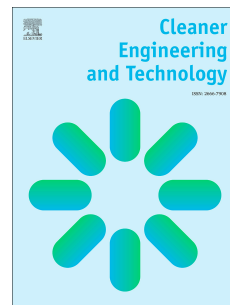


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Pilot scale production of *Hermetia illucens* (L.) larvae and frass using former foodstuffs

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

1 **7647 words**

2 **Abstract**

3 The food and feed sector requires new sustainable sources of protein and innovative solutions
4 for upcycling of food waste (former foodstuffs), which today is downcycled into energy or
5 even wasted. This study aimed at evaluating the use of former foodstuff waste streams as feed
6 substrate for *Hermetia illucens* (L.) larvae (black soldier fly larvae, BSFL) under long-term
7 and semi-industrial conditions. Different foodstuff-based mixtures and different stocking
8 BSFL densities were used during 20 batches, and quality and safety assessments were
9 performed on the main outputs, namely BSFL production performance, frass impurities,
10 larval and frass nutrient profiles and heavy metal content. About 1400 kg of former
11 foodstuffs (fresh weight) were used to produce 239 kg BSFL and 230 kg frass. The
12 production of BSFL reared on former foodstuffs was highly efficient, with feed conversion
13 rates (FCR) ranging between 2.3 and 5.5 (dry matter basis). The optimization experiment
14 revealed that former foodstuffs-based mixture and high larval density (10 larvae/cm²) lead to
15 highly efficient (FCR: 2.6) and heavy metal-free production of BSFL and frass. The quality
16 of the derived BSFL meal was high in terms of protein and amino acids. Furthermore, the
17 quality of the technical frass was high in terms of N, P, and K levels and minimal packaging
18 material residuals (<2.65%). This investigation suggests that nutrients in former foodstuffs
19 can be successfully and safely recycled in production of BSFL.

20

21 **Keywords**

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

24

25

26 1. Introduction

27 A third of the total food produced is known to end up as waste (FAO, 2019). Moreover, it is
28 estimated that by 2050 the global population will increase to 9.7 billion people (FAO, 2019),
29 while the amount of waste generated will reach 3400 billion kg (Silpa et al., 2018), making food
30 waste an unstructured, cross-cutting and persistent problem (Närvänen et al.,
31 2020). The food sector requires innovative and sustainable production systems that can reduce
32 waste and increase nutrient recovery by nutrient cycling. Such a solution can be found in the
33 form of insect bioconversion systems, which can upcycle a high amount of heterogeneous
34 substrates from organic waste streams into high value protein and lipids in form of insect larval
35 biomass, suitable as food and feed. The obtained insect frass (residual substrate from the
36 production) could be successfully used as fertilizer (Bortolini et al., 2020).

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

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

51 The performances of BSFL depend on the quality of the feed and on the production conditions
52 (pH, density, temperature, humidity, etc.) (Singh and Kumari, 2019). As
53 other holometabolous insects, the BSFL are adjusting their growth rate and nutrient accretion
54 with the main goal of accumulating enough reserves for the adult stage and ensuring
55 reproduction (Gold et al., 2018). According to Gold et al. (2018), the rearing of BSFL on a
56 more balanced waste-based feed with ratio of 1:1 of protein (14-19%) to non-
57 fiber carbohydrate (13-15%), showed high improvement in the performances of BSFL
58 compared to the individual waste streams (mill by-products, canteen waste, human feces,
59 slaughterhouse waste, cow manure, vegetable canteen waste). Differences in BSFL
60 performance are also associated to different waste streams, while the protein content (dry
61 matter, DM) of BSFL was found to be similar, ranging between 39% to 44% when reared on
62 food waste, human feces, slaughterhouse waste, fruit and vegetable waste (Lalander et al.,
63 2019). On the contrary, the BSFL lipid content seems to be strongly affected by the feed quality
64 and by the larval density (Barragan- Fonseca et al., 2018). Pre-treated feeding substrates with
65 high amounts of tannins and total phenolic compounds resulted in low-weight larvae, and
66 consequently in a low biomass conversion ratio (Isibika et al., 2019). The utilization

67 of balanced feeds developed on different waste streams could stabilize the BSFL rearing and
68 assure sustainable production of both insect protein and insect oil.

69 The frass from BSF has potential as a sustainable soil amendment (Schmitt and de Vries, 2020),
70 as shown by Setti et. (2019). When compared with composting of untreated food waste,
71 bioconversion by BSFL is more environmentally and economically efficient (Ites et al.,
72 2020). However, when reared on waste streams such as former foodstuffs, the insect frass
73 might contain packaging materials or potential hazardous compounds (e.g. heavy metals)
74 (Lievens et al., 2021). In case of high moist content of such waste stream, the separation of
75 BSFL from the frass might be challenging and time consuming (Lalander et al., 2020).
76 Moreover, one quarter of the biomass production can be lost during this step (Guo et al., 2021).
77 In this study, former foodstuffs are distinguished from general food waste and household waste
78 by being defined as pre-consumer food products of plant or animal origins, which were
79 intended to be used as food. This may be an important feature for feed substrates in the
80 legislative regulation of future BSFL production.

