedure: i) Initially, a pre-weighed sample (approx. 10 g) of technical frass was visually inspected under a stereoscope, and ii) based on this, the BSFL frass was separated into two categories: Purified BSFL frass and packaging residue, and iii) finally, the BSFL frass was individually weighed and used to determine the impurity content following eq. 3.

$$Impurity\ content\ (\%) = \frac{packaging\ residue\ (g) * 100}{Technical\ frass\ sampel\ (g)}$$

*2.4 Statistics*

The production of BSFL reared on former foodstuffs during 20 batches is presented graphically, and thus only basic statistics (means values) and box plots (only for FCR data) were presented. Similarly, as in the case of the sub-experiment on feed mixtures and density, production data on BSFL crude protein and lipid content, BSFL meal crude protein content, technical frass quality (N, P, and K) and BSFL and frass safety (heavy metals) data were presented graphically with mean values only, since these were determined at treatment level, using a pooled samples procedure, while the impurities content of the BSFL frass was presented as mean and standard deviation.

The BSFL biomass, meal, oil, survival rate, FCR, substrate reduction, total and technical frass data sets were analyzed with two-way ANOVA tests with feed mixture and density as factors followed by Tukey’s post-hoc tests for multiple comparisons. Prior to the statistical analysis, the data belonging to technical frass and FCR were log10 transformed to obtain normal distribution. All data were tested for normality using Shapiro-Wilk test and for the homogeneity of variance using Levene’s test. The statistical analysis was performed using SYSTAT 13 (Systat Software Inc., Chicago, USA).

## 3. Results

Overall, the pilot scale production of BSFL reared on former foodstuffs during multiple batches was successful. During the 1.5 years, a total of 1,400 kg of former foodstuffs was used in the pilot production. The production of both BSFL and BSFL frass on former foodstuffs was high, counting for 239 kg of BSFL and 230 kg of frass during 20 batches (Fig. 1).

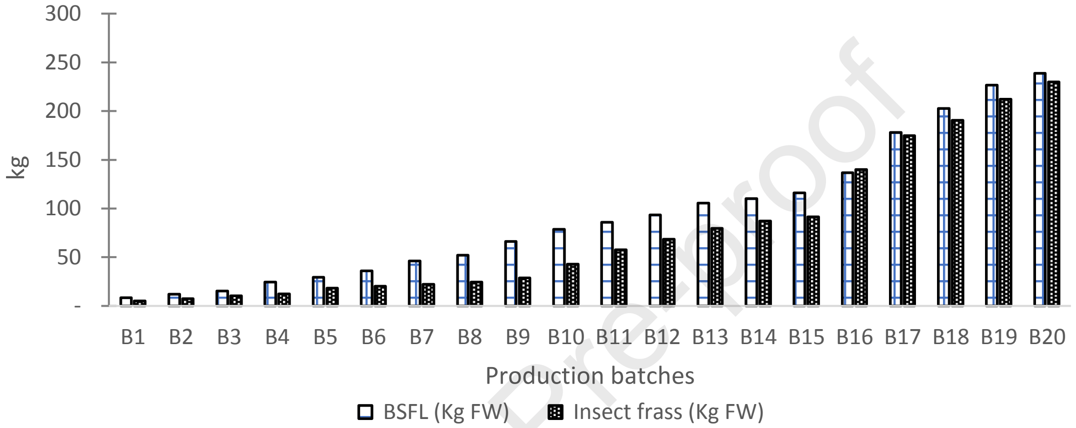
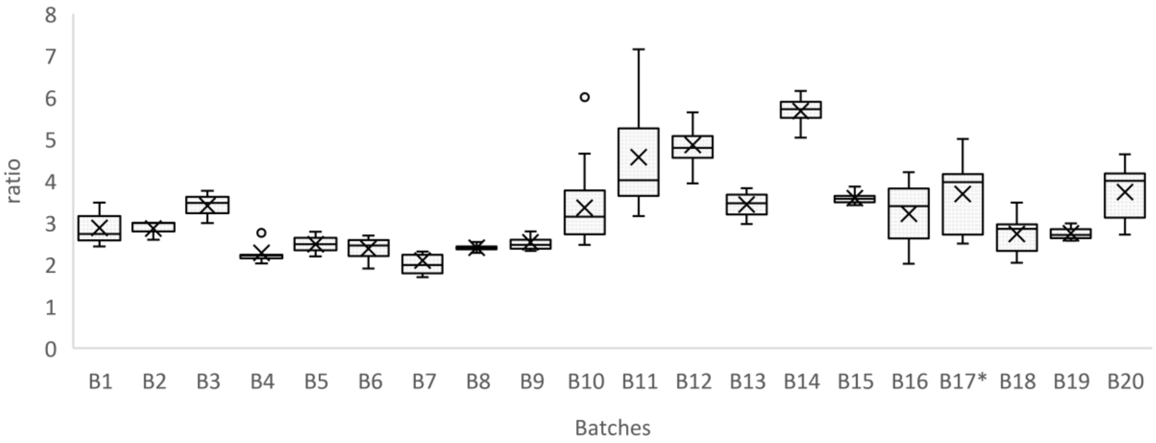


Fig. 1. Cumulative BSFL and frass biomasses produced in total over 20 batches (B1-B20). The BSFL biomass and BSFL frass are presented on a fresh weight (FW) basis.

The FCR was found to vary across the production batches, ranging between 2.3 for B4 and 5.5 for B14. Moreover, a high variation was seen across multiple batches (B10, B11, B16, B17, B18 and B20) due to different experimental parameters being altered (i.e., larval density and former foodstuffs quality) during the production optimization period (Fig. 2).



*The FCR belonging to B17 is an average value from six different treatments evaluating different feed mixtures and BSFL densities.

Fig. 2. Feed conversion rate (FCR) obtained during the production of 20 batches of BSFL reared on former foodstuffs. The FCR values are displayed on a dry matter basis. Boxes show median, 10th, 25th, 75ht, and 90th percentiles, and crosses show the mean.

Overall, the production performances were affected by different treatments. As such, the larval density had a great impact on the production of larval biomass ($p < 0.01$), BSFL meal ($p < 0.01$), BSFL frass ($p < 0.01$), technical frass ($p = 0.01$) and on the substrate reduction ($p < 0.01$). The feed mixture and the interaction of feed mixture and larvae density had a highly significant impact on all the production performances ($p < 0.01$).

The production of BSFL biomass at all density and feed mixture treatments ranged between 2,000 g/tray for mixture A at high density, and 950 g/tray for mixture C at low density. A higher larval density resulted in significantly larger BSFL biomass production. Moreover, the largest biomass production was obtained on mixture A, followed by mixture B and lastly mixture C. There was an interaction between density and waste mixture on the biomass production: The biomass production obtained on mixtures A and B at high density were high compared to the biomass production on the same mixtures at lower density. Such difference was less evident when the biomass production on mixture C was compared between the two different densities (Fig. 3).

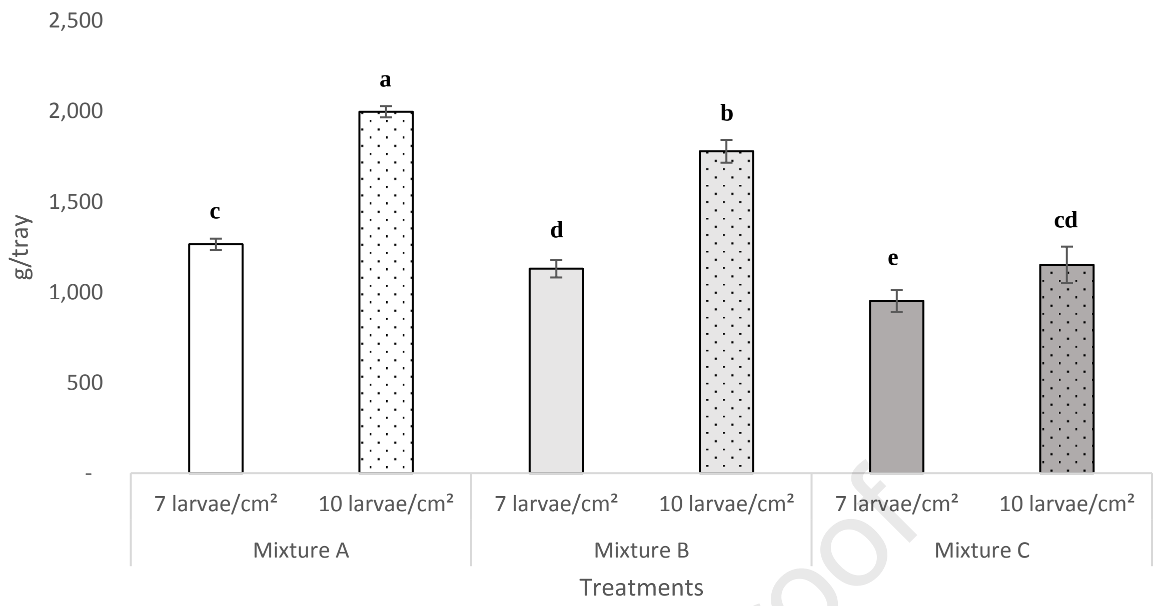


Fig. 3. Larval biomass production of BSFL reared on three former foodstuff-based mixtures (A, B and C) and at 7 or 10 larvae/cm² densities (mean ± s.d.). Columns with the same letter are not significantly different.

The FCR varied across different treatments ranging from 2.6 for mixture A and 4.6 for mixture C at high density. There was an interaction between density and waste mixture, with mixtures A and B unaffected by density, while mixture C showed higher FCR at higher density than at the lower density (Fig. 4).