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

89

90 **2. Materials and methods**

91 *2.1 BSF colony*

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

97

98 *2.2 Long-term BSFL production*

99 Twenty consecutive batches (B1-B20) of BSFL and frass were produced on former foodstuffs
100 over a period of 1.5 years at the Danish Technological Institute, Aarhus. The production was
101 performed at pilot scale, using trays of 60 x 40 x 20 cm, under controlled condition at 27±1°C
102 and a relative humidity 45±9%. Fresh former foodstuff was multiple collected from Daka
103 ReFood (Hedensted, Denmark). The biomasses originate typically from food production
104 industry, central warehouse, supermarkets, and dairy production. Currently the former
105 foodstuff is processed into a pulp to be used for biogas production. During the fractionation

106 processing step, the former foodstuffs, including packaging materials, are loaded into a pulper
 107 and mixed with water. Consequently, the biomass originate typically from food production
 108 industry, central warehouse, supermarkets, and dairy production are squeezed through
 109 perforated separation plate (2 mm sieves), and thus divided into 2 fractions: large fragments
 110 (>2mm), consisting mainly of fibers and packaging residues, and small fractions (<2mm)
 111 consisting mainly of organic materials called bio-pulp.

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

115 **Table 1.**

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

117

| 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 |

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

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

126 feeding strategies, feed enhancement with other waste streams, substrate porosity and viscosity,
 127 separation procedures etc.), and to optimize the production.

128

129 2.3 Feed optimization and BSFL density experiment

130 2.3.1 Experimental design

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

142

143 **Table 2.**

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

146

| | 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 |
|------------------|------|------|------|
|------------------|------|------|------|

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

148 About 14,000 and 20,000 6 days old BSFL belonging to the two larval densities (7 and 10
149 larvae/cm²) were placed in plastic trays (40 x 60 x 20 cm, N=30) and fed with a total of 0.4 g
150 fresh substrate per larva for 10 days until the first prepupae were observed. The mixtures
151 were administrated in two tranches: 75% at the beginning of the experiment and the remaining
152 25% during day 8 of the experiment. The trays were maintained under controlled laboratory
153 conditions (26.88 ± 0.84°C and 60 % relative humidity) and rotated every second day until
154 harvest, to ensure similar conditions in all trays.

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

171

172 2.3.2 Production performances

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

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

184

$$185 \text{ Substrate reduction (\%)} = 100 - \left(\frac{\text{Total frass (g)}}{\text{Total feed (g)}} * 100 \right) \quad \text{eq. 1}$$

186

187 *2.3.3 Product quality*

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

191

$$192 \text{ Protein content of BSFL meal (\%)} = \frac{\text{BSFL protein content (\%)} * \text{BSFL biomass (g DM)}}{\text{BSFL meal (g DM)}} \quad \text{eq. 2}$$

193

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

197

198 *2.3.4 Product safety*

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

$$206 \text{ Impurity content (\%)} = \frac{\text{packaging residue (g)} * 100}{\text{Technical frass sampel (g)}}$$

207

208 *2.4 Statistics*

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

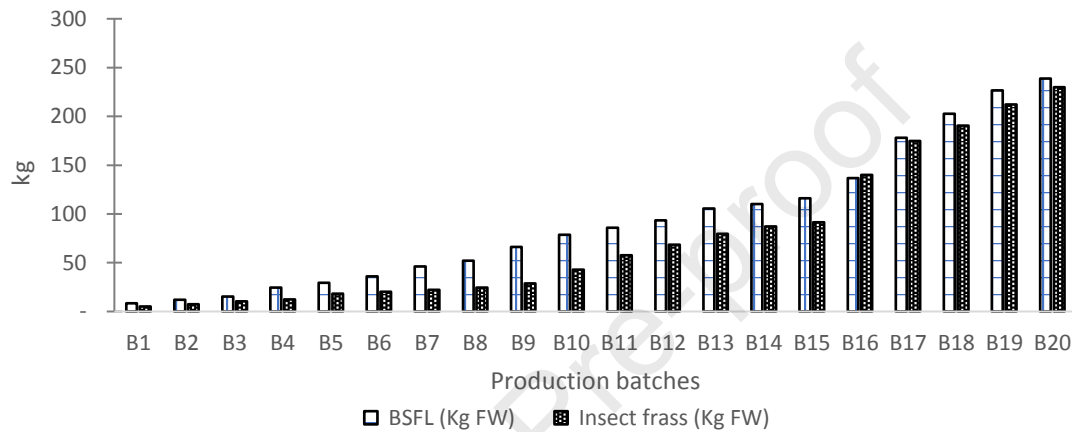
217 The BSFL biomass, meal, oil, survival rate, FCR, substrate reduction, total and technical
218 frass data sets were analyzed with two-way ANOVA tests with feed mixture and density as
219 factors followed by Tukey's post-hoc tests for multiple comparisons. Prior to the statistical
220 analysis, the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

224

225 **3. Results**

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

230

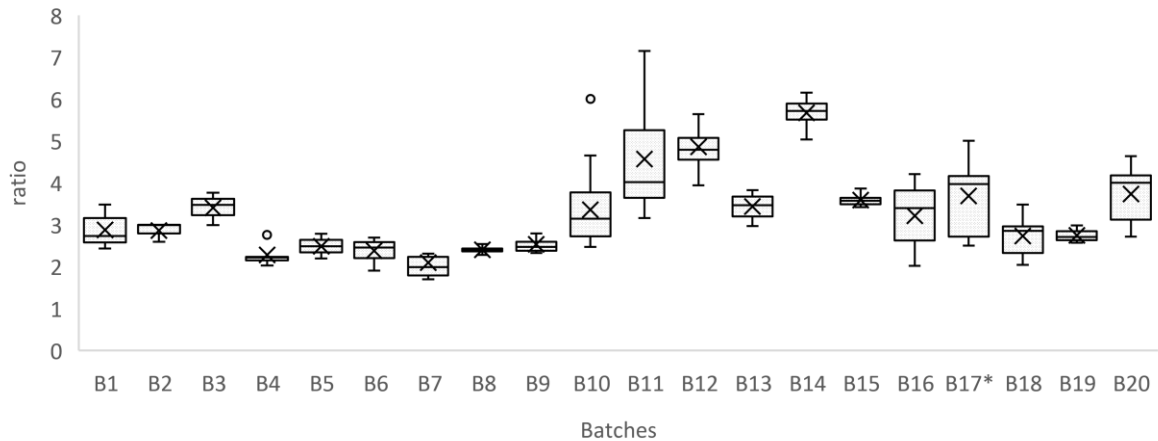


231

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

234

235 The FCR was found to vary across the production batches, ranging between 2.3 for B4 and
 236 5.5 for B14. Moreover, a high variation was seen across multiple batches (B10, B11, B16,
 237 B17, B18 and B20) due to different experimental parameters being altered (i.e., larval density
 238 and former foodstuffs quality) during the production optimization period (Fig. 2).



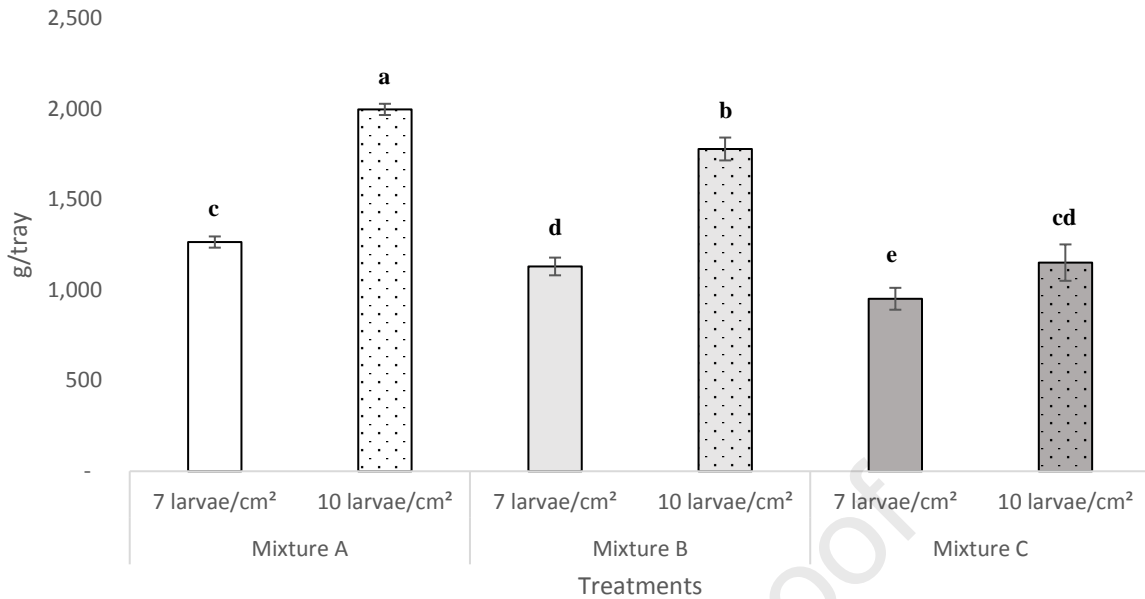
239

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

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

245 Overall, the production performances were affected by different treatments. As such, the larval
 246 density had a great impact on the production of larval biomass ($p < 0.01$), BSFL meal ($p <$
 247 0.01), BSFL frass ($p < 0.01$), technical frass ($p = 0.01$) and on the substrate reduction ($p <$
 248 0.01). The feed mixture and the interaction of feed mixture and larvae density had a highly
 249 significant impact on all the production performances ($p < 0.01$).