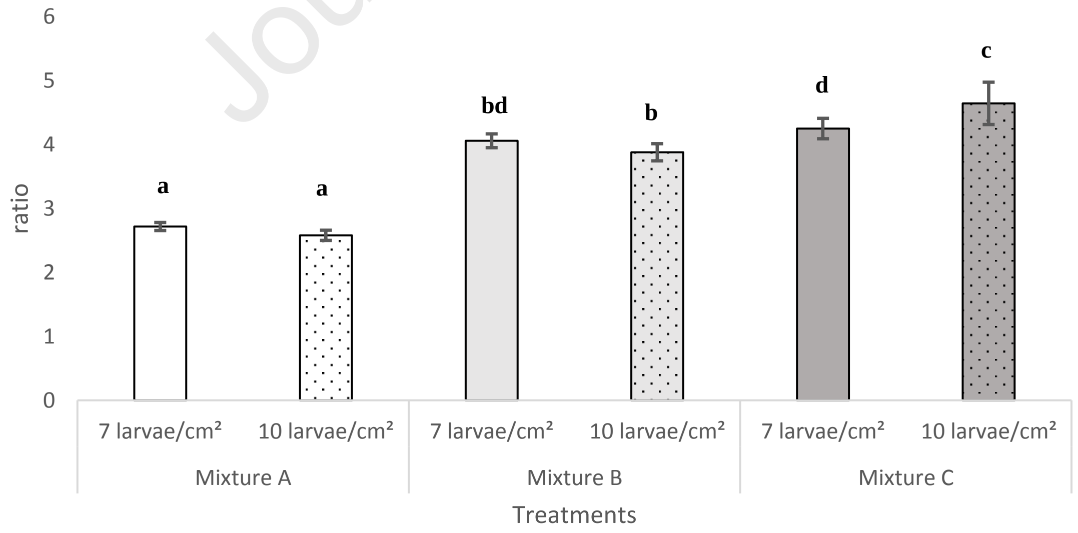


Fig. 4. Feed conversion rate of BSFL reared on three former foodstuff-based mixtures (A, B and C) at 7 or 10 larvae/cm² densities (mean ± s.d.). Columns with the same letter are not significantly different.

The substrate reduction was high across the different treatments, with the greatest reduction (86-87%) being obtained when BSFL were reared on mixture A at both densities, and the lowest reduction (75%) being achieved when BSFL were reared on mixture C at low density. The substrate reduction was affected by both density, waste mixtures and the interaction between these treatments (Fig. 5).

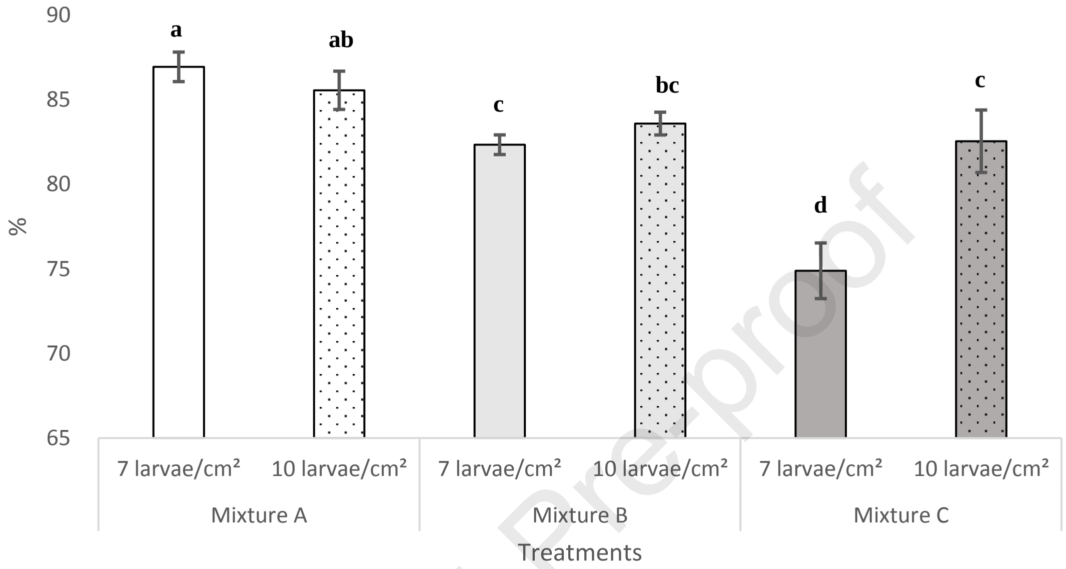


Fig. 5. Substrate reduction from BSFL reared on three former foodstuff-based mixtures (A, B and C) at 7 and 10 larvae/cm² densities (mean ± s.d.). Columns with the same letter are not significantly different.

The survival rate of BSFL was high across different treatments varying between 92% for mixture A and 69% for mixture C at low densities. No significant impact on larval survival rate was attributed to density, while on the other hand, the survival rate was significantly affected by the difference in mixture, as well as by the interaction of mixture and density (Fig. 6).