250 The production of BSFL biomass at all density and feed mixture treatments ranged between
 251 2,000 g/tray for mixture A at high density, and 950 g/tray for mixture C at low density. A higher
 252 larval density resulted in significantly larger BSFL biomass production. Moreover, the largest
 253 biomass production was obtained on mixture A, followed by mixture B and lastly mixture C.
 254 There was an interaction between density and waste mixture on the biomass production: The
 255 biomass production obtained on mixtures A and B at high density were high compared to the
 256 biomass production on the same mixtures at lower density. Such difference was less evident
 257 when the biomass production on mixture C was compared between the two different densities
 258 (Fig. 3).

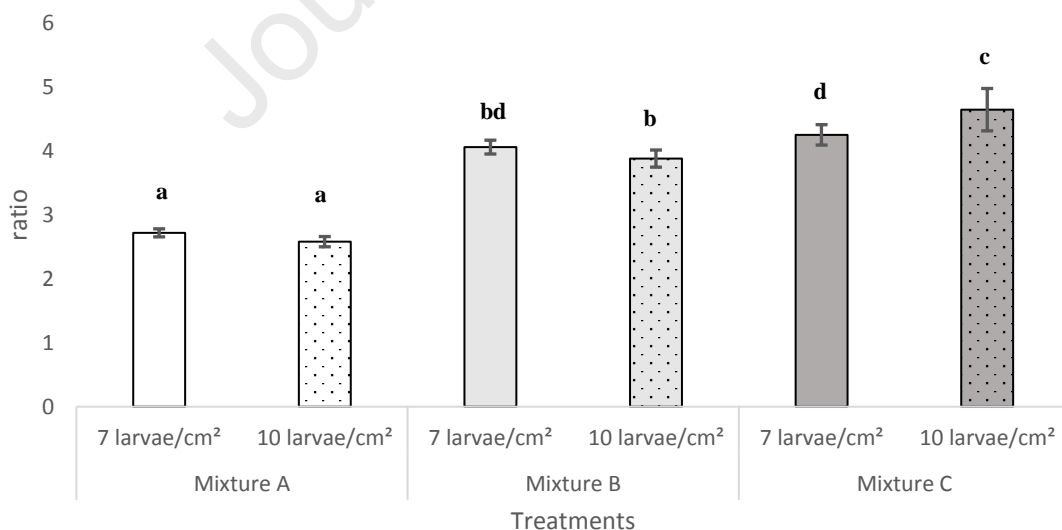


259

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

263

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

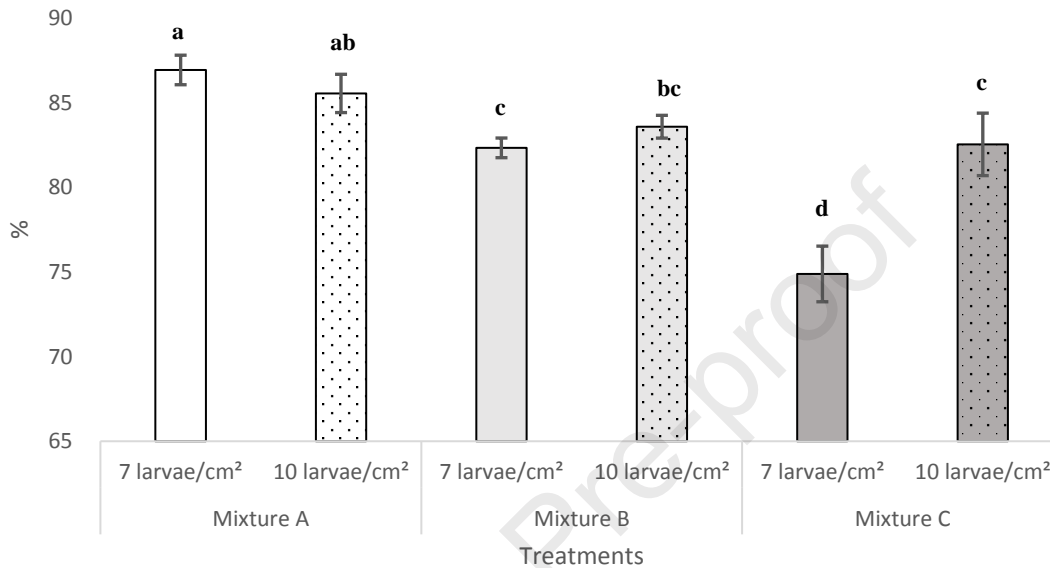


268

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

272

273 The substrate reduction was high across the different treatments, with the greatest reduction
 274 (86-87%) being obtained when BSFL were reared on mixture A at both densities, and the
 275 lowest reduction (75%) being achieved when BSFL were reared on mixture C at low density.
 276 The substrate reduction was affected by both density, waste mixtures and the interaction
 277 between these treatments (Fig. 5).

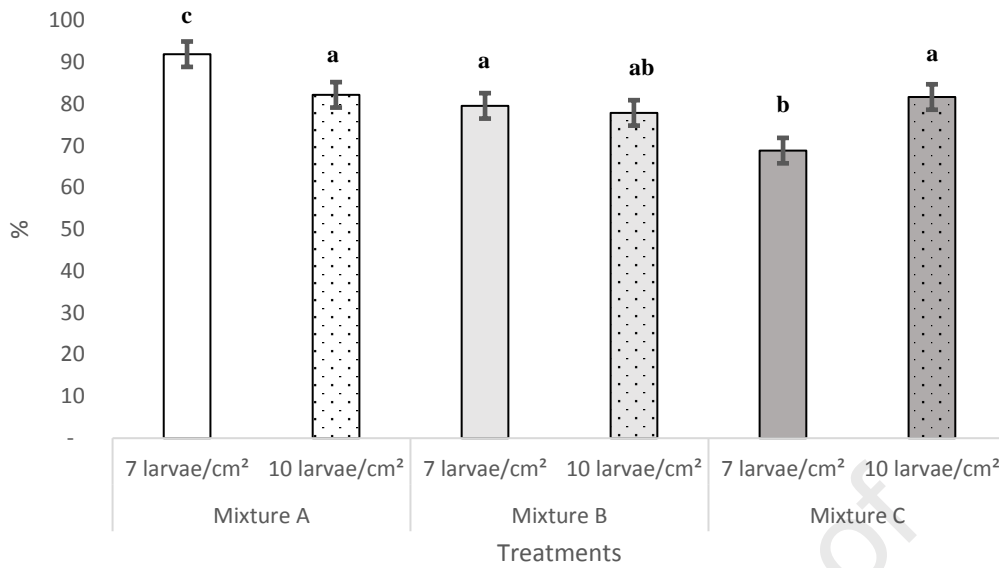


278

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

282

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

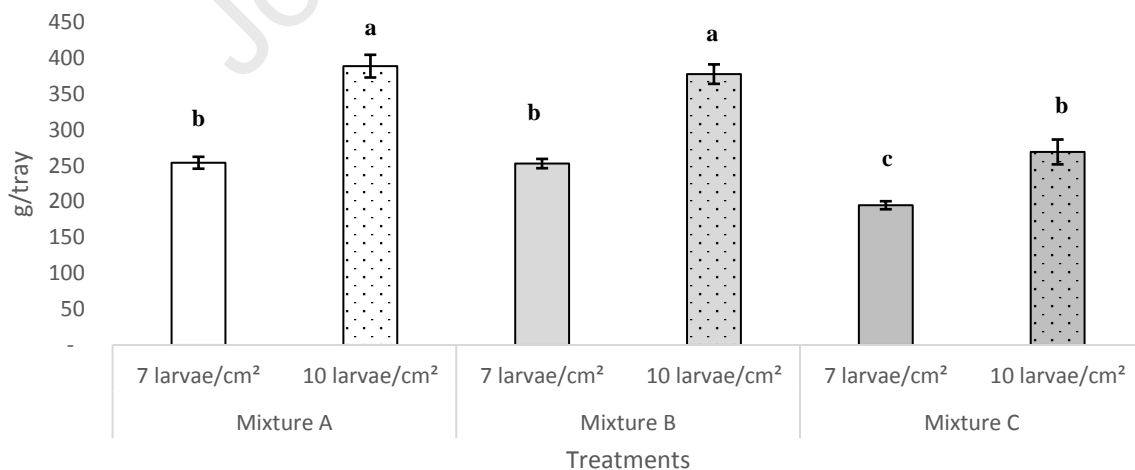


288

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

292

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

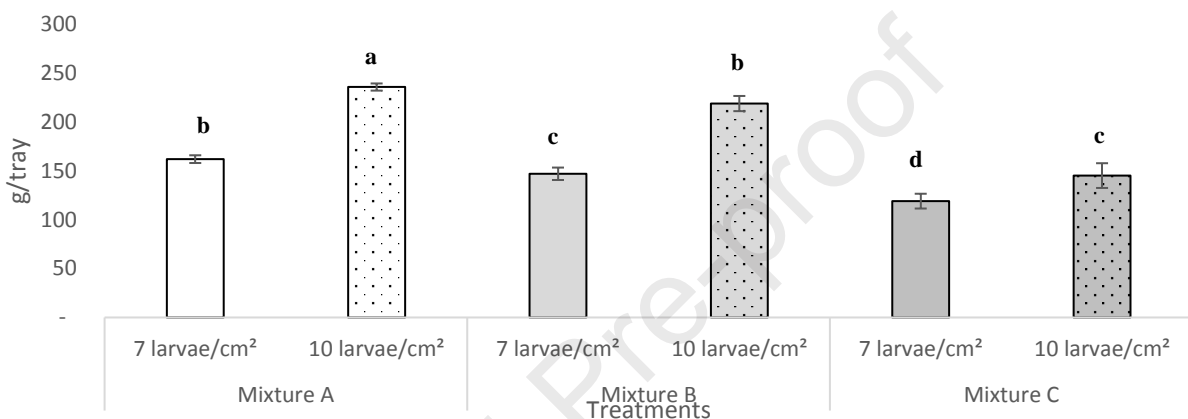


299

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