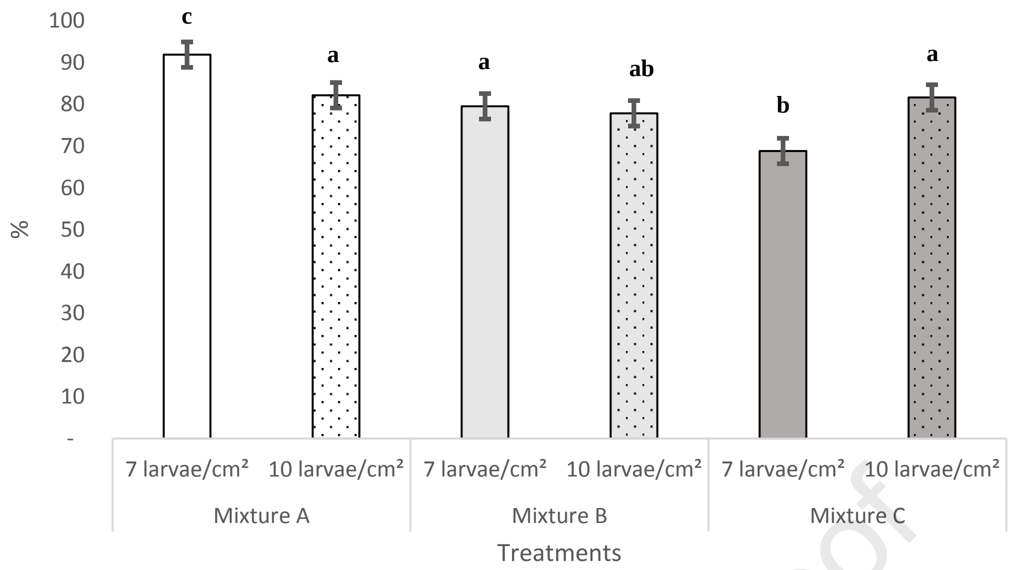


Fig. 6. Survival rate of BSFL reared on three former foodstuff-based mixtures (A, B and C) and at 7 or 10 larvae/cm² densities (mean ± s.d.). Columns with the same letter are not significantly different.

The calculated BSFL meal production varied from 390 g/tray for mixture A at high density, to 195 g/tray for mixture C at low density. Both the density and feed mixture treatments significantly affected BSFL meal production, with a higher BSFL meal production at higher density. An interaction effect of density and mixture significantly affected BSFL meal production, as seen by the similar production of BSFL meal on mixtures A and B at low density, and mixture C at high density (Fig. 7).

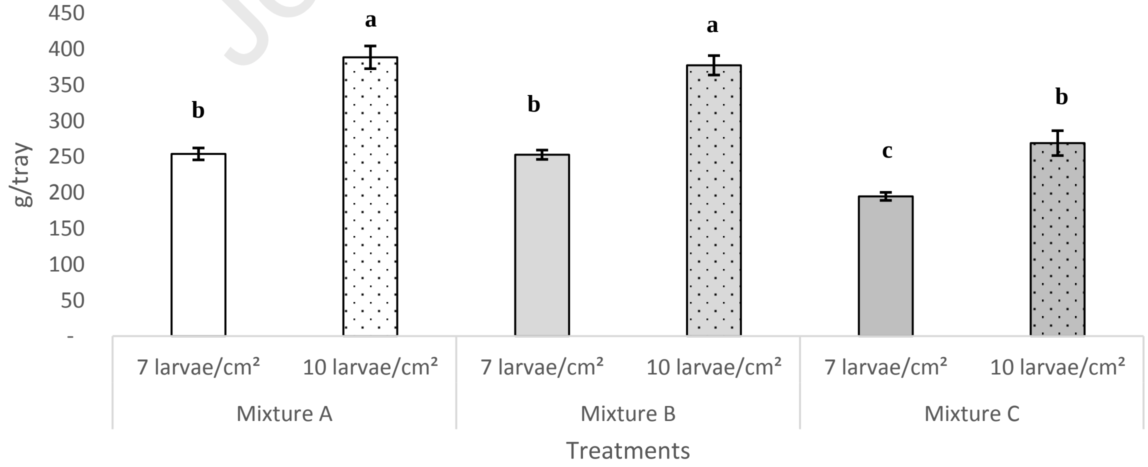


Fig. 7. Estimated BSFL meal production when reared on three former foodstuff-based mixtures (A, B and C) at a density of 7 or 10 larvae/cm² (mean ± s.d.). Columns with the same letter are not significantly different.

The production of BSFL oil was estimated to vary from an average of 236 g/tray in mixture A at high larval density to on average 119 g/tray for mixture C at low density. Density was found to influence the production of BSFL oil, with the higher density resulting in larger BSFL oil production. While the BSFL oil production was larger for mixture A, followed by mixture B and lastly by mixture C, an interaction between density and mixture was seen on the BSFL oil production, as indicated by similar BSFL oil production between mixture A at low density and mixture B at high density, as well as similar oil production between mixture B at low density and mixture C at high density (Fig. 8).

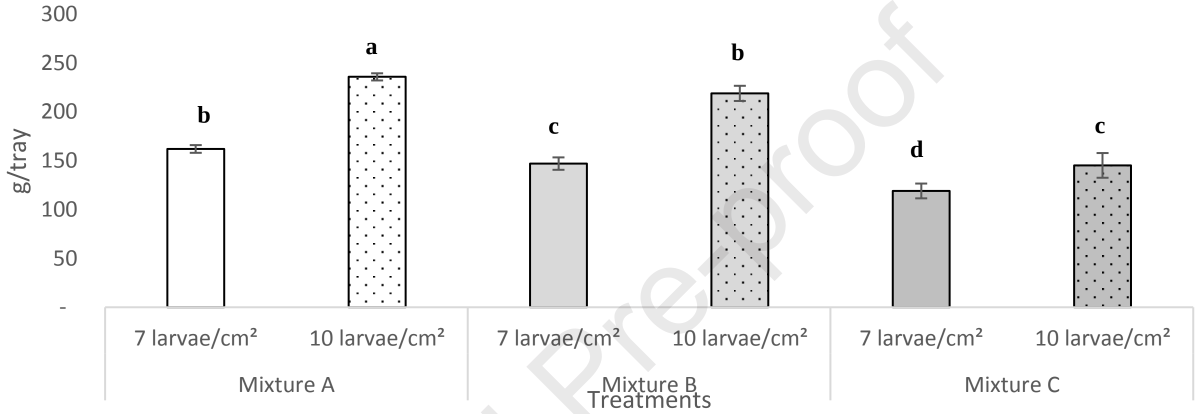


Fig. 8. Estimated BSFL oil production when reared on three former foodstuff-based mixtures (A, B and C) at a density of 7 or 10 larvae/cm² (mean ± s.d.). Columns with the same letter are not significantly different.