303

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

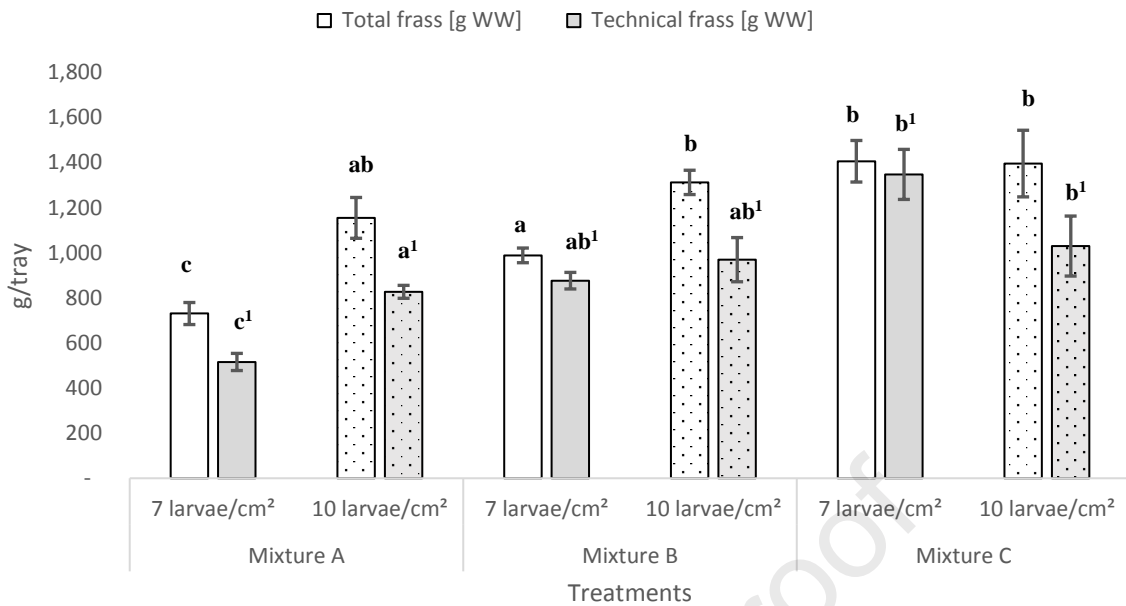


312

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

316

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

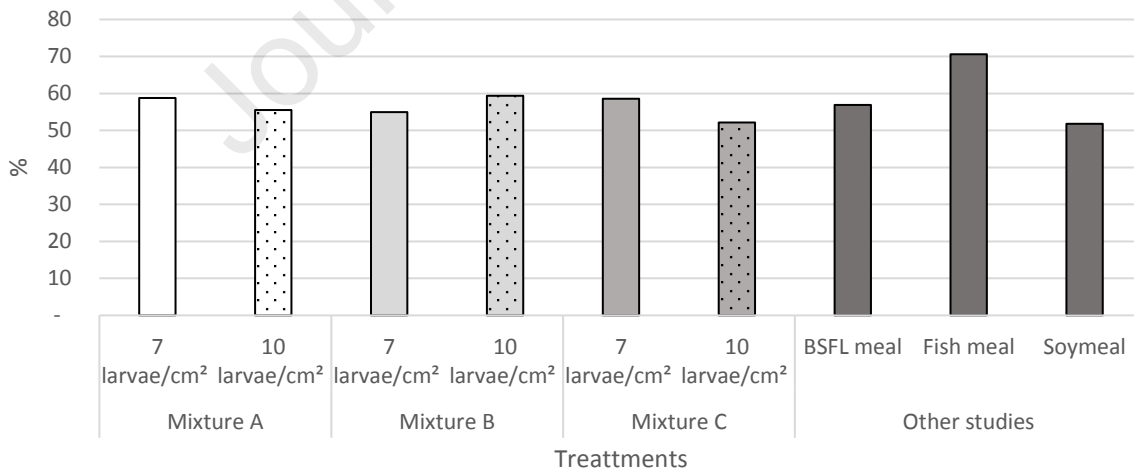


323

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

327

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



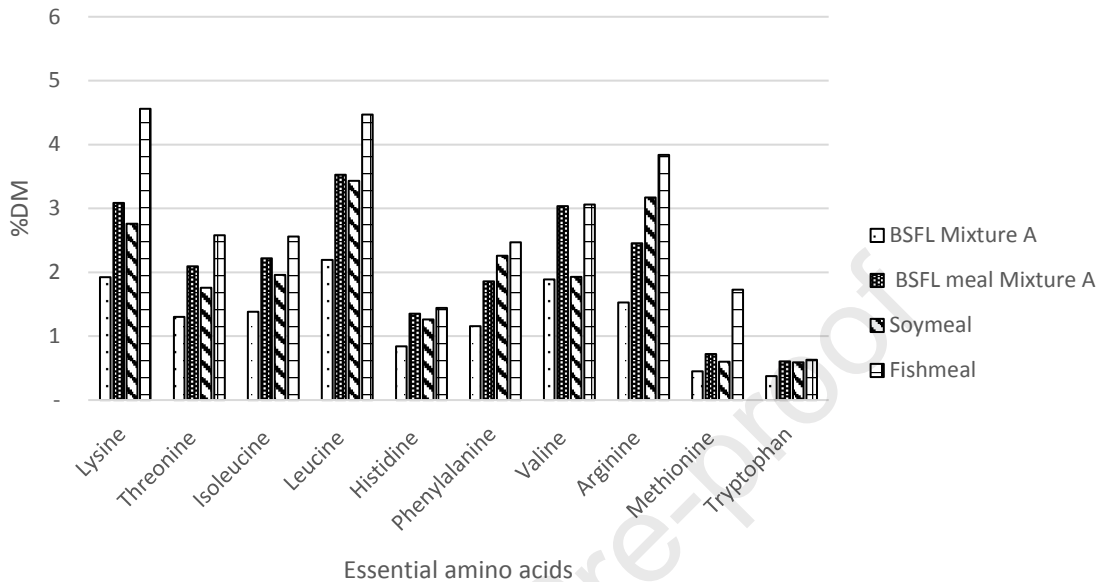
331

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

335

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

339

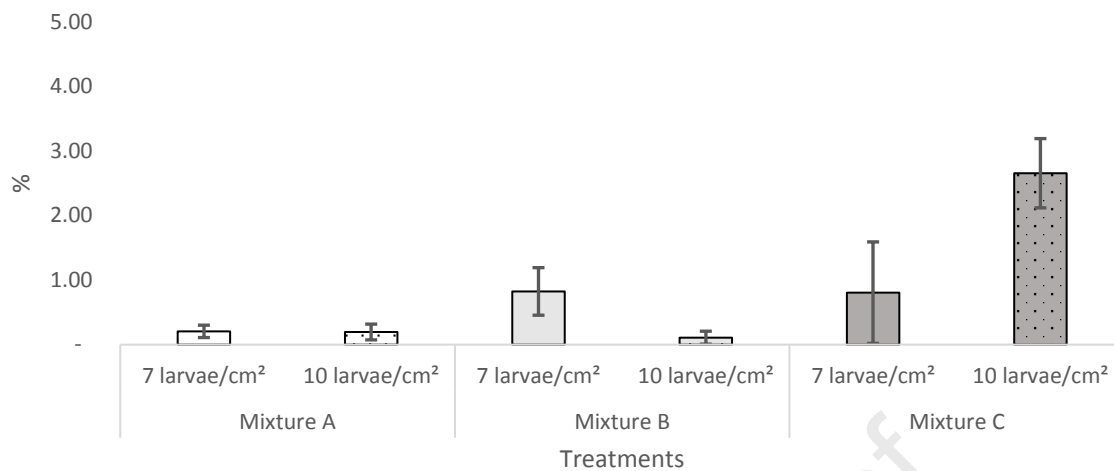


340

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

343

344 The impurity content in the technical frass was small across the treatments, ranging from
 345 0.11% for mixture B at high density, to 2.65% for mixture C high density treatments (Fig.
 346 12).