The total frass production varied from an average around 1400 g/tray on mixture C at both densities to 731 g/tray on mixture A at low density. Both density and feed mixture treatment, as well as their interaction affected the total frass production (Fig. 9). The technical frass was varied between 1400 g/tray for mixture C at high density and 516 g/tray for mixture A at low density. As in the case of total frass, this was significantly affected by density, waste mixture, and the interaction between these treatments (Fig. 9).

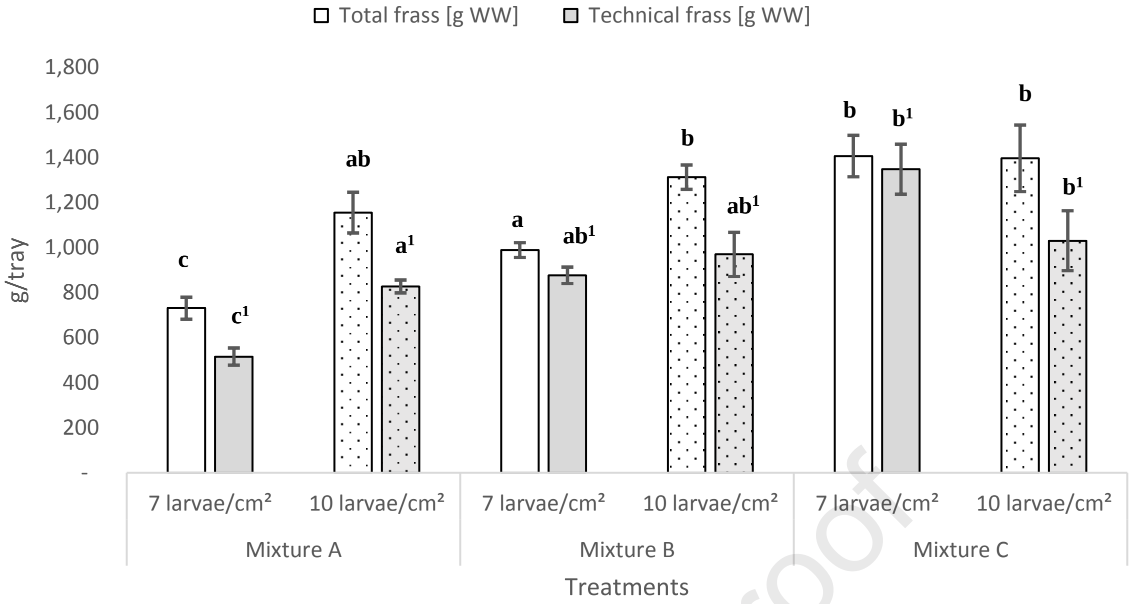


Fig. 9. Total and technical frass production of BSFL reared on three former foodstuff-based mixtures (A, B and C) at 7 or 10 larvae/cm² densities (mean ± s.d.). Columns with the same letter belonging to either total frass or to technical frass$^{(1)}$ are not significantly different.

The crude protein content (DM basis) of BSFL meal estimated for the different treatments ranged from 52% for mixture C at high density to 59% for the treatments: mixture A at low density, mixture B at high density and mixture C at low density (Fig. 10).

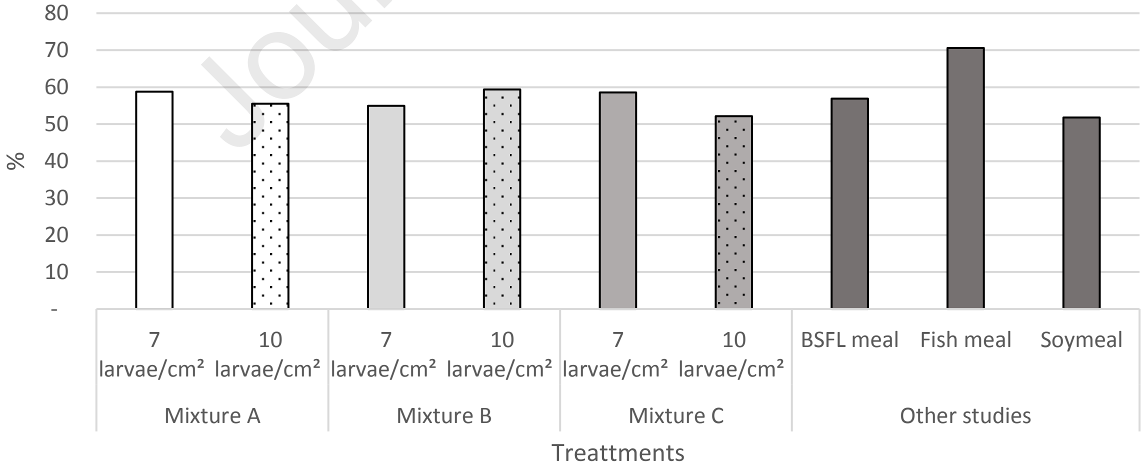


Fig. 10. Crude protein content of BSFL reared on three former foodstuff-based mixtures (A, B and C) at 7 and 10 larvae/cm² densities and in comparison to BSFL meal, fish meal and soybean meal as reported by Makkar et al. (2014).

Overall, the content of essential amino acid content of BSFL meal produced on former foodstuff (mixture A) during this study was higher than rearing on soybean meal (except for phenylalanine and arginine), and lower than rearing on fishmeal (Fig. 11).

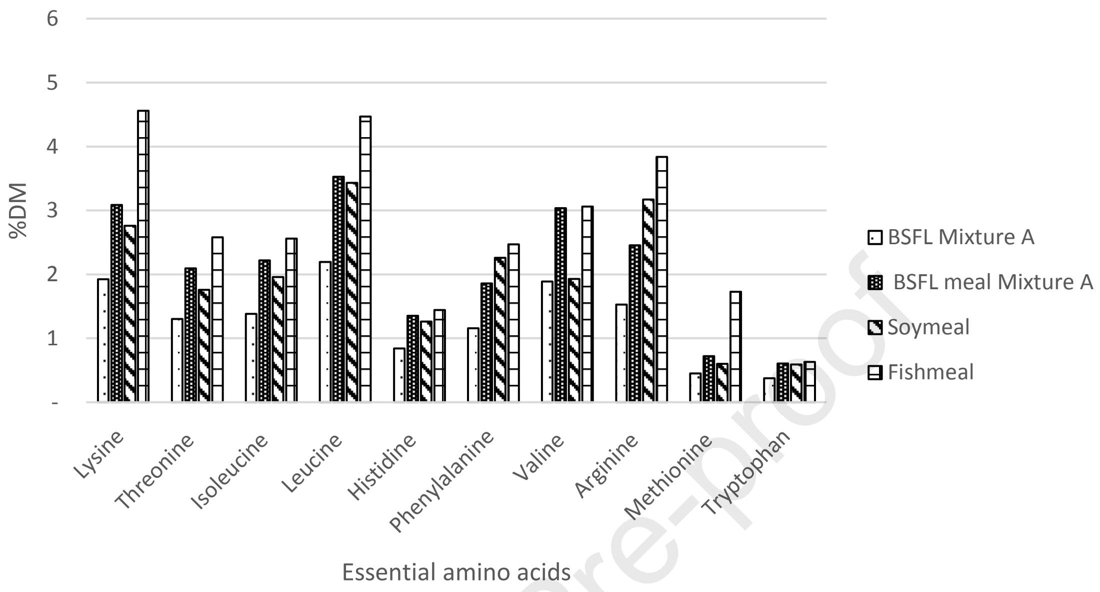


Fig. 11. The essential amino acid content of BSFL and BSFL meal produced on mixture A in comparison to fish meal and soybean meal as reported by Surendra et al. (2016).

The impurity content in the technical frass was small across the treatments, ranging from 0.11% for mixture B at high density, to 2.65% for mixture C high density treatments (Fig. 12).

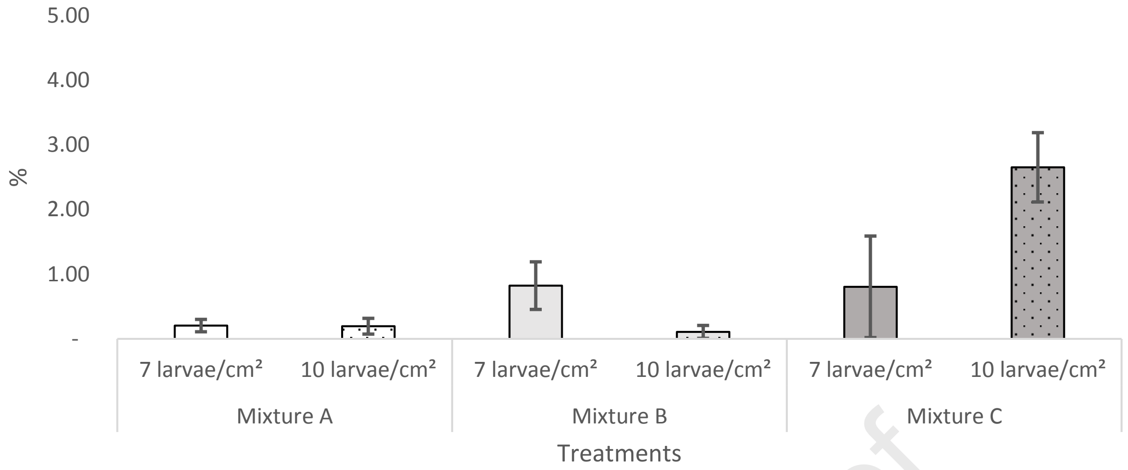


Fig. 12: Impurity content in technical frass from BSFL reared on three former foodstuff-based mixtures (A, B and C) at 7 or 10 larvae/cm² densities (mean ± s.d.).

Results showed that when compared to poultry, swine or cattle manure, the technical frass obtained by administrating mixture B at both larval densities presented higher concentrations of N, P and K (Table 3); nevertheless, the N content of frass was superior in all treatments.