347

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

350

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

354 Table 3.

355 Nitrogen (N), phosphorus (P) and potassium (K) in frass in comparison with different types
 356 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 |

357

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

360 **Table 4.**

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

| | | <i>Arsenic</i> (mg/kg DM) | <i>Cadmium</i> (mg/kg DM) | <i>Mercury</i> (mg/kg DM) | <i>Lead</i> (mg/kg DM) |
|---------------|---|---------------------------------|---------------------------------|---------------------------------|------------------------------|
| <i>Larvae</i> | 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 |
| <i>Frass</i> | 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 |

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

364

365 4. Discussion

366 The production of BSFL on former foodstuffs showed high performances in terms of both
 367 larval biomass production and FCR. The FCRs obtained when feeding BSFL with former
 368 foodstuffs (FCR: 2.3 to 5.5) were more efficient compared to rearing BSFL on vegetable waste
 369 (FCR: 9.29) (Giannetto et al., 2020), municipal organic waste (FCR: 5.8) (Diener et al., 2011),
 370 industrial by-products (bakery waste, fish trimmings, fish waste, brewery grain and yeast, sugar
 371 beet pulp and cheese waste) (FCR: 4.84 to 20.54) (Magee et al., 2021), animal manure
 372 (FCR:5.6 to 10.3 on poultry and dairy manure) (Rehman et al., 2017), and it was similar when
 373 feeding BSFL with catering waste and household waste (FCR:1.7 to 3.6) (Gligorescu et al.,
 374 2020). It is important to underline that lower BSFL performances (in terms of FCR) were
 375 registered during production of batches B10-15. These batches were produced at the beginning
 376 of the lockdown period caused by the Covid-19. A high degree of BSFL migration was
 377 observed during the production of these batches, where the bio-pulp had a high lipid content
 378 and increased viscosity (Table 1). This could have an impact on BSFL, since increased
 379 viscosity can impede the larvae to move freely or even breath inside the feeding substrate
 380 (Klammsteiner et al., 2021). Further studies are required to understand the impact of viscosity
 381 and other physical characteristics (substrate porosity) on the performances of BSFL.

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

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

407 The highest estimated meal production was obtained on both mixtures A and B at higher larval
408 density, while the greatest BSFL oil production was supported by mixture A at high density.
409 The crude protein content of BSFL reared on the different former foodstuff formulations was
410 similar, ranging from 52 to 59%, suggesting it was diet-independent (Ravi et al., 2020;
411 Spranghers et al., 2017). However, according to Fuso et al. (2021), when BSFL were reared
412 exclusively on vegetable by-products, the total protein content varied between 35% and 49%
413 and was mainly correlated to fibre and protein content in the diet. The crude protein content of
414 BSFL meal estimated for mixture A at high density (56%) was comparable to other BSFL meal
415 (57%), higher than soybean meal (52%) but lower than fishmeal 71% (Makkar et al., 2014).
416 The essential amino acid content revealed that BSFL meal obtained on mixture A had an overall
417 higher essential amino acid content than soybean meal but lower content than fishmeal. These
418 results indicate that a high quality BSFL meal and BSFL oil suitable for feed can be obtained
419 when rearing BSFL on former foodstuffs. For high-performing laying hens, the BSFL meal
420 and oil can replace completely the soybean-based feeds (Heuel et al., 2021), while as a potential
421 fish meal substitute, a diet composed by 25% BSFL meal and 75% fish meal was recommended
422 for suitable growth performance of birds, or a 100% BSFL meal inclusion rate for the most
423 cost-effective feed (Sumbule et al., 2021). The outlook for BSFL as a protein replacement for
424 fishmeal in salmonids diets is more sustainable, presenting an optimal growth of up to 200 g/kg

425 diet (English et al., 2021); the dietary inclusion of BSFL meal on other fish species also presents
426 encouraging results (Mousavi et al., 2020). New research looking at the application of BSFL
427 meal derived from the production of BSFL on former foodstuffs should be considered in the
428 future.

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

443 The current legislation that applies to BSF production can generally be divided into two parts,
444 more precisely in legislation on feed for BSFL, and legislation regarding BSFL as feed
445 ingredient or as a source of biochemical compounds (Lievens et al., 2021). Although
446 bioaccumulation of metals has been observed (Proc et al., 2020), the present study showed
447 levels of heavy metals of both BSFL and technical frass much lower than the EU maximum
448 limits for feed and for organic fertilizer (European Parliament, 2002; European Commission,
449 2006). However, further studies are required to assess the safety aspect of utilizing former
450 foodstuffs across the EU, since different processing methods are applied across different
451 countries when recycling such resources.

452

453 **5. Conclusions**

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

460 The production of BSFL on former foodstuffs-based mixtures and at two different densities
461 was successfully assessed. The use of high fiber bio-pulp or husk to enhance the physical
462 properties of the former foodstuffs (lowering viscosity and enhancing porosity), while securing
463 a high nutrient content, were found to lead to the production of high quality and safe BSFL
464 meal, BSFL oil and technical BSFL frass, which is suitable for further applications, such as
465 feed and soil amendment. A density of 10 larvae/cm² performed better than 7 larvae/cm². The

466 utilization of low value waste streams for stabilizing the physical properties of former
 467 foodstuffs might have high implications for the production, since this could secure high
 468 quantity and quality production outputs. Similar, the increase in larval density, can increase
 469 production output per production area. The utilization of former foodstuffs as feed in BSFL
 470 production will open for new opportunities and further consolidate this new sector.

471

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479

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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

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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:

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