**Table 3.**

Nitrogen (N), phosphorus (P) and potassium (K) in frass in comparison with different types of manure (% DM) as reported by Adekiya et al. (2020).

| | **N** | **P** | **K** |
|---|---|---|---|
| Frass: Mixture A, low density | 4.06 | 0.49 | 0.97 |
| Frass: Mixture B, low density | 6.13 | 1.10 | 2.86 |
| Frass: Mixture C, low density | 3.04 | 0.33 | 0.89 |
| Frass: Mixture A, high density | 2.65 | 0.49 | 1.04 |
| Frass: Mixture B, high density | 5.27 | 1.35 | 3.38 |
| Frass: Mixture C, high density | 2.36 | 0.22 | 0.60 |
| Poultry manure | 2.91 | 0.84 | 3.79 |
| Swine manure | 2.16 | 0.80 | 2.16 |
| Cattle manure | 1.86 | 0.82 | 2.11 |

Overall, the content of heavy metals of both BSFL and BSFL frass produced during the study were much lower than the EU maximum limits for feed and for organic fertilizer (Table 4).

**Table 4.**

Heavy metal content in BSFL and BSFL frass in comparison with maximum limits for feed and organic fertilizer

| | | ***Arsenic (mg/kg DM)*** | ***Cadmium (mg/kg DM)*** | ***Mercury (mg/kg DM)*** | ***Lead (mg/kg DM)*** |
|---|---|---|---|---|---|
| *Larvae* | Mixture A, 7 larvae $cm^2$ | 0.27 | 0.10 | 0.01 | 0.36 |
| | Mixture B, 7 larvae $cm^2$ | 0.25 | 0.25 | 0.01 | 0.22 |
| | Mixture C, 7 larvae $cm^2$ | 0.27 | 0.11 | 0.01 | 0.20 |
| | Mixture A, 10 larvae $cm^2$ | 0.29 | 0.08 | 0.01 | 0.64 |
| | Mixture B, 10 larvae $cm^2$ | 0.27 | 0.29 | 0.01 | 0.24 |
| | Mixture C, 10 larvae $cm^2$ | 0.25 | 0.10 | 0.01 | 0.19 |
| *Frass* | Mixture A, 7 larvae $cm^2$ | 0.40 | 0.02 | 0.03 | 1.23 |
| | Mixture B, 7 larvae $cm^2$ | 0.20 | 0.05 | 0.01 | 0.53 |
| | Mixture C, 7 larvae $cm^2$ | 0.14 | 0.14 | 0.01 | 0.27 |
| | Mixture A, 10 larvae $cm^2$ | 0.23 | 0.16 | 0.01 | 0.83 |
| | Mixture B, 10 larvae $cm^2$ | 0.25 | 0.05 | 0.01 | 0.63 |
| | Mixture C, 10 larvae $cm^2$ | 0.13 | 0.13 | 0.01 | 0.17 |
| | EU Max. limits for feed * | 2 | 2 | 0.1 | 10 |
| | EU Max. limits for organic fertilizers ** | 10 | 1 | 1 | 100 |

**European Parliament (2002); **European Commission (2006).*

## 4. Discussion

The production of BSFL on former foodstuffs showed high performances in terms of both larval biomass production and FCR. The FCRs obtained when feeding BSFL with former foodstuffs (FCR: 2.3 to 5.5) were more efficient compared to rearing BSFL on vegetable waste (FCR: 9.29) (Giannetto et al., 2020), municipal organic waste (FCR: 5.8) (Diener et al., 2011), industrial by-products (bakery waste, fish trimmings, fish waste, brewery grain and yeast, sugar beet pulp and cheese waste) (FCR: 4.84 to 20.54) (Magee et al., 2021), animal manure (FCR:5.6 to 10.3 on poultry and dairy manure) (Rehman et al., 2017), and it was similar when feeding BSFL with catering waste and household waste (FCR:1.7 to 3.6) (Gligorescu et al., 2020). It is important to underline that lower BSFL performances (in terms of FCR) were registered during production of batches B10-15. These batches were produced at the beginning of the lockdown period caused by the Covid-19. A high degree of BSFL migration was observed during the production of these batches, where the bio-pulp had a high lipid content and increased viscosity (Table 1). This could have an impact on BSFL, since increased viscosity can impede the larvae to move freely or even breath inside the feeding substrate (Klammsteiner et al., 2021). Further studies are required to understand the impact of viscosity and other physical characteristics (substrate porosity) on the performances of BSFL.

The optimization experiment revealed that former foodstuffs can be enhanced with other waste streams to reduce potential viscosity or low porosity. The best larval performances (larval biomass, FCR and substrate reduction) were obtained when BSFL were maintained at high larval density (10 larvae/cm²) and fed with former foodstuff-based feed mixture (mixture A), indicating that such mixture can be successfully used in BSFL production. The content of macronutrients and amino acids was highest for mixture A (Table 2), which also supported the greatest larval performances, when compared with the other two tested mixtures. The second highest larval biomass production was observed at high larval density when BSFL were fed on mixture B, consisting of 8% husk inclusion, indicating that such feed can be successfully implemented in the production. The addition of 20% coffee grounds in mixture C reduced larval performances, and consequently this limits the application of coffee ground for BSFL production. Similarly, Permana and Putra, (2018) found that the utilization of coffee grounds in BSFL production prolongs the development time of the larvae and consequently limits the growth and the production of BSFL.

The survival rate was high across the treatments and is comparable with the survival rate of BSFL reared on similar feeds (Gold et al., 2020; Lalander et al., 2019). However, despite the similarities between the nutrient content of mixtures B and C, a lower survival rate was observed when BSFL were feed on the coffee grounds mixture (mixture C) and maintained at low density. These results may indicate that coffee grounds contained substances limiting BSFL growth and survival when fed at high concentrations. Similar findings were found by Saadoun et al., (2020), observing no survival of BSFL after feeding spent coffee grounds as the only substrate for 15 d. The negative effects by coffee grounds may be due to the high content of indigestible fibers, alkaloids and Maillard reaction products (Saadoun et al., 2020), which will reduce the available metabolizable energy, have general inhibitory effects (Jan et al., 2021) and reduce potential protein synthesis (Almeida et al., 2014).

The highest estimated meal production was obtained on both mixtures A and B at higher larval density, while the greatest BSFL oil production was supported by mixture A at high density. The crude protein content of BSFL reared on the different former foodstuff formulations was similar, ranging from 52 to 59%, suggesting it was diet-independent (Ravi et al., 2020; Spranghers et al., 2017). However, according to Fuso et al. (2021), when BSFL were reared exclusively on vegetable by-products, the total protein content varied between 35% and 49% and was mainly correlated to fibre and protein content in the diet. The crude protein content of BSFL meal estimated for mixture A at high density (56%) was comparable to other BSFL meal (57%), higher than soybean meal (52%) but lower than fishmeal 71% (Makkar et al., 2014). The essential amino acid content revealed that BSFL meal obtained on mixture A had an overall higher essential amino acid content than soybean meal but lower content than fishmeal. These results indicate that a high quality BSFL meal and BSFL oil suitable for feed can be obtained when rearing BSFL on former foodstuffs. For high-performing laying hens, the BSFL meal and oil can replace completely the soybean-based feeds (Heuel et al., 2021), while as a potential fish meal substitute, a diet composed by 25% BSFL meal and 75% fish meal was recommended for suitable growth performance of birds, or a 100% BSFL meal inclusion rate for the most cost-effective feed (Sumbule et al., 2021). The outlook for BSFL as a protein replacement for fishmeal in salmonids diets is more sustainable, presenting an optimal growth of up to 200 g/kg diet (English et al., 2021); the dietary inclusion of BSFL meal on other fish species also presents encouraging results (Mousavi et al., 2020). New research looking at the application of BSFL meal derived from the production of BSFL on former foodstuffs should be considered in the future.

The highest BSFL frass production was obtained when BSFL were fed on mixture C followed by mixture B and lastly by mixture A. However, the amount of separable frass (technical frass) was overall high across different treatments, counting for at least 71% of the total frass produced. These results indicate that a high fraction of technical frass can be obtained when BSFL are fed on former foodstuff-based mixtures. In general, organic waste streams frequently contain impurities (e.g. packaging materials, plastics and microplastics) and possible hazardous chemicals from food packaging materials (e.g. plasticisers, flame retardants, etc.) (Lievens et al., 2021). These can potentially affect the quality and safety of both BSFL derived BSFL meal and BSFL oil, as well as the quality and safety of frass. In the present study, the content of impurities in the frass was extremely low. The N, P, and K profile of technical frass was comparable with other manure types. These results indicate that high quality BSFL frass can be obtained when former foodstuffs are utilized in BSFL production. Although not addressed in the current study, the presence of microplastics does not influence the BSFL growth or waste reduction (Romano and Fischer, 2021).

The current legislation that applies to BSF production can generally be divided into two parts, more precisely in legislation on feed for BSFL, and legislation regarding BSFL as feed ingredient or as a source of biochemical compounds (Lievens et al., 2021). Although bioaccumulation of metals has been observed (Proc et al., 2020), the present study showed levels of heavy metals of both BSFL and technical frass much lower than the EU maximum limits for feed and for organic fertilizer (European Parliament, 2002; European Commission, 2006). However, further studies are required to assess the safety aspect of utilizing former foodstuffs across the EU, since different processing methods are applied across different countries when recycling such resources.

## 5. Conclusions

The implementation of new efficient technologies and practices that enable reduction of food waste and ensure upcycling of nutrients is crucial for a sustainable management of resources in a circular economy paradigm. Currently, former foodstuffs are used for energy production (e.g. biogas), composting or otherwise wasted. The production of BSFL on former foodstuffs conducted at semi-industrial conditions over a period of 1.5 years was highly successful with a very efficient production (FCR: 2.3 to 5.5) of BSFL biomass and technical frass.

The production of BSFL on former foodstuffs-based mixtures and at two different densities was successfully assessed. The use of high fiber bio-pulp or husk to enhance the physical properties of the former foodstuffs (lowering viscosity and enhancing porosity), while securing a high nutrient content, were found to lead to the production of high quality and safe BSFL meal, BSFL oil and technical BSFL frass, which is suitable for further applications, such as feed and soil amendment. A density of 10 larvae/cm$^2$ performed better than 7 larvae/cm$^2$. The utilization of low value waste streams for stabilizing the physical properties of former foodstuffs might have high implications for the production, since this could secure high quantity and quality production outputs. Similar, the increase in larval density, can increase production output per production area. The utilization of former foodstuffs as feed in BSFL production will open for new opportunities and further consolidate this new sector.

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**Highlights**

- Innovative sustainable solutions are needed to upcycle food waste
- Production of *Hermetia illucens* larvae (BSFL) was set up on former foodstuff
- Production of BSFL was more efficient at 10 compared to 7 larvae per cm²
- Heavy metals of BSFL and frass were much lower than the EU maximum limits
- Rearing larvae on former foodstuff leads to high quality insect meal and frass

**Declaration of interests**

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